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Full Length Research Paper

Evaluation of the antioxidant activity, antimicrobial effect and acute toxicity of leaves from *Allophylus edulis* (A. St.-Hil., A. Juss. Cambess &.) Hieron. ex Niederl

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***Allophylus edulis* is a Brazilian plant commonly used in the mid-west region of Brazil for treatment of disorders related to oxidative stress such as diabetes, inflammation, hypertension and digestive diseases. The aims of the present study were to quantify flavonoids and phenolic compounds, evaluate the antioxidant activity, antimicrobial effect and acute toxicity of leaves of *A. edulis*. Ethanolic (EEAE) and aqueous (AEAE) extracts of *A. edulis* were prepared. The antioxidant activity was determined by 2.2-diphenyl-2-picrylhydrazyl (DPPH) free radical scavenging, lipid peroxidation and oxidative hemolysis induced by 2.2'-azobis (2-amidinopropane). The antimicrobial assays of diffusion on solid media and broth microdilution were performed against *Staphylococcus aureus*, *Escherichia coli* and *Candida albicans.* The acute toxicity was assessed in Wistar rats treated with doses of 2 and 5 g/kg of body weight. The EEAE presented higher concentrations of flavonoids and phenolic compounds, and higher activity of scavenge DPPH free radicals. In addition, it was more effective against *S. aureus* compared to AEAE. The extracts were unsuccessful against *E. coli* and *C. albicans*. The EEAE prevented the lipid peroxidation in human erythrocytes and inhibited oxidative hemolysis in all the concentrations assessed. During the evaluation of acute toxicity, the dose of 5 g/kg of body weight increased hepatic mass. Together, these results demonstrated that the EEAE of leaves of *A. edulis* is more effective than the AEAE, showing antioxidant activity and antimicrobial effect against *S. aureus*, as well as low toxicity.**

Key words: Cocum, native Brazilian plant, lipid peroxidation, DPPH, AAPH, thiobarbituric acid reactive substances (TBARS), malondialdehyde, Sapindaceae.

INTRODUCTION

Medicinal plants have been used for centuries to treat numerous diseases around the world (Garg et al., 2012). The biological activity of extracts from medicinal plants is associated with the presence of phytochemical components, many of which have been studied to develop new drugs (Calixto, 2005). The ethnopharmacological approach is an important method of investigating the properties of medicinal plants. Indeed, it is reported that almost 80% of the compounds isolated from medicinal plants by the pharmaceutical industry were obtained from information from folk medicine (McClatchey et al., 2009).

There is a growing interest in substances derived from medicinal plants with antioxidant capacity, such as tannins, flavonoids and other phenolic compounds that can eliminate free radicals (Burda and Oleszek, 2001; Casagrande et al., 2014). Free radicals are highly reactive substances that can induce the oxidation of molecules, leading to cellular and tissue damage. The presence of these substances increases the risk of developing several diseases (as diabetes, cancer, inflammatory and cardiovascular diseases) and promotes the premature aging process (Burton and Jauniaux, 2011). Antioxidant compounds can protect the organism through different mechanisms, such as reducing the lipid peroxidation of cell membranes and the damage to proteins and DNA (Farber, 1994; Halliwell, 1992).

Although antioxidant activity has been extensively studied (Raposo et al., 2014), the scientific community's interest in medicinal plants has also increased due to their antimicrobial properties. Indeed, it is known that various components of plants are potential antimicrobial agents (Cowan, 1999). However, in order to ensure the safety of the medicinal use of any plant species, its toxicity should first be investigated. *Allophylus edulis* is a native Brazilian plant that is popularly known as *"chal chal","cocum*", "*vacum*" and "*fruto do pombo*" it also occurs in the Uruguay, Bolivia, Argentina and the Guayanas (Díaz et al., 2014). Its leaves are used for their antihypertensive, digestive, anti-inflammatory and healing purposes, particularly in the Midwest region of Brazil (Abreu et al., 2005; Alves et al., 2008). Although this plant has been frequently used by the population, there are no existing reports on their biological properties. Therefore, the aim of the present study was to evaluate the antioxidant and antimicrobial properties, as well as the toxicological risk, of the extract of leaves of *A. edulis*.

MATERIALS AND METHODS

Reagents

Folin-Ciocalteau and ethanol PA (Chemical Dynamic®); sodium carbonate, butyric alcohol, gallic acid, aluminum chloride, trichloroacetic acid (Vetec®); quercetin (Sigma-Aldrich®); malondialdehyde and thiobarbituric acid (Merck®); 2,2`-azobis (2-amidinopropane) (AAPH) and and 2,2-diphenil-1-picrylhydrazil (DPPH•) (Sigma-Aldrich®); ascorbic acid (Proquímios®) and butyl hydroxy toluene (BHT) (Via farma®); Mueller Hinton agar and Mueller Hinton broth (Merck Brasil®).

Plant material and preparation of extracts

The leaves of *A. edulis* were collected in April, 2011, in native Cerrado located at 424 m altitude, latitude 22° 05' 545'' and longitude 055° 20' 746'', in the countryside of Dourados, Mato Grosso do Sul/Brazil. The species was identified by botanist and deposited in the Herbarium of the Federal University of Grande Dourados (UFGD). The species has been cataloged in the voucher specimen number 4676. After drying in air-circulation oven (40°C) for seven days, the leaves were pulverized in a Wiley mill. For each gram of powder 10 ml of 80% ethanol or distilled water in the ratio of 1:10 was added. The ethanolic extract from *A. edulis* was kept on maceration, under constant stirring and at room temperature, for four weeks; the aqueous extract of *A. edulis* kept macerating under constant stirring at 4°C for one week. After that, the extracts were filtered through filter paper, rotaevaporated and lyophilized. The yield of extracts (in percentage) was calculated by the expression: R (%): (dry extract mass/dry plant material mass) × 100. The dried extracts were kept in a freezer (-20°C) for subsequent studies. The dry extract obtained from the ethanol solvent was named EEAE and the one obtained from water was called AEAE.

Determination of total polyphenols

The content of total polyphenols was determined by Folin-Ciocalteau method described by Meda et al. (2005). An aliquot of 0.5 ml (200 µg/ml of EEAE and AEAE) was mixed with 2.5 ml of Folin-Ciocalteau reagent prepared in water (1:10). After 5 min incubation, 2 ml of aqueous solution of sodium carbonate (14%) was added to the solution. After 2 h at room temperature, the reading on spectrophotometer (T 70 UV/VIS spectrometer PG Instruments®) was performed at a wavelength of 760 nm. The quantification was performed using a calibration curve with gallic acid standard (0.4 to 11 µg/ml). Ethanol was used as a blank. The analytical curve was plotted using GraphPad Prism 3.0 software being implemented as the linear regression and the equation of the straight line was obtained (y = a + b.x) by correlating the concentration of gallic acid and the absorbance of each sample. The results were expressed in mg of gallic acid equivalents (GAE) per 100 mg of extract. The assay was performed in triplicate.

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Determination of total flavonoids

To determine the levels of total flavonoids, the methodology was kept by using the aluminum chloride described by Liberio et al. (2011), with some modifications. An aliquot of 0.5 ml (200 µg/ml of EEAE and AEAE) solubilized was added to 4.5 ml of a solution of hexahydrated aluminum chloride solution (2%). After 30 min rest at room temperature, the absorbances were read on spectrophotometer (T 70 UV/VIS spectrometer PG Instruments®) at a wavelength of 415 nm. To determine the concentration of flavonoids, a calibration curve was prepared using quercetin as standard (0.4 to 11 µg/ml). Methanol was used as a blank. The analytical curve was plotted using GraphPad Prism 3.0 software being implemented as the linear regression and the equation of the straight line was obtained (y = a + bx) by correlating the concentration of quercetin and the absorbance of each sample. The results were expressed in mg of quercetin equivalents (QE) per 100 mg of extract. The assay was performed in triplicate.

Determination of the presence of saponins

For this test, 10 mg of each extract (EEAE and AEAE) was solubilized in 2 ml of 80% ethanol, and then 5 ml of boiling water was added to the mixture. After cooling, it was vigorously stirred, and was followed by a rest period of 20 min. The presence or absence of foam was visually observed. The presence of foam indicates the presence of saponins in the extracts (Barbosa et al., 2004).

Antioxidant assays

DPPH free radical scavenging activity

The method of scavenging of free radical 2,2-diphenyl-1-picrylhydrazyl (DPPH) was used to determine the antioxidant activity, as described by Gupta and Gupta (2011), with some modifications. A volume of 0.2 ml of the extracts (EEAE and AEAE), solubilized in 80% ethanol at different concentrations (0.1 to 1000 g/ml), was homogenized in 1.8 ml of a DPPH (0.11 mM) solution. After 30 min, at room temperature and protected from light, the reading on spectrophotometer at wavelength of 517 nm was performed. Antioxidants, ascorbic acid and butylated hydroxytoluene (BHT), were used as standard in the same concentrations of the extracts. 80% ethanol was used as a blank. To determine the percentage of antioxidant activity, the following equation was used:

Inhibition of DPPH free radical (%) = (1 - Absorbance sample/Absorbance control) × 100

For the absorbance control DPPH (0.1 mM) was used. Three independent experiments were performed in duplicate. The IC_{50}, concentration capable of reducing by 50% the initial concentration of DPPH, was calculated by nonlinear regression after determining the antioxidant activity curve.

Inhibition of lipid peroxidation assay

After approval by the Ethics Committee of the University Center of Grande Dourados (Unigran), Brazil (CEP No. 123/12), 5 ml of blood from nonsmoking healthy adults was collected, and lipid peroxidation was determined by measurement of malondialdehyde (MDA) formed as described in Campos et al. (2014). Erythrocytes induced to lipid peroxidation by AAPH were used to assess the protective effects of EEAE and of ascorbic acid. The erythrocytes were washed three times with saline (0.9% NaCl). A suspension of these erythrocytes was prepared (5% final hematocrit) and an aliquot of 0.25 ml was homogenized with 0.25 ml extract (EEAE) and ascorbic acid at different concentrations (100 to 175 µg/ml). After 30 min in water bath at 37°C, 0.5 ml of AAPH solution (50 mM) was added. After 3 h in water bath at 37°C with constant stirring, an aliquot of 0.5 ml of supernatant was added to 0.5 ml of trichloroacetic acid (20%). Then, 0.5 ml of the solution was added to 1 ml of thiobarbituric acid (TBA) (10 nM) solution. The homogenate was kept in a water bath at 94°C for 45 min. After 45 min the samples were kept at room temperature for 15 min for cooling, followed by addition of 4 ml of butane with subsequent stirring and centrifugation. The reading of the supernatant absorbance was performed on spectrophotometer (532 nm). Lipid peroxidation was determined by quantification of MDA. The calculation for the amount of MDA in the sample was obtained by the formula:

MDA = Absorbance of samples × (20 × 220.32/Absorbance 1,1,3,3-tetrahydroxipropane standard)

The results were expressed in nmol/ml. The experiment was accomplished in duplicate.

Inhibition oxidative hemolysis induced by 2,2- diphenyl-2-picryl hydrazyl assay

For this, the method for inducing hemolysis by 2,2- diphenyl-2-picryl hydrazyl (AAPH), described by Valente et al. (2011), with some modifications was used. We used an erythrocyte suspension with a final hematocrit of 2.5%. Erythrocytes were preincubated at 37°C for 30 min in the presence of different concentrations of EEAE and ascorbic acid (100 to 175 µg/ml). After this period, 0.5 ml of AAPH solution (50 mM) was added. The mixture was incubated for 240 min in a water bath at 37°C, with periodic stirring. The hemolysis was determined spectrophotometrically at 540 nm, and aliquots for the determination of hemolysis were taken every 60 min of incubation, diluted in saline and centrifuged at 3600 rpm for 10 min. The percentage of hemolysis was determined using the formula: A/B × 100 (A) abs. of the sample and (B) total hemolysis (erythrocytes with distilled water). All experiments included: negative control (erythrocytes in 0.9% saline), control of extracts and ascorbic acid (erythrocytes in 0.9% saline solution with EEAE and ascorbic acid in different concentrations in the presence and absence of AAPH), solvent control (erythrocytes in 0.9% saline solution with 1% ethanol solvent). Two independent experiments were performed in duplicate.

Antimicrobial assay

For the antimicrobial activity of the extracts (EEAE and AEAE) the diffusion assay method was kept in a solid medium from the hole, and also the broth microdilution method, as described by Mokale et al. (2011) with some modifications and observing the recommendations of the standard M100 S5 of the National Committee for Clinical Laboratory Standards (NCCLS/CLSI) (2005).

All strains were purchased from the American Type Culture Collection (ATCC). The identification of microorganisms was confirmed by the Laboratory of Mycology, Department of Mycology of the University Center of Grande Dourados, Unigran, Dourados, MS, Brazil. Three microorganisms were used: gram-positive bacterium *Staphylococcus aureus* (ATCC: 25923), a gram-negative bacterium *Escherichia coli* (ATCC: 8739) and the fungus *Candida albicans* (ATCC: 10231). Microbial inocula were prepared in 0.9% physiological solution and its density was adjusted according to McFarland turbidity standard scale 0.5 (5×10^5 CFU/ml). Fungal and bacterial suspensions were homogenized and sown with a sterile swab and on the surface of Petri plates containing the culture medium agar Mueller Hinton (AMH). With the aid of sterile stainless steel tubes, holes of 6 mm diameter were made in culture medium. The holes were filled with 0.1 ml of the extract (300 mg/ml). The plates were kept for 1 h at room temperature for diffusion of the extracts. Then the plates were incubated in an oven maintained at 37°C for 24 and 48 h. As positive controls, we used tetracycline for bacteria and ketoconazole for fungus, both at a concentration of 4 mg/m. The solvent ethanol 80% was used as a negative control. The experiment was performed in triplicate.

Minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC)

The extracts were submitted to a broth microdilution assay for determining the minimum inhibitory concentration and minimum bactericidal concentration according to Bussmann et al. (2010), with some modifications and observing the recommendations of standard M100 S5 of the National Committee for Clinical Laboratory Standards (NCCLS/CLSI) (2005). Sterile plastic microplates containing 96 wells with 100 µl of the culture medium AMH were used. The initial wells of the microtiter plate received a 100 µl aliquot of the extracts (300 mg/ml). Then, a serial dilution of the extract was preformed, resulting in concentrations achieved from 1.50 to 1.56 mg/ml. At the end, 100 µl of the bacterial inoculum in the concentration 5×10^5 CFU/ml (0.5 McFarland scale) was added to all wells except to the sterility control of the medium (HAM only). In each microplate was used a negative control (80% ethanol). The microplates were incubated in greenhouse at 37°C for 24 h. After this period of time, the resulting turbidity was evaluated in a microplate reader (TP Reader NM, Thermo Plate®) at 620 nm. The $CIM_{100\%}$ was defined as the lowest concentration of the extract able to inhibit microbial growth. Two independent experiments were performed in triplicate. To determine the MBC, an aliquot of 20 µl was removed from the wells of the determined concentration as MIC and, at least, two upper levels to it, and transferred to Petri plates with HAM medium. The plates were incubated for 24 h at 37°C. MBC was defined as the lowest concentration that produced negative subculture. This method determined bacteriostatic (lowest concentration that inhibit of bacterial growth) and bactericidal (lowest concentration that kills a bacteria) effects of the antimicrobial agents (Bakker-Woudenberg et al., 2005).

Acute toxicity assay

Animals

After approval by the Ethics Committee on the use of animals (CEUA) of Unigran, number 015/12, this study followed international protocols of the guide for animal testing of chemical substances of the Organization for Economic Co-operation and Development (OECD) number 425 (2008). The animals were obtained from School of Environmental and Biological Sciences of UFGD, and were kept under conditions of controlled temperature and humidity, fed with ration (purine-Labina®) and water *ad libitum*.

Experimental model

Twelve Wistar rats weighing an average of 226.7 ± 4.6 g were used. The animals were randomly divided into 4 experimental groups, each of them containing 3 animals. The experimental groups were: C = control group that received water; TC = control group that received a solution of Tween 80 (20%); LD = experimental group that received 2 g/kg and HD = experimental group that received 5 g/kg of EEAE solubilized in Tween 80 (20%). A single dose by gavage was administrated. After the administration, and daily throughout the experimental period, the presence or absence of clinical toxicity signs (piloerection, tremors, excitability, irritability, muscle contraction, salivation, and death) was observed. The variation in body weight and food and water consumption was evaluated three times a week. On the 15th day, after fasting for 12 h, euthanasia was performed. Organs and tissues were macroscopically examined and stored at -20°C. Blood samples were obtained for haematological and biochemical analysis (Asare et al., 2011).

Statistical analysis

Data were expressed as mean ± standard error of the mean (SEM). Analyses were performed using variance analysis (ANOVA) followed by Tukey's test or Student *t* test. The level of significance was set at $p < 0.05$. GraphPad Prism 3.0 software was used.

RESULTS

Preparation of the extracts, determination of phenolic compounds, flavonoids and saponins

The EEAE and AEAE presented a yield of 8.8 and 10.0%, respectively, after extraction with the solvent. The concentrations of phenolic compounds were 17.6 ± 0.6 and 9.0 ± 0.2 mg GAE/100 mg, respectively. Conversely, the concentrations of flavonoids were 2.0 ± 0.3 and 1.0 ± 0.2 mg QE/100 mg, respectively. Saponins were not detected in any of the extracts.

DPPH free radical scavenging activity

The extracts and controls were assessed at different concentrations (0.1 to 1000 µg/ml). The 50% inhibitory concentration (IC_{50}) and the maximal activity of DPPH free radical scavenging are shown in Table 1. These data showing that the EEAE exhibited an antioxidant capacity 4.5 times lower than that of ascorbic acid and 0.6 times higher than that of BHT. On the other hand, the AEAE presented an antioxidant capacity 1.4 times lower than

Table 1. DPPH radical scavenging activity (%) of ethanolic (EEAE) and aqueous extracts (AEAE) of A. edulis at different concentrations (µg/ml) and IC_{50} values.

Treatments	0.1	1	5	10	50	100	500	1000	IC_{50}
Ascorbic acid	1.8±1.2	10.2±4.2	48.5±3.7	94.2±2.3	95.2±1.3	97.7±0.3	96.0±0.9	96.3±0.8	3.9±0.8
BHT	6.0±3.6	8.2±2.4	19.3±4.6	31.7±7.4	69.3±9.5	81.0±5.3	95.2±0.7	95.0±0.6	31.5±1.9
EEAE	6.2±3.0	6.8±2.3	13.8±3.2	27.8±2.3	91.8±1.9	96.0±0.8	91.3±1.8	94.5±2.5	17.7±2.6
AEAE	2.8±1.0	6.0±1.4	10.0 ±4.9	13.8±1.4	47.2±2.5	78.3±2.9	91.8±2.7	89.0±1.3	45.8±4.6

The results were expressed as mean ± standard error of the mean (SEM), n = 3, duplicate. IC_{50} represents the half-maximal inhibitory concentration and the values obtained were different between all samples.

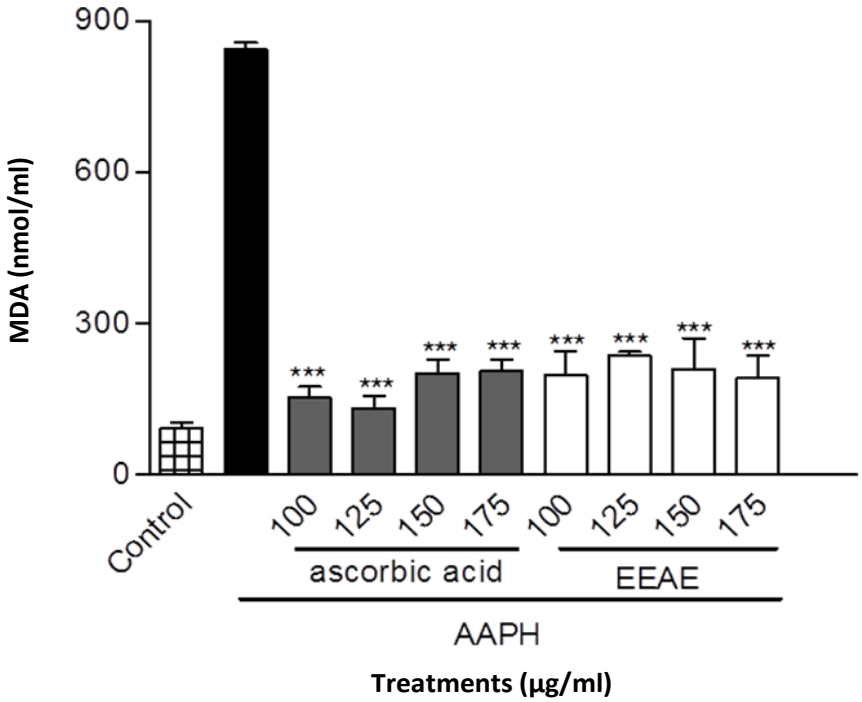

Figure 1. Effects of ethanolic extract of A. edulis (EEAE) on the AAPH-induced lipid peroxidation of erythrocytes. Control group (erythrocytes incubated with saline solution) and AAPH-treated group (erythrocytes incubated with 50 mM of AAPH). Values are expressed as the mean ± SEM. *p < 0.001, compared with AAPH at respective time.

that of ascorbic acid and 11.7 times lower than that BHT. The EEAE exhibited an antioxidant activity 0.4 times higher than that of AEAE, reaching maximal activity at the concentration of 50 µg/mL, which is 10 times higher than the AEAE concentration (500 µg/ml) (Table 1).

Inhibition of lipid peroxidation

In this test, the EEAE was able to prevent lipid peroxidation of the erythrocytes induced by the AAPH in all of the concentrations assessed (100 to 175 µg/ml). Levels of MDA were between 72 and 77% lower than those in the group of erythrocytes with AAPH alone. This result was similar to that of ascorbic acid, with levels of MDA between 76 and 84%, which were lower than the control group (Figure 1).

Inhibition of hemolysis induced by AAPH

All concentrations of the extract tested were able to protect against hemolysis at 60 and 120 min after the beginning of the test. Despite this, none of the concentra-

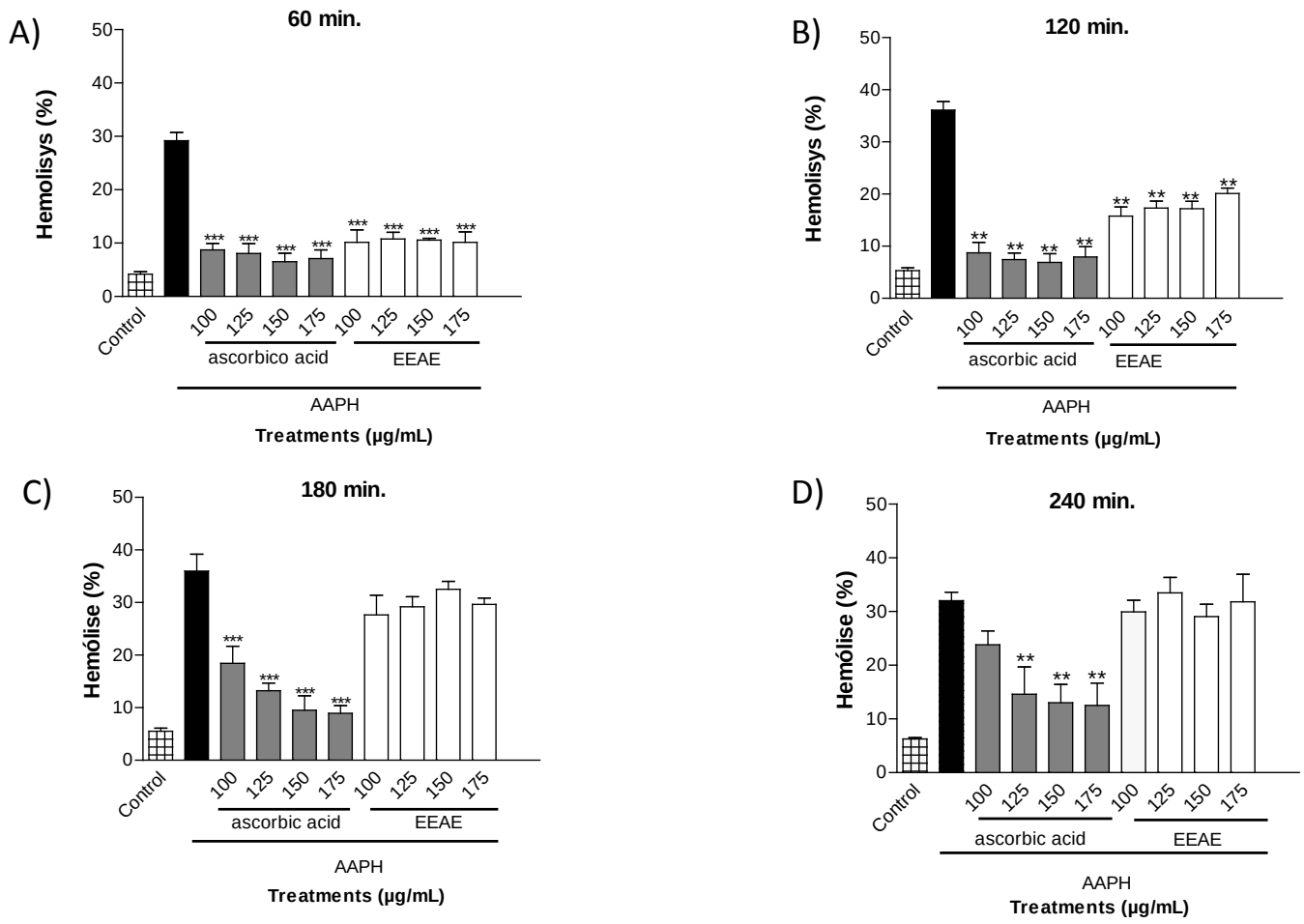

Figure 2. Effects of ethanolic extract of A. edulis (EEAE) on the AAPH- induced hemolysis of erythrocytes. Control group (erythrocytes incubated with saline solution) and AAPH-treated group (erythrocytes incubated with 50 mM of AAPH). A) Erythrocytes incubated for 60 min (B) 120 min, (C) 180 min and (D) 240 min. The results were expressed as mean ± standard error of the mean (SEM), n = 2, duplicate. **p< 0.01; ***p < 0.001 versus. AAPH at respective time.

tions of the extract were able to protect against hemolysis at 180 and 240 min (Figure 2). The ascorbic acid was able to provide protection at almost all of the concentrations and time-points analyzed, with the exception of the 100 µg/ml concentration at 240 min. None of the concentrations of EEAE, ascorbic acid or solvent significantly affected the rate of basal hemolysis throughout the study period.

Antimicrobial activity and MIC

The extracts were effective against the bacterium *S. aureus*. The EEAE exhibited an inhibition zone of 20.3 ± 0.3 mm. In addition, bactericidal activity was detected at the concentration of 150 mg/ml. Similarly, the AEAE presented an inhibition zone of 17.3 ± 1.2 mm, but only bacteriostatic activity at the same concentration of 150 mg/ml. None of the extracts were effective against *E. coli* and *C. albicans* (Table 2).

Test of acute toxicity

In this test, no clinical signs of toxicity, macroscopic changes in the organs or death were observed throughout the study. In addition, no changes in the water/food intake and body weight of the animals were

Table 2. Diameter of inhibition zones, MIC and MBC/MFC of ethanolic (EEAE) and aqueous (AEAE) extracts of A. edulis.

Treatments		*Staphylococcus aureus*					*Escherichia coli*			*Candida albicans*		
		DI	MIC	MBC	Activity	F. effectiveness (%)	DI	MIC	MBC	DI	MIC	MBC
Ketoconazole	-	-	-	-	-	-	-	-	-	24±1.2	-	-
Tetracycline	-	24.0±1.0	-	-	-	100	30.0±2.0	-	-	-	-	-
A. edulis	EEAE	20.3±0.3	150	150	bactericidal	85	0	-	-	0	-	-
	AEAE	17.3±1.2*	150	>150	bacteriostatic	72	0	-	-	0	-	-

DI: diameter of inhibition zone. Ketoconazole and tetracycline (4 mg/ml), F: effectiveness: samples / Tetracycline × 100, EEAE and AEAE (300 mg/ml), MIC and MBC (150 to 1.56 mg/ml). -: not tested. The results were expressed as mean ± standard error of the mean (SEM), n = 2, triplicate. *p<0.05 versus tetracycline treatment.

Table 3. Body weight, food/water intake, hematological and biochemical indices of the Control group (C) and the Tween control group (TC). Low dose group (LD = 2 g/kg BW) and High dose group (HD = 5 g/kg BW) on day 15 after the administration of ethanolic extract (EEAE) of A. edulis in Wistar rats.

Variable	C	TC	LD	HD
Food intake (g/24 h)	22.0±1.0	22.0±1.0	20.0±0.6	20.0±1.7
Water intak (ml/24 h)	44.0±3.1	38.0±1.4	42.0±1.3	42.0±2.2
B.w 1° day (g)	226. 7±4.6	231.4±7.1	232.0±7.8	220.2±7.5
B.w 15° day (g)	246. 3±3.8	250.0±8.1	251.7±8.4	233.8±12.7
Liver (g/100 g)	3.2±0.1	3.0±0.1	3.30±0.1	3.6±0.1*
Kidney (g/100 g BW)	0.66±0.1	0.69±0.1	0.66±0.1	0.70±0.0
Heart (g/100 g BW)	0.38±0.1	0.39±0.0	0.41±0.1	0.41±0.0
Lung (g/100 g BW)	0.58±0.0	0.57±0.1	0.54±0.1	0.61±0.1
RBC (10^6/ml)	8.9±0.1	8.6±0.1	8.1±0.6	8.5±0.1
WBC (10^3/ml)	7.4±0.5	5.9±1.4	6.3±1.4	6.1±0.7
HGB (g/dl)	13.8±0.1	13.5±0.2	13.5±0.3	13.8±0.2
HCT (%)	47.1±0.4	45.6±0.4	44.3±1.7	47.0±0.3
PLT (10^3/ml)	977.7±60.2	996.0±100.3	902.3±68.2	790.5±46.5
URE (mg/dl)	31.6±4.5	30.2±0.9	31.9±0.4	31.5±1.1
CR (mg/dl)	0.2±0.03	0.3±0.06	0.3±0.03	0.3±0.03
γ-GT (U/L)	0.7±0.3	0.7±0.3	0.5±0.5	0.7±0.3
ALT (U/L)	57.0±8.2	60.0±2.9	56.0±3.1	57.0±4.5
AST (U/L)	152.7±1.8	271±32.9*	183.3±20.3	198.3±22.4

BW = body weight WBC = White Blood Cells; RBC = Red Blood Cells; HGB =Hemoglobin; HCT = Hematocrit; PLT = Platelet; URE = urea; CR= creatinine; γ- GT = γ-glutamyltranspeptidase; ALT = alanine aminotransferase; AST = aspartate aminotransferase. p<0.05 versus C group.

detected. At a dose of 2 g/kg, the extract did not change any of the parameters assessed. However, the dose of 5 g/kg caused an increase in the liver weight of treated animals, when compared with the control group. Finally, the control group treated with tween exhibited an increase in serum levels of aspartate aminotransferase, when compared with the control group treated with water (Table 3).

DISCUSSION

In recent years, the number of studies on alternative therapies for several diseases has increased, with

medicinal plants as the main target for scientific research seeking to develop more efficient new drugs and reduce side effects. In Brazil, several species of plants with medicinal properties are used, although less than 5% of plants have been phytochemically and biologically studied (Calixto, 2005; Simões et al., 2004). Among these plant species, *A. edulis* contains compounds with anti-insect activity (Diaz et al., 2014), flavonoids and phenolic molecules, which are directly associated with their biological activities. Previous studies have demonstrated that essential oils, alkaloids (Bandoni et al., 1972; Yajia et al., 1999) and polyol L-quebrachitol are the main constituents of this vegetal (Diaz et al., 2008). The presence of flavonoids in *A. edulis* extract could be associated with DPPH free radical scavenging and antioxidant activities.

Flavonoids present a chemical structure which favors the inactivation of free radicals, since free hydroxyl groups are able to donate hydrogen and electrons which will neutralize the free radicals (Burda and Oleszek, 2001). Several phenolic and flavonoid compounds with antioxidant activity have been identified and isolated from plant extracts (Lee et al., 1998). These constituents are represented by various molecules which are considered natural antioxidants (Dryden et al., 2006; Middleton, 1998).

The higher antioxidant activity exhibited by EEAE compared to AEAE could be related to higher concentrations in the ethanol extract of phenolic and flavonoid compounds. The antioxidant activity demonstrated by EEAE was 2.7 times higher than that reported in a previous study using fruit of the same species (Umeo et al., 2011). Other antioxidant properties of EEAE have been demonstrated by its ability to prevent the lipid peroxidation of the membranes of human erythrocyte, as evidenced by the reduction of malondialdehyde production, and its ability to prevent oxidative hemolysis.

These activities could be attributed to the phenolic compounds of EEAE, which are able to eliminate the peroxyl radicals produced by the thermal decomposition of AAPH. This reaction may occur before the action on the lipid molecules of the erythrocyte membrane, breaking the chain reaction of free radicals, which inhibits lipid peroxidation and consequently, hemolysis (Silva et al., 2011). Previous studies have reported that phenolic compounds are the main components associated with the anti-hemolytic ability of natural products (Valente et al., 2011; Campos el., 2014; Casagrande et al., 2014).

Besides the presence of phenolic compounds, other metabolites have been described in terms of their protective ability against oxidative hemolysis. For example, polyol L-quebrachitol, previously described for this species (Diaz et al., 2008), could be responsible for the antioxidant activity observed. A previous study demonstrated the antioxidant activity of L-quebrachitol in other plant species belonging to the same family of the *A. edulis* (Nobre Junior et al., 2006). These protective activities are of great importance, since the effects of oxidative stress on the organism include damage to the cell membrane by lipid peroxidation (Halliwell, 1992). This oxidative process is present in various pathologies, such as diabetes, cancer, cardiovascular and inflammatory diseases (Burton and Jauniaux, 2011).

Antimicrobial activity is another significant biological property. Indeed, it is known that there is an increase in the number of new multi-resistant strains to conventional drugs, which cause high morbidity and mortality rates among patients and pose a threat to public health (Kamicker et al., 2008). The antimicrobial activity of EEAE and AEAE was assessed against *S. aureus*, *E. coli* and *C. albicans*, with effective results associated with the treatment of *S. aureus*. This is an important finding, since *S. aureus* has been reported as a multi-resistant bacterium of medical concern (Russell, 2002). This agent is responsible for several syndromes, such as food poisoning, toxic shock syndrome, skin lesions and atopic dermatitis (Guay, 2003). Among the molecules related to the antimicrobial activity of natural products, flavonoids are one of the most important due to their ability to interfere in the synthesis of nucleic acid and the energetic metabolism of microorganisms. In addition, they can also bind to the proteins of cell membranes, causing their death (Cowan, 1999; Cushnie and Lamb, 2005, 2011). However, the extracts were not able to inhibit the growth of the gram-negative bacterium *E. coli* and the fungus *C. albicans*. Gram-negative bacteria and fungi exhibit a complex cell membrane, which is difficult to penetrate (Braun, 2009). Most likely, the different composition of the cell membrane of these organisms is the cause of the resistance observed herein. The resistance of these microorganisms to plant extracts has already been reported (Engels et al., 2011; Hendra et al., 2011).

Toxicological studies of medicinal plants are important to understand the eventual toxic effects that could reduce its medicinal value. Many studies are performed in Wistar rats to evaluate the toxicity of the leaves extract from medicinal plants. In these animals the most important signs of toxicity are characterized by reduction of body weight, hind limb paralysis, increase in creatinine, aspartate aminotransferase, sodium and potassium serum levels, reduction of urea and albumin, leucopenia and small alteration in color and consistency of viscera (Félix-Silva et al., 2014). Considering the popular use of *A. edulis* as a medicinal plant, the knowledge of its toxicological effects is essential. In the test of acute toxicity, only the highest dose of 5 g/kg of EEAE caused an increase in liver weight, suggesting hepatotoxicity at

this dose. However, in the group treated with EEAE (2 g/kg), no alterations were observed in the organs or in the haematological, biochemical and toxic parameters assessed. Therefore, based on the guidelines of the Organization for Economic Co-operation and Development, the lethal dose (LD_{50}) of *A. edulis* is higher than 5 g/kg and EEAE can be considered an extract of low toxicity.

Conclusion

The results of the present study show that the extract of leaves of *A. edulis* has antioxidant activity *in vitro* by scavenging free radicals and inhibiting hemolysis and lipid peroxidation in human erythrocytes incubated with an oxidizing agent. It was active against the bacterium *S. aureus*, as well as showed low toxicity. The antioxidant and antimicrobial activities of this extract can be attributed to the presence of flavonoids and phenolic compounds. Therefore, these results suggest that this natural product may be used for the treatment and/or prevention of various diseases related to microorganisms and oxidative stress.

Conflict of interest

The authors declare that there are no conflicts of interest.

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In vivo hypoglycemic and alloxan induced antidiabetic activity of *Xeromphis uliginosa* Retz

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***Xeromphis uliginosa* Retz. is an indigenous plant of Bangladesh. Traditionally this plant is used to treat many diseases. In order to explore the medicinal quality of this plant systematically, the crude methanol extract of roots was screened for antidiabetic activity in rats model. The antidiabetic action was determined by using oral glucose tolerance test (OGTT) and alloxan induced antidiabetic test. In OGTT, both doses (200 and 400 mg/kg body weight) of methanol extract reduced the blood glucose significantly ($p < 0.05$) after 1 h on administration and continued to remain lower up to 3 h. However, the extract significantly ($p < 0.05$) attenuated the blood glucose level in diabetic rats at a dose of 500 mg/kg body weight which was comparable to the standard drug used (glibenclamide).**

Key words: *Xeromphis uliginosa*, antidiabetic, blood glucose.

INTRODUCTION

Plants have being used as a source of medicine by man since ancient times globally (Bargali et al., 2003). In the beginning, these were the main source of the folk or ethnomedicine (Parihaar et al., 2014). Subsequently, in Bangladesh, a considerable amount of this traditional knowledge was formulated and documented into an organized system of medicine parallel to the modern medicine. The use of plants as the principal form of medicine is increasing throughout the developed world. In fact, about 80% population of developing countries still utilizes traditional medicines derived from herbs for their health care (Bargali and Shrivastava, 2002; Shrivastava and Bargali, 2005). As Bangladesh has numerous plants, proper scientific evaluations are required to explore the potentiality of these plants for treating various diseases (Rahmatullah et al., 2010; Banglapedia, 2012; Ashraf et al., 2014).

Diabetes is a chronic disease that occurs either when the pancreas does not produce enough insulin or when the body cannot effectively use the insulin it produces (World Health Organization (WHO), 1999). Diabetes is associated with long-term micro- and macro-vascular complications and is widely recognized as a leading cause of mortality and morbidity (Hossain et al., 2007).

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The number of people with diabetes is increasing day by day due to population growth, aging, urbanization and increasing prevalence of obesity and physical inactivity (Wild et al., 2004). To achieve glycemic control, therapeutic agents like insulin, sulfonylureas, biguanides and thiazolidinedione derivatives etc are used. However, on chronic usage most of these agents produced several side effects including hypoglycemic coma, insulin resistance, hyper-sensitivity, abdominal pain, anorexia and metallic test (Sharma and Kumar, 2011; Chaudhary, 2001). Moreover, it is difficult to afford and use these medicines for prolonged period as the cost and treatment failure rate are high. Until the time insulin was invented, this disorder was managed principally by using medicinal plants due to their low cost, easy accessibility and less side effects (Sharma and Kumar, 2011). There are numerous traditional medicinal plants reported to have hypoglycemic properties such as *Allium sativum* (Garlic), *Azadirachta indica* (Neem), *Vinca rosea* (Nayantara), *Momordica charantica* (Bitter ground) and *Ocimum santum* (Tulsi) (Sharma and Kumar, 2011; Grover et al., 2002).

Xeromphis uliginosa Retz. is an underutilized plant of the family Rubiaceae. The plant is distributed in dry and moist deciduous forests. It is native to Bangladesh, India, Sri Lanka and Thailand. The ethnic communities use the various parts of the plant as a vegetable and curing various illness like cholera, diarrhoea, dysentery, eye complaints, pimples, diuretic, tonic properties, and biliousness amongst others. The unripe fruit acts as astringent. Bark powder with egg, turmeric and calcium is used for bone fracture healing (Srivastava and Pandey, 2013). The stem bark of *Helicteres isora* Linn. along with that of *Xeromphis uliginosa* and a whole plant of *Bacopa monnieri* Wettst. are used to treat colds and coughs. A decoction of wood is used in the treatment of diabetes mellitus (Khare, 2004; Chuakul et al., 2011; Kirtikar and Basu, 1933).

Since this plant has important medicinal properties, the present study has been undertaken as part of our regular research program (Ara et al., 2006; Begum et al., 2010; Dey et al., 2014), and we, herein, report the hypoglycemic and antidiabetic properties of the roots of *X. uliginosa* for the first time.

MATERIALS AND METHODS

Plant

The roots of *X. uliginosa* were collected on August, 2013 from Tangail, Bangladesh and a voucher specimen has been deposited at Bangladesh National Herbarium, Mirpur, Dhaka for future reference.

Extraction and fractionation

The collected roots were sun dried for several days and then oven dried for 24 h at 40°C to facilitate grinding. The powdered roots (565 g) of *X. uliginosa* was extracted with 2.4 L methanol for 7 days and then filtered through a cotton plug followed by Whatman filter paper number 1. The extract was then concentrated by using a rotary evaporator at reduced temperature (40 to 45°C) and pressure. The concentrated methanol extract was used for different biological screenings.

Animals

Evan's rats of both sexes, weighting 150 to 200 g, bred in the animal house of Department of Pharmacy, Jahangirnagar University, Savar, Dhaka, Bangladesh were used for the experiments. All the animals were acclimatized one week prior to the experiments. The animals were housed under standard laboratory conditions (relative humidity 55 to 65%, room temperature 25.0 ± 20°C, and 12 h light dark cycle). The animals were fed with standard diet (ICDDRB, B formulated) and had free access to tap water but were fasted 12 h prior to each experiment. The Federation of European Laboratory Animal Science Associations (FELASA) guidelines and recommendations were followed to reduce the pain and stress of the experimental rats.

Drugs

The drugs and chemicals used in this study include glucose solution (10%), glibenclamide (Square Pharmaceuticals Ltd., Bangladesh), dimethyl sulphoxide (DMSO, Merck Chemicals Ltd., Germany) and alloxan (Sigma Aldrich, Germany).

Oral glucose tolerance test (OGTT) in normal rats

The OGTT was performed by the method described by Durschlag et al. (1996). Here, the lowering of blood glucose level of the experimental animals was measured by tail tipping method. Rats were divided into 4 groups of 3 rats each. The control group received 1% Tween 80 in normal saline (10 ml/kg body weight), the standard group received glibenclamide (10 mg/kg body weight) and the experimental groups received crude extract of 200 and 400 mg/kg body weight. In the evaluation of the hypoglycemic effect of the crude methanol extract of *X. uliginosa*, the blood glucose level of the experimental animals was measured at zero hour using a glucometer (Bioland G-423 S). Then the control, standard and methanolic crude extract (200 and 400 mg/kg body weight) were administered orally to the experimental animals with the help of feeding needle. At 1st, 2nd and 3rd hour after administration, the blood glucose level of the experimental animals was measured to observe the hypoglycemic effect of the test samples relative to control and standard groups.

Alloxan Induced Antidiabetic activity

The alloxan induced antidiabetic activity was evaluated by the method described by Semwal et al. (2008) and Ahmed et al. (2010). Rats were divided into 3 groups of 4 rats each.

Group I: Normal saline treated control (20 ml/kg body weight).
Group II: Alloxan treated control (150 mg/kg body weight i.p.).
Group III: Alloxan (150 mg/kg body weight i.p.) + Standard drug, Glibenclamide (10 mg/kg body weight).
Group IV: Alloxan (150 mg/kg body weight i.p.) + Methanol extract of *X. uliginosa* (500 mg/kg body weight).

The root extracts, glibenclamide (10 mg/kg body weight) and normal saline were administered with the help of feeding cannula.

Table 1. Effect of methanol extract of *X. uliginosa* on oral glucose tolerance test.

Group	Blood glucose level (mmol/L)				
	Doses	0 h	1 h	2 h	3 h
1% Tween 80 in normal saline (Control)	10 ml/kg	5.9 ± 0.6	7.3 ± 0.7	6.4 ± 0.6	5.6 ± 0.4
Glibenclamide (standard drug)	10 mg/kg	6.0 ± 0.5	3.5 ± 0.3**	2.3 ± 0.2**	2.5 ± 0.1**
Roots extract of *X. uliginosa*	200 mg/kg	5.9 ± 0.4	5.9 ± 0.5*	4.6 ± 0.5	4.1 ± 1.0
	400 mg/kg	5.6 ± 0.5	5.6 ± 0.8*	4.6 ± 0.7	3.9 ± 1.1

All values are expressed as mean ± SEM; n=4, *p < 0.05, **p < 0.01, significant compared to control.

Table 2. Effect of roots extracts of *X. uliginosa* in alloxan induced diabetic rats.

Groups	Dose (ml/kg body weight)	Blood glucose level (mmol/L)							
		Days							
		0	1	2	3	4	5	6	7
1% Tween 80 in normal saline (Normal Control)	20	6.5 ± 0.9	5.7 ± 0.5	6.2 ± 0.3	5.1 ± 0.3	6.3 ± 0.7	5.2 ± 0.5	5.0 ± 0.9	4.4 ± 0.5
1% Tween 80 in normal saline (Diabetic Control)	20	6.0±0.2	17.4±1.0	17.3 ± 1.0	16.1±1.0	15.8±1.0	15.5±0.4	15.5±1.0	14.7±0.4
Glibenclamide (standard drug)	10	5.9±0.1	13.1±0.5*	6.8±0.3**	6.8±0.2**	3.6±0.4**	3.8±0.3**	4.3±0.4**	4.2±0.3**
Roots extract of *X. uliginosa*	500	6.1±0.3	17.2 ± 0.5	13.6±0.8*	11.9±0.8*	11.1±1.0*	4.4±0.3**	7.1±0.1.5**	6.3±1.5**

All values are expressed as mean ± SEM; n = 4, *p < 0.05, **p < 0.01, significant compared to control diabetic control.

In this method, Group I to III animals were allowed to fast for 12 h. Diabetes was induced by injecting intraperitoneally a freshly prepared solution of alloxan (150 mg/kg) in normal saline after base line glucose level determination. The alloxan treated animals were allowed to feed over night to overcome drug induced hyperglycemia. After 48 h blood glucose content was measured from the tail vein by using a glucometer (Bioland G-423 S). When the diabetic model rats was established with blood glucose level above 11.1 mmol/L, the animals was selected for the study. The blood glucose level was tested in 0, 1, 2, 3, 4, 5, 6 and 7 days after the oral administration of glibenclamide and methanol extracts.

Statistical analysis

The values are presented as mean ± standard error of mean (SEM) and one way analysis of variance (ANOVA) was used to determine a significant difference between the control group and experimental groups. A p-value of < 0.05 was considered to be statistically significant.

RESULT

The methanol extract of *X. uliginosa* was subjected to assay for OGTT at doses of 200 and 400 mg/kg body weight and alloxan induced antidiabetic activity at a dose of 500 mg/kg body weight. The extract of *X. uliginosa* when administered orally at 200 and 400 mg/kg body weight exhibited significant glucose lowering effects when compared to control. The glucose lowering effects was found to be dose dependant. However, maximum effect was seen at the dose of 400 mg/kg body weight and was comparable with the standard drug (glibenclamide) (Table 1). Single intraperitonial administration of alloxan monohydrates (150 mg/kg body weight) led to elevation of blood glucose level. The methanol extract of *X. uliginosa* showed significant (indicate p-value) antidiabetic property at a dose of 500 mg/kg body weight. The antidiabetic effects of methanol extract and Glibenclamide on blood sugar levels on diabetic rats was significant. However, the antidiabetic effect of root extract was comparable with the standard drug (Table 2).

DISCUSSION

Treatment of diabetes with the compound that has no side effects is still difficult in the field of medical

system.

As a result, nowadays demand of natural products which have significant antidiabetic activity with fewer side effects is increasing rapidly. Alloxan causes diabetes through its ability to destroy the insulin producing beta cells of the pancreas (Lenzen and Panten, 1988, Oberley, 1988). In vitro studies have shown that alloxan is selectively toxic to pancreatic beta cells, leading to the induction of cell necrosis (Jorns et al., 1997, Ledoux et al., 1986). The cytotoxic action of alloxan is mediated by reactive oxygen species, with a simultaneous massive increase in cytosolic calcium concentration, leading to a rapid destruction of beta cells (Szkudelski, 2001).

According to the earlier studies, plant extracts cause anti-hyperglycemic effect by promoting regeneration of β cells or by protecting these cells from destruction, by restricting glucose load as well as by promoting unrestricted endogenous insulin action. Anti-hyperglycemic effect may also be caused by the effect of plant extract on β cells to release insulin or activate the insulin receptors to absorb the blood sugar and stimulate the peripheral glucose consumption (Jadav et al., 2009). It has been published in the literature that the plant extracts have antioxidant potential, flavonoids and tannins (Hossain et al., 2014; Srivastava and Pandey, 2013). Presence of flavonoids and tannins in the extracts is known to possess antidiabetic activity (Sharma et al., 2010). In our present study, the exhibited antidiabetic activity of the plant extract may be due to the presence of similar phytoconstituents.

Conclusion

The methanol extract was very effective in diabetes management which indicates that the plant has potential antidiabetic property. The present study justifies the use of this plant in diabetes mellitus. Further extensive studies are required to isolate the bioactive compounds and to explore the underlying mechanisms to treat these diseases.

Conflict of interests

The authors did not declare any conflict of interest.

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Evaluation of neuro-pharmacological activities in six homeopathic drugs

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The effects of extracts of *Digitalis purpurea* L., *Sambucus nigra* L., *Thuja occidentalis* L., *Urtica urens* L., *Arctostaphylos uva–ursi* L. and *Apis mellifica* L. were analyzed *in vivo* in mice. The analysis of neuro-pharmacologiacl activity was recorded at different doses such as 100, 300 and 500 mg/kg and compared with control and standard drug, Diazepam. The tested extracts exhibited significant neuro-pharmacological activities in open field, dip cage, light and dark, cage cross and forced swimming activities. Our research supports the safe and effective use of the extracts in low doses.

Key words: Neuro-pharmacological, open field activity, dip cage activity, light and dark activity, cage cross activity, forced swimming test.

INTRODUCTION

This study was carried out on *Apis mellifica* L., *Digitalis purpurea* L., *Sambucus nigra* L., *Thuja occidentalis* L., *Urtica urens* L., *Arctostaphylos uva–ursi* L., with the objective to explore the neuro-pharmacological activities of these drugs which are in current use of cardiac diseases, skin problems (dermatitis), urinary tract infection, gastro-intestinal tract disorders, allergic conditions, maintaining normal blood pressure, in the removal of kidney stone etc. These activities are reported by different researchers (Klass et al., 2002; Navarro et al., 2000; Bisset and Wichtl, 2001; Chang et al., 2000; Chrubasik et al., 2007; Matsuda et al., 1992).

MATERIALS AND METHODS

The homeopathic mother tincture of drugs (sealed packs from William Schwabe, Germany) were purchased from local supplier (*Apis mellifica* L. Lot # 0730707; *Digitalis purpurea* L. Lot # 2010207; *Sambucus nigra* L. Lot # 0012188808; *Thuja occidentalis* L. Lot # 7100710; *Urtica urens* L. Lot # 3030909 and *Arctostaphylos uva–ursi* L. Lot # 0512208827). To obtain dried, solid extract, the ethyl alcohol was evaporated by keeping open bottles in sterilized condition in lab for 1 to 2 days at room temperature. Later the residue (dry extract) of each drug was used for experiment. The doses of extract were prepared in 0.5 ml distilled water that is, 300 and 500 mg/kg/0.5 ml (Farah-Saeed, 2014)

Experimental animals

Wistar male mice (25 to 30 g) were obtained from Animal House, Dow University of Health Sciences, Karachi, Pakistan. Animals were kept in Animal House, DUHS, Karachi. They were kept in a climate and light controlled room at least 7 days before initiating neuro-pharmacological activities on them and were provided

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with food and water *ad libitum*.

Assessment of neuro-pharmacological activity

Neuro-pharmacological activities (field test, head dip test, cage cross, light and dark and forced swimming activities) were carried out in calm and peaceful environment. Each group comprised of 5 animals (n = 5). Diazepam as 2 mg/kg orally was used as standard. The crude drug and the diazepam were diluted in distilled water and administered orally. The control animals were treated orally with same volume of saline as the crude extract. In all the tests, observations were made after 30 to 40 min of oral dose of the test substance.

Open field activity

The open field apparatus designed in the laboratory consists of 76 × 76 cm square area with opaque walls 42 cm high. The floor is divided by lines into 25 equal squares (Ahmad et al., 2013). Mice were placed in the center square of the open field (one at a time). Number of squares crossed with all four paws was counted for 30 min. Activities of control and drug treated mice were monitored in a balanced design to avoid order effect.

Light and dark test

Light and dark test is one of the apparatus designed to test neuro-pharmacological behavior in mice. The apparatus consists of a plastic box with two compartments one of which is made of transparent plastic and the other of black color plastic. Each animal is placed at the center of the transparent compartment and then the number of entries in each space, as well as time spent in each compartment is recorded for 30 min (Crawley and Goodwin, 1980).

Head dip test

Head dip box is used to check exploratory behavior of mice. A specially designed square shaped having several holes at bottom were used in this study. The observation was to count the number of head dips by the animal through these holes in specified time (Durcan and Lister, 1988; Hossain and Uma-Devi, 2001; Sultana and Najam, 2012). The control and drug treated animals were placed individually in the head dip box and the observations were made for 30 min.

Cage crossing movements

The test was performed on mice in a specifically designed box having rectangular shape. Both control and treated mice were placed into the cage and their cage crossing movements were noted in 30 min. The test was performed to evaluate motor activity of mice. This test was performed according to the method described by Najam and Anser (2011).

Forced swimming test

Forced swimming test was performed according to Porsolt (1977, 1978). This test determines the muscle and central nervous system (CNS) activity of the crude extract. Mice were placed individually for six minutes in the specially designed plastic cylinder filled with water at room temperature up to the marked level. Mouse when placed in water suddenly starts to move its front and hind paws. The activity time of animal is determined with the help of stopwatch out of total observation time of six minutes.

Statistical analysis

All values were compared with the control and standard drug reading by taking out mean and standard error of mean. Level of significance was determined by student t-test (Alcaraz et al. 1989).

RESULTS AND DISCUSSION

The neuro-pharmacological activity of six crude extracts were assessed using open field, head dip, light and dark, cage cross and forced swimming activities (Table 1).

Neuro-pharmacological effects of *A. mellifica* L. on mice

The neuro-pharmacological effects were observed at the dose of 100 mg/kg of *A. mellifica* extract as follows; in open field activity (28 ± 2.84) counts in 30 min were observed, while in head dip test, the mice dipped head (13.33 ± 2.61) times. At the dose of 300 mg/kg the pronounced depressed effects were observed in case of light and dark, cage cross and swimming activities. Number of entries in light compartment is 9.33 ± 2.93 times. The readings of cage cross is (23.33 ± 2.44) times. In forced swimming test (FST) the mean forced mobility time was (1.25 ± 0.04) s. Locomotor and exploratory activity was observed to be considerably reduced in comparison to control and standard Diazepam (2 mg kg^{-1}) (Figure 1).

Neuro-pharmacological effects of *D. purpurea* L. on mice

The most significant neuro-pharmacological effect was observed at the dose of 500 mg/kg of *D. purpurea* extract as follows; in head dip test, the mice dipped head (19.16 ± 4.92) times. Number of entries in light compartment is 4 ± 1.35 times. The readings of cage cross is (6.67 ± 1.48) times. In forced swimming test (FST) the mean forced mobility time was (4.14 ± 0.48) s. At the dose of 300 mg/kg, in case of open field activity (44 ± 4.54) counts in 30 min were observed. Locomotor and exploratory activity was observed to be substantially reduced in comparison to control and standard diazepam (2 mg kg^{-1}) (Figure 2).

Neuro-pharmacological effects of *S. nigra* L. on mice

At the dose of 100 mg/kg of *S. nigra,* CNS inhibitory effects were observed in following activities; in open field activity (75.16 ± 3.68) counts in 30 min, in light and dark

Table 1. Neuro-pharmacological activity of some crude extracts in comparison with the control and the standard drug – Diazepam.

Treatment	Concentration mg/kg	Open field activity (Counts in 30 min.)	Dip cage activity (No. of times in 30 min.)	Light and dark activity (No. of entries in light portion in 30 min.)	Cage cross activity (No. of times in 30 min.)	FST (mobility time s)
Control		392.83±11.79	272.5±4.84	21.5±2.90	72.5±4.10	3.69±0.12
Apis mellifica L.	500	220.5±34.10	112.83±21.33	20±3.93	115.83±4.33	8.98±0.52
	300	101.66±4.50	35.33±4.14	9.33±2.93*	23.33±2.44*	1.25±0.04*
	100	28±2.84*	13.33±2.61*	13.33±3.83	32.66±2.85	2.47±0.03
Digitalis purpurae L.	500	283±17.10	19.16±4.92*	4±1.35*	6.67±1.48*	4.14±0.48*
	300	44±4.54*	52.83±3.23	10.33±1.80	54.16±4.46	4.24±0.04
	100	215.5±2.31	40.16±4.05	6.16±1.55	55.33±4.32	5.28±0.04
Sambucus nigra L.	500	103.16±6.68	7.16±0.77*	5±0.63	22±4.69	1.62±0.21
	300	175.83±4.12	9.67±0.96	4.5±0.83	27±3.64	0.24±0.03*
	100	75.16±3.68*	9±1.16	3.83±0.65*	8.33±1.01*	0.508±0.02
Thuja occidentalis L.	500	223.83±29.72	94.83±9.70	15±3.48	50.16±3.92	1.23±0.05
	300	60.33±3.94	3.5±0.96	3±0.93	13.16±1.27	0.43±0.02
	100	10±1.41*	2.83±0.65*	1.16±0.52*	5.67±0.96*	0.29±0.01*
Urtica urens L.	500	253.16±50.43	152.33±23.56	49.83±11.89	101±10.91	2.74±0.38
	300	127.33±6.01	4.67±1.28*	4.83±1.03*	77.16±3.26	1.37±0.02*
	100	85.83±3.53*	32.67±3.47	11.83±1.36	67.5±3.15*	3.46±0.02
Arctostaphylos uva–ursi L.	500	253.5±31.77	18.83±5.11*	10.83±2.30	28.5±6.13*	4.48±0.78
	100	541±21.59	109.83±3.95	18.5±2.52	49.33±3.99	1.22±0.03*
	50	444.83±4.99	83.78±3.82	18.5±2.54	116±3.84	3.17±0.09
	30	177.5±3.17*	33.16±2.52	10±2.11*	59.33±2.72	2.59±0.09
	10	347±6.80	44.67±3.99	13.33±1.48	93.67±3.40	4.18±0.14
Standard-Diazepam 2 mg/kg		12.5±0.83	11.5±0.83	1±0.4	19.5±0.83	1.60±0.06

Significant CNS inhibitory effect observed in each activity amongst different doses of extracts is indicated by*

activity 3.83 ± 0.65 times in cage cross test, 8.33 ± 1.01 times. Whereas, in forced swimming test (FST) the mean force mobility time recorded was 0.24 ± 0.03 s, at 300 mg/kg dose of *S. nigra* extract. At 500 mg/kg of *S. nigra*, the mice dipped its head 7.16 ± 0.77 times. Locomotor and exploratory activity was observed substantively reduced in comparison to control and standard drug, diazepam (2 mg kg^{-1}) (Figure 3).

Neuro-pharmacological effects of *T. occidentalis* L. on mice

The most significant neuro-pharmacological effect was observed at the dose of 100 mg/kg of *T. occidentalis* extract as follows; in open field activity 10 ± 1.41 counts in 30 min were observed. In head dip test, the mice dipped its head 2.83 ± 0.65 times. In light and dark activity, number of entries in light compartment were found 1.16 ± 0.52 times. The observations of cage cross were found 5.67 ± 0.96 times. In forced swimming test (FST) the mean forced mobility time was 0.29 ± 0.01 s. Locomotor and exploratory activities were noticeably reduced in comparison to control and standard drug, diazepam (2 mg kg^{-1}) (Figure 4).

Neuro-pharmacological effects of *U. urens* L. on mice

The neuro-pharmacological effects were observed at the dose of 100 mg/kg of *U. urens* extract in case of open field activity, 85.83 ± 3.53 counts in 30 min and cage cross 67.5 ± 3.15 times activity. At the dose of 300 mg/kg of *U. urens*; maximum CNS depression was observed in head dip cage, the mice dipped its head 4.67 ± 1.28 times. Number of entries in light compartment, in light and dark activity was 4.83 ± 1.03 times. In forced swimming test (FST) the mean forced mobility time was

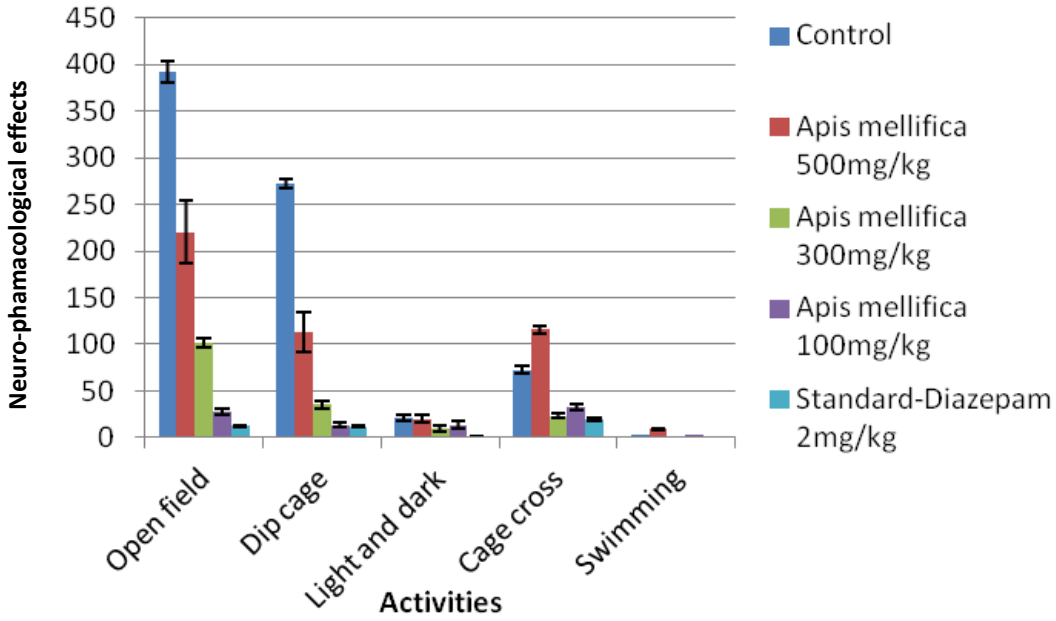

Figure 1. The neuro-pharmacological effects of 100, 300 and 500 mg/ml concentrations of *A. mellifica* extract on mice in comparison with control and standard drug.

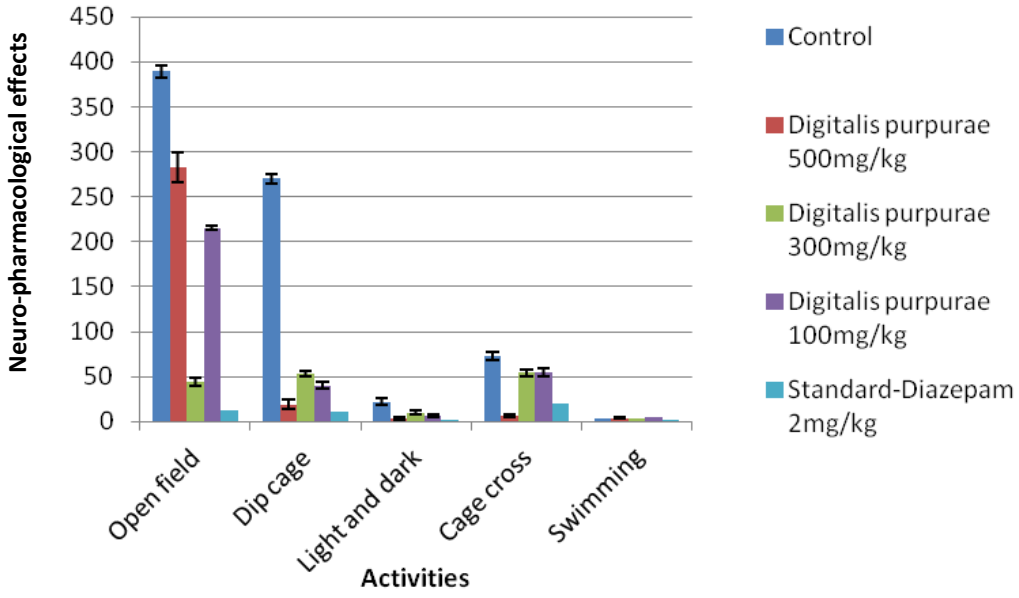

Figure 2. The neuro-pharmacological effects of 100, 300 and 500 mg/ml concentrations of *D.purpurae* extract on mice in comparison with control and standard drug.

1.37 ± 0.02 s. Locomotor and exploratory activity was observed considerably reduced in comparison to control and standard drug, Diazepam (2 mg/kg) (Figure 5).

Neuro-pharmacological effects of *A. uva-ursi* L. on mice

At the dose of 30 mg/kg of *A. uva-ursi* during open field activity 177.5 ± 3.17 counts in 30 min were observed. Whereas, number of entries in light compartment were 10 ± 2.11 times, in light and dark activity. In forced swimming test (FST) the mean forced mobility time was 1.22 ± 0.03 s at the dose of 100 mg/kg. On administration of 500 mg/kg of *A. uva-ursi*, the mice dipped its head 18.83 ± 5.11 times, during head dip activity and the observations on cage cross were found 28.5 ± 6.13. Neuropharmaco-

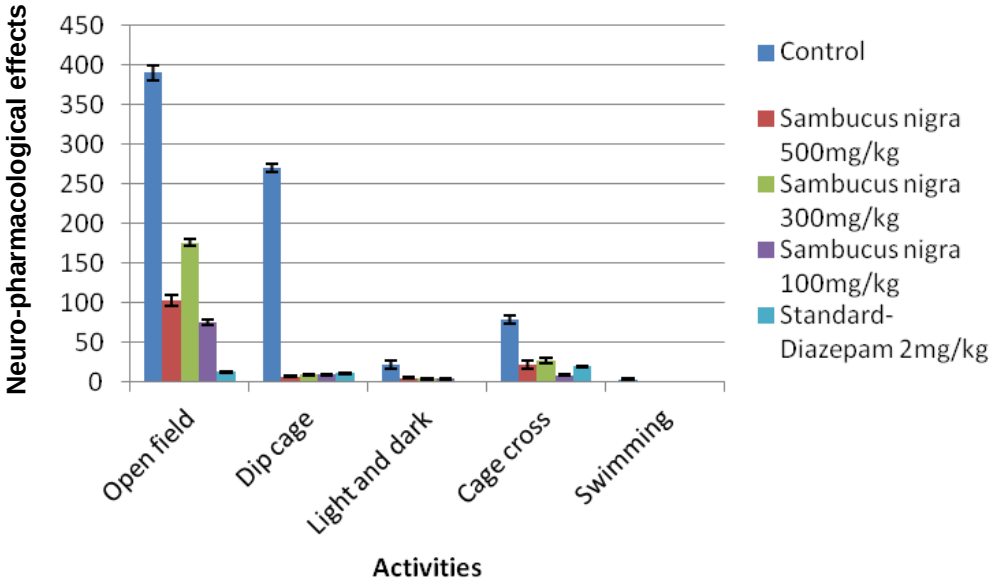

Figure 3. The neuro-pharmacological effects of 100, 300 and 500 mg/ml concentrations of S.*nigra* extract on mice in comparison with control and standard drug.

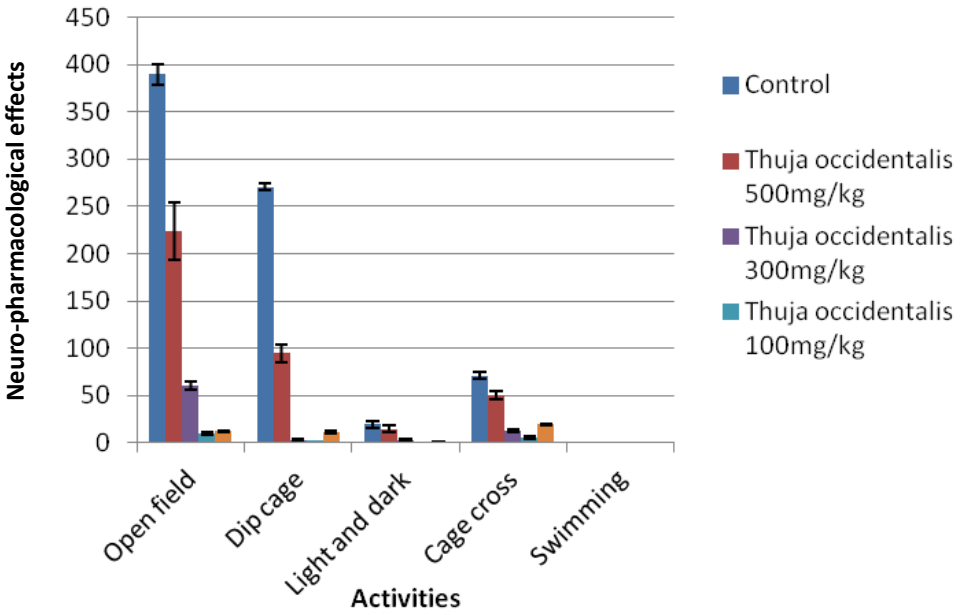

Figure 4. The neuro-pharmacological effects of 100, 300 and 500 mg/ml concentrations of *T. occidentalis* extract on mice in comparison with control and standard drug.

logical activities were observed to be significantly lowered in comparison to control and standard drug Diazepam (2 mg kg^{-1}) (Figure 6). The purpose of carrying out neuro-pharmacological studies is to explore the biological basis of the efficacy of crude extracts for the treatment of central nervous system disorders. These studies are mandatory for the formulation of safe and effective drugs. Most of the crude extracts with neuro-pharmacological activity possess quite non-specific action, affecting several different target receptors, ions, channels and transporters, likewise, the currently available most of the neuroactive drugs (Rang et al., 2007). The main neuro-pharmacological effects exhibited by crude extracts were as follows: reduction of anxiety and aggression,

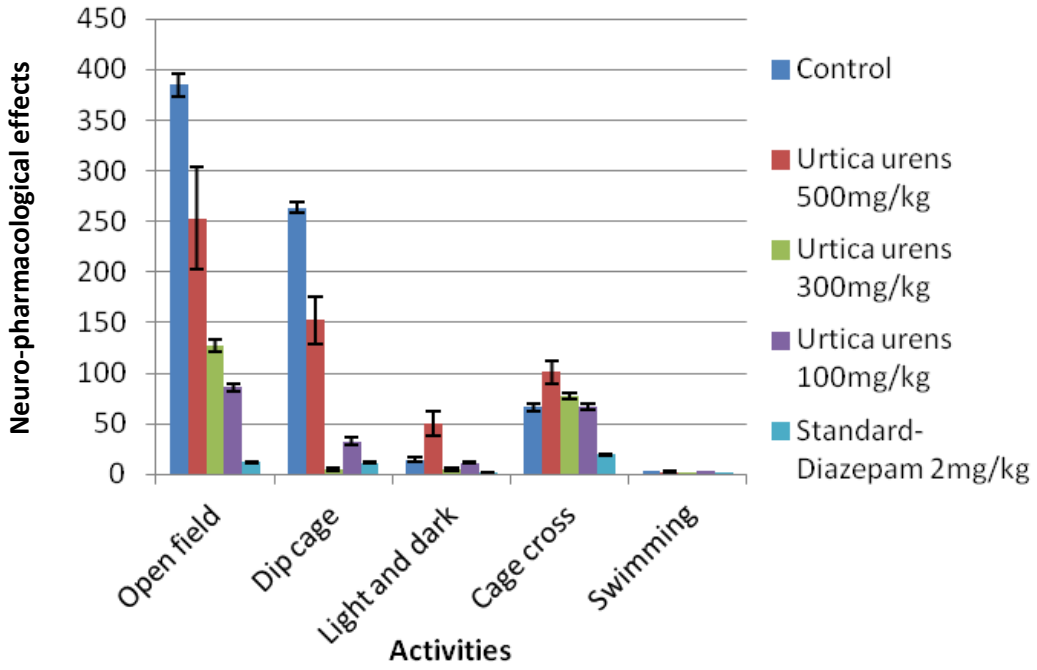

Figure 5. The neuro-pharmacological effects of 100, 300 and 500 mg/ml concentrations of *U. urens* extract on mice in comparison with control and standard drug.

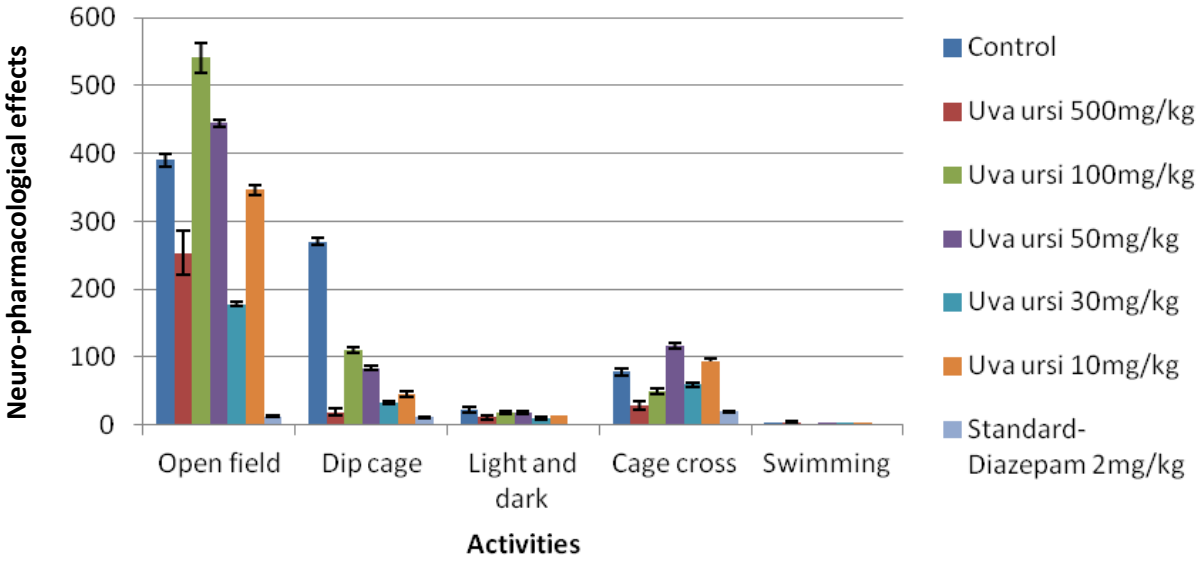

Figure 6. The neuro-pharmacological effects of 10, 30, 50, 100 and 500 mg/ml concentrations of *A. uva-ursi* extract on mice in comparison with control and standard drug.

sedation and induction of sleep as well as reduction of muscle tone and coordination (Argyropoulos et al., 2000) The neuro-pharmacological effects were observed at the dose of 100 and 300 mg/kg of *A. mellifica* extract. Extensive research was carried out on this insect drug by various researchers (Asafova et al., 1986; Ludyanskii, 1994). The pharmacologically active constituents reported are useful in the treatment of different diseases, for example Melittin is reported to influence the central nervous system; Phospholipase A, prevents neuronal cell death caused by prion peptides; Phospholipase B has detoxicating activity; Apamine stimulates the CNS; MCD stimulates CNS; Adolapin inhibits the specific brain enzymes (Shkenderov and Ivanov, 1983; Son et al., 2007; Urtubey, 2005).

Pharmacological effects of *D. purpurea* have been

reported by Ayuso et al. (1993) and Navarro et al. (1994). Our neuro-pharmacological results are found similar to the previously reported results by various researchers that may be due to the presence of digitoxin and minerals present in it (Benli et al., 2009; Negi et al., 2012).

Locomotor and exploratory activities of mice were significantly reduced by administration of *S. nigra* extract in comparison to control and standard drug that indicates that the extract of *S. nigra* acts through GABA receptor and this action may occur due to presence of following chemical constituents of *S. nigra*, that is, zicrin, prunasin, holocalin, potassium nitrate and choline. In general the change in pharmacological action occur in model experiments on introduction of *S. nigra* extract are reported due to rutin, quercetin, cyanicrin-3-glucoside, cyanidin-3-sumbubioside, sambunigrin, viburnic acid and vitamin A and C (Mahmoudi et al., 2014).

Anxiolytic effect of *T. occidentalis* was explored and the results were found to be similar to the research work carried out previously by Lokesh et al. (2011), Jahan (2010) and Alam (2009). Our research work revealed that *T. occidentalis* possessed pronounced depressive response in comparison to the other crude extracts, control and standard drug treated mice. Neuro-pharmacological action was observed in mice treated with *U. urens* extract may be due to the occurrence of butyric acid, acetylcholine and acetophenone (Tita et al., 1993).

Neuro-pharmacological activities on mice treated with *A. uva-ursi* extract showed significant depressive action in comparison to the control group. This response may be contributed by arbutin, methyl arbutin, hydroquinone and galloyl derivative of arbutin or related compounds (Beaux et al., 1999).

Conclusion

The conclusive remark on neuropharmacological activities of six crude drugs is that the chemical composition of each drug is different from each other but all are effective in reducing the hypothalamus (CNS) activity, therefore, a quick change/alteration in system behavior takes place which is neither toxic nor destructive in action. On the basis of these and other reported results it is recommended as a safe and effective drug.

Conflict of interest

Authors declared no conflict of interest.

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Effects of *Jatropha gossypiifolia* L. on the blood pressure and vascular reactivity of rats

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This work investigated the effects of the ethanolic extract (EEJg) of aerial parts of *Jatropha gossypiifolia* L. and its aqueous (AFJg) and chloroformic (CFJg) fractions on the blood pressure and vascular reactivity (VR) in normotensive Wistar rats (NWR) and spontaneously hypertensive rats (SHR). In anaesthetized NWR, the EEJg and its fractions reduced mean blood pressure (MAP). The oral administration of EEJg (100 mg/kg/bw), for 8 weeks, did not alter MAP and heart rate in the non-anesthetized SHR. VR was determined in mesenteric artery rings, with the EEJg and fractions inhibiting the contractile responses to noradrenaline (NA, 10^{-9} to 10^{-4} M) in NWR, but not in SHR. In addition, the CFJg inhibited the contractile response to calcium ($CaCl_2$, 10^{-6} to 10^{-2} M). These results suggest that *J. gossypiifolia* L. has no effect on the hypertensive factors in SHR, which is a model of polygenic hypertension, but indicate the presence of substances with hypotensive activity, which act directly on the adrenoceptor and/or decrease calcium mobilization in NWR.

Key words: *Jatropha gossypiifolia* L., vascular reactivity, hypertension, calcium, mesenteric artery.

INTRODUCTION

For centuries plants offered the only source of therapeutic agents for humans. At the beginning of the 19th century, with the development of pharmaceutical chemistry, plants became the main source of raw materials for the development of drugs. Currently, natural products are involved in the development of 44% of all new drugs. Folk knowledge on the use of medicinal plants makes a very relevant contribution to the dissemination of the therapeutic benefits of plants and aids researchers in the selection of species for botanical, pharmacological and phytochemical studies. However, some factors such as adverse reactions,

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contraindications and drug interactions are often disregarded, thereby creating a risk to health. This problem reinforces the need for more extensive studies of medicinal plants to enable the public and health care professionals to use them more efficiently, safety and in as rational a way as possible (Hostettmann et al., 2003).

Jatropha gossypiifolia L. (Family: Euphorbiaceae), popularly known as 'pião-roxo' and 'erva-purgante' (Pio Corrêa, 1984; Wiersema and León, 2013) is used for the treatment of hypertension and as a diuretic and anti-dysenteric. In the laboratory, substances isolated from other species in the same genus exhibit pharmacological activities. For example, Jatrophone, substance isolated from tubers of *Jatropha elliptica*, presented a non-competitive and concentration-dependent inhibition, of the contractile responses to neurotransmitters, electrical stimulation, potassium (K^+) or Bay K 8644 in cardiac muscle, as well as vascular and non-vascular smooth muscle from rats, guinea pigs, rabbits and dogs (Calixto and Sant'ana, 1987; Trebien et al., 1988). The same compound exhibited a non-competitive inhibition of the contractile responses to calcium (Ca^{++}) in rat aorta or uterus, depolarized with potassium (Calixto and Sant'ana, 1990).

Meanwhile the alkaloid tetramethylpyrazine, isolated from *Jatropha podagrica* stems, presents neuromuscular blocking activity *in vitro* and a hypotensive effect in anesthetized rats and cats (Ojewole and Odebiyi, 1980). This alkaloid also inhibits the contractile responses induced by electrical stimulation or by noradrenaline (NA) in vascular and non-vascular smooth muscle from rats, guinea pigs and rabbits (Ojewole and Odebiyi, 1981).

Silva et al. (2011) have investigated the effect of *J. gossypiifolia* L. aerial parts on intestinal transit velocity and on isolated. It was observed that the chloroformic fraction of *J. gossypiifolia* L. ethanolic extract had a calcium-antagonist effect, whereas both chloroformic and aqueous fractions had anticholinergic effect, suggesting that the antispasmodic effect of *J. gossypiifolia* L. may be due to a combination of anticholinergic and calcium-antagonist mechanisms. Another study show that ethanol extract and the chloroformic fraction reduced the calcium-evoked contractile response of the uterine smooth muscle, promoting a rightward displacement of calcium cumulative curves, as well as reducing the maximal contractions. So the mechanisms by which they promote this effect possibly involve the impairment of Ca^{++} influx through membrane voltage-operated channels (Paes et al., 2012).

Additionally, Abreu et al. (2003) observed the hypotensive effect of the ethanolic extract (EE) from the aerial parts of *J. gossypiifolia* L., which presented a significant, dose-dependent reduction, in the systolic blood pressure in non-anesthetized normotensive Wistar rats (NWR). The percentage of inhibition was 11 and 13% for groups treated orally with EE at 125 and 250 mg/kg/bw, respectively. This effect was confirmed by the ability of the extract to produce relaxation in rat isolated mesenteric artery preparations pre-contracted with NA or $CaCl_2$, and by inhibiting, in a competitive and non-competitive, concentration-dependent manner, the contraction induced by these agonists, without demonstrating any Ca^{++} chelating property (Abreu et al., 2002). These results suggest a possible inhibitory mechanism of action on adrenoceptors and/or on the mobilization of calcium ions. Importantly, acute toxicological studies on the EE of *J. gossypiifolia* L. in rats indicate a low acute oral toxicity (Mariz et al., 2008; Mariz et al., 2006).

Based on evidence from previous scientific studies on the hypotensive effects of *Jatropha*, including *J. gossypiifolia* L., the present study was carried out to investigate the effects of the EE of *J. gossypiifolia* L. and its fractions on the blood pressure and reactivity of smooth muscle from NWR and spontaneously hypertensive rats (SHR) resistance arteries.

MATERIALS AND METHODS

Plant

The aerial parts of *J. gossypiifolia* L. (Euphorbiaceae) were collected from urban areas in São Luís, Maranhão, Brazil, in the month of August, 2011. Plant identification was done in the Atico Seabra herbarium and a voucher specimen (n° 1006) was deposited at the same herbarium for reference.

Preparation of ethanolic extract and fractions

The air-dried, powdered plant material was macerated in an ethanolic solution (95%), then concentrated in a rotary evaporator under reduced pressure, to obtain the ethanolic extract of *J. gossypiifolia* L. (EEJg) with a yield of 7.6%. After this process, the EEJg was fractioned using chloroform and water as solvent, obtaining the aqueous (AFJg) and chloroformic (CFJg) fractions, with a yield of 71 and 29%, respectively (Abreu et al., 2003; Silva et al., 2011).

Experimental animals

In these experiments, adult (200 to 250 g), NWR and SHR, obtained from the Animal House of the Federal University of Maranhão, São Luís, Brazil were used. Animals were housed under conditions of controlled temperature (25 ± 1°C) and lighting (lights on: 06:00 to 18:00 h) and had free access to food and tap water. All procedures described in the present study were approved by the Animal Research Ethics Committee of the State University of Maranhão, Brazil (License number 05/2008).

Measurement of mean blood pressure in normotensive wistar rats – direct method

For measurement of mean blood pressure (MAP), NWR were anaesthetized with urethane 20% and nembutal 1% (1.8 g/kg/bw and 40 mg/kg/bw, respectively, i.p.). Catheters were inserted into the external iliac vein for the administration of drugs and into the

carotid artery for pressure recordings. The arterial catheter was connected to a pre-calibrated pressure transducer (P-1000B, Narco Biosystems, Inc., Houston, Texas, USA) and pressure outputs were recorded on a physiograph (Narcotrace 40, Narco BioSystems, Inc., Texas, USA). After the hemodynamics parameters had stabilized, MAP was recorded before (baselines values) and after i.v administration, of the randomized doses of ACh (1 µg/kg), EEJg (1 to 100 mg/kg/bw), AFJg (5 to 30 mg/kg/bw) and CFJg (1 to 100 mg/kg/bw), which did not exceed a volume of 0.4 ml. The successive injections were separated by a period of time sufficient to allow full recovery of baseline blood pressure (Ghayur et al., 2005).

Measurement of blood pressure and heart rate in spontaneously hypertensive rats – indirect method

Mean arterial pressure (MAP) and heart rate (HR) were measured in two groups of non-anaesthetized SHR (n = 8/group), three times a week, during a basal period of 15 days. The tail of pre-warmed (10 min, at 45°C) non-anesthetized rats was placed in a cuff coupled to an isometric force transducer (Korotkoff) for the measurement of arterial pressure. This in turn was coupled to a non-invasive blood pressure system (LE5001-Pressure Meter, LSI Letica, Panlab, Barcelona, Spain) (Queiroz-Madeira et al., 2010). After the basal period, the treated groups received, orally (0.1 ml/100g/bw), a dose of EEJg (100 mg/kg/bw), five times a week, for 8 weeks. The MAP and HR were obtained three times a week. Control groups received only the vehicle (water), in the same volume as the ethanolic extract.

Preparation of isolated rings of rat superior mesenteric artery

The superior mesenteric artery was removed carefully and placed in freshly prepared Krebs solution containing (mM): NaCl, 118; KCl, 5; $MgCl_2$, 1.2; NaH_2PO_4, 1.2; $NaHCO_3$, 15.5; $C_6H_{12}O_6$ (glucose), 11; $CaCl_2$, 2.0. Each ring (0.5 cm long) was suspended between two wire hooks and mounted in a 5.0 ml organ chamber containg Krebs solution at 37°C, pH 7.4, continuously aerated with a carbogenic mixture of 95% O_2 and 5% CO_2 under a resting tension of 1.0 g. The tissue isometric tension was recorded by a force-displacement transducer (F-60, Narco) and recorded on a polygraph (Vidrio et al., 2003).

After the stabilization period (1 h), cumulative concentration – response curves for noradrenaline (NA, 10^{-9} to 10^{-4} M) were obtained in the absence and presence of EEJg (250 µg/ml), AFJg (250 µg/ml) or CFJg (250 µg/ml) in SHR, and in the presence of AFJg (100 or 250 µg/ml) or CFJg (50, 100 or 250 µg/ml) in NWR. In some set of experiments with NWR mesenteric artery the incubation medium was replaced by depolarizing Krebs' solution (60 mM of K^+; 63 mM of Na^+ and without Ca^{++}). After the basal tonus was recovered, cumulative curves for calcium ($CaCl_2$, 10^{-6} to 10^{-2} M) were obtained, in the absence or presence of AFJg (100 or 250 µg/ml) or CFJg (100 or 250 µg/ml).

EEJg, AFJg and CFJg were incubated for a period of 10 min before the addition of NA or $CaCl_2$, remaining in the incubation medium until the maximum contraction produced by these agonists had been recorded. The size of the contractions was measured and depicted on graphs after statistical analysis.

Drugs

Acetylcholine chloride, noradrenalin hydrochloride and urethane were purchased from Sigma Chemical Co. (St Louis, MO, USA). Nembutal (Cristália, Brasil) and all other chemicals (Merck Darmstadt, Germany) were of high analytical grade.

Statistical analysis

Values are expressed as mean ± standard error of the mean (SEM) of n experiments. Student's t-test for blood pressure tests and One-way analyses of variance (ANOVA) followed by a Newmans-Keuls test for vascular reactivity experiments were conducted in order to evaluate the significance of differences between means. All statistical analyses were done using Graph Pad Prisma™ version 5.00 software with $p \leq 0.05$ considered significant.

RESULTS

Ethanolic extract (EEJg) and fractions produced important reduction on MAP of normotensive wistar rats – directed method

Anaesthetized rats, treated with EE presented an initial mean blood pressure (MAP) of 92.98 ± 7.9 mmHg. In these animals, the intravenous administration of EEJg (1 to 100 mg/kg/bw) induced dose-dependent hypotension with a variation of 3.8 to 39.4 mmHg, which represents a reduction of 4.1 to 58% (Figure 1a). In the other animals that received the fractions, AFJg and CFJg presented a baseline MAP of 84.55 ± 4.9 mmHg. The i.v administration of the AFJg (5 to 30 mg/kg/bw) induced transitory, dose-dependent hypotension with a variation of 22.1 to 35.5 mmHg, and reducing of 26 to 28% (Figure 1b); the i.v. injection CFJg (1 to 100 mg/kg/kg) induced transitory dose-dependent hypotension with a variation of 6.6 to 26.7 mmHg, which represents a fall of 5.8 to 23.1%, respectively (Figure 1c).

Ethanolic extract (EEJg) not altered the MAP and HR in non-anesthetized spontaneously hypertensive rats – indirect method

In the beginning of the study (Day 0), the values of MAP and heart rate (HR) were 144 ± 2.3 mmHg and 388 ± 2.6 bpm, respectively, an indication of established hypertension in these animals. Treatment with EEJg did not alter MAP and HR compared to the control group (Figure 2a and b). MAP of EEJg treated and control animals showed a fluctuating trend reaching 139.3 ± 9.2 and 147.2 ± 7.6 mmHg, respectively, at the end of the study (after 8 weeks), as well as HR, which reached the values of 347.8 ± 18.6 and 358.0 ± 18.2 bpm, respectively.

Ethanolic extract and fractions antagonized contractile responses in mesenteric resistance artery in NWR, but not SHR

In isolated rings of mesenteric artery from NWR, the

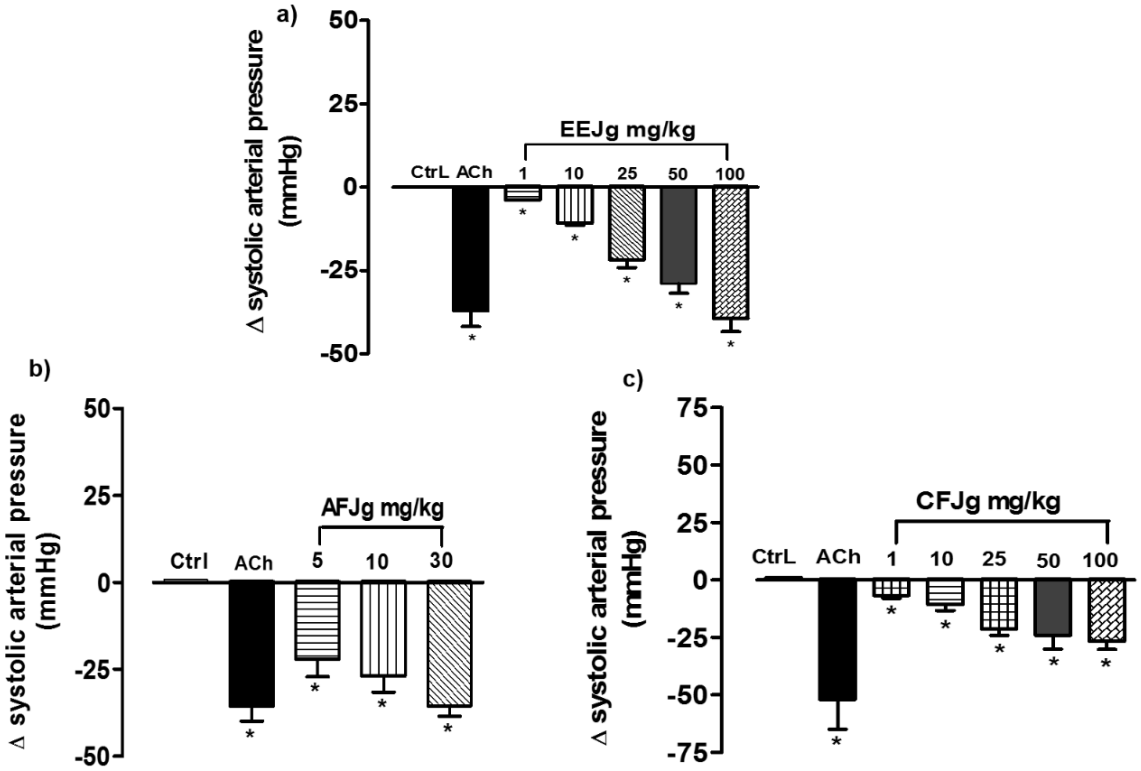

Figure 1. Ethanolic extract (EEJg) and aqueous (AFJg) and chloroformic (CFJg) fractions from *Jatropha gossypiifolia* L. reduces the on mean arterial pressure of anaesthetized Normotensive Wistar Rats (NWR). The intravenous administration of EEJg (1 to 100 mg/kg/bw, a), AFJg (5 to 30 mg/kg/bw, b) or CFJg (1 to 100 mg/kg/bw, c) significantly lowered MAP in NWRs, compared to the control. Symbols expressed MAP mean ± standard error of the mean (SEM, n = 8). *p < 0.001 vs control.

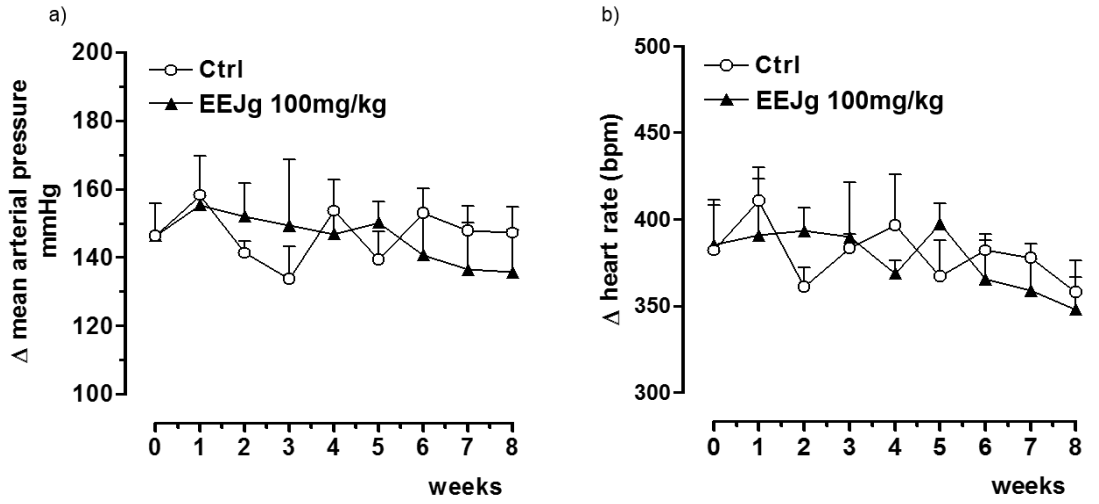

Figure 2. Ethanolic extract (EEJg) of *Jatropha gossypiifolia* L. not altered BP in SHRs. (a) MAP (mmHg) values for control or EEJg treated (100 mg/kg/bw) SHRs over a period of 8 weeks. (b) HR (bpm) of SHRs in treatment groups over 8 weeks. Symbols expressed mean ± standard error of the mean (SEM, n = 8).

AFJg (100 and 250 µg/ml) shifted the dose-response curves to the right 2.3 and 2.0 times, respectively; thus, there was a reduction in the maximum effect (E_{max}), in the presence of AFJg (250 µg/ml), of 31% (Figure 3a).

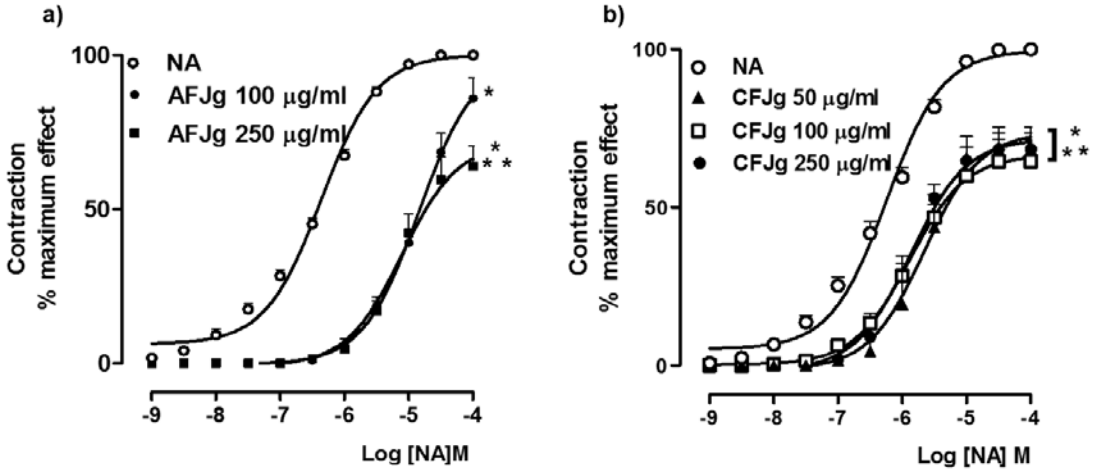

Figure 3. Aqueous (AFJg) and chloroformic (CFJg) fractions from *Jatropha gossypiifolia* L. antagonized NA-induced contractile response in mesenteric artery from Normotensive Wistar Rats (NWR). * p< 0.05 shift to the right; **p< 0.05 reduction the maximum effect vs control (n = 5).

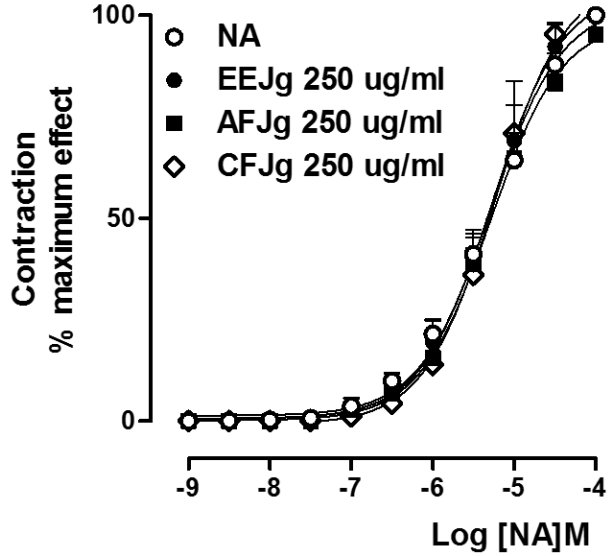

Figure 4. Ethanolic extract (EEJg) and aqueous (AFJg) and chloroformic (CFJg) fractions from *Jatropha gossypiifolia* L. not altered NA-induced contractile response in mesenteric artery from Spontaneously Hypertensive Rats (SHR) (n = 5).

Similarly, the CFJg (50, 100 and 250 µg/ml) shifted the curves to the right 2.5, 2.5 and 3.1 times, respectively, also reducing the E_{max} by 35.4, 31.5 and 31.4%, respectively (Figure 3b). These effects were reversed after 30 min of repeated washes. By contrast, in mesenteric artery preparations from SHR, the EEJg, AFJg and CFJg did not alter the contraction induced by NA (Figure 4). The AFJg (100 or 250 µg/ml) did not alter the contractile responses to $CaCl_2$ (Figure 5a), while the CFJg (100 and 250 µg/ml) shifted the $CaCl_2$ dose-response curves to the right by a factor of 2.2 and 3.1, respectively (Figure 5b).

DISCUSSION

In this work, we evaluated the effects of EEJg and its

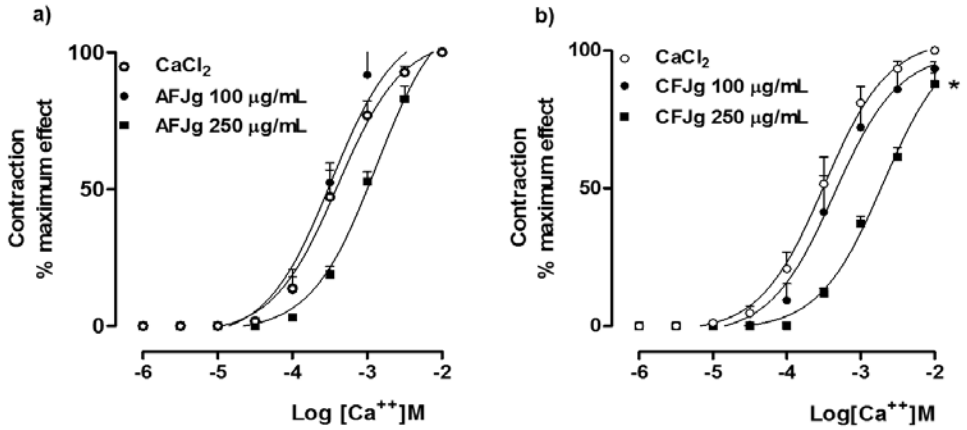

Figure 5. Effects of the aqueous (AFJg) and chloroformic (CFJg) fractions from *Jatropha gossypiifolia* L. on the reactivity to Ca^{2+} ions in mesenteric artery from Normotensive Wistar Rats (NWR). Only chloroformic fraction antagonized in $CaCl_2$-induced contractile response. * $p< 0.05$ shift to the right; (n = 5).

fractions on the mean blood pressure (MAP) in anaesthetized normotensive rats. Baseline MAP values were in agreement with those previously reported in other studies (Santos et al., 2007; Badzynska et al., 2014; Dantas et al., 2014), showing anesthesia and possible stress do not influence. In these animals, acute intravenous administration of EEJg, AFJg and CFJg induced significant transitory dose-dependent hypotension, with effects compatibly between the fractions at the administered doses (Figure 1). These findings are consistent with results reported by Abreu et al. (2003), where the ethanolic extract of *J. gossypiifolia* L. reduced the MAP in non-anaesthetized NWR in older work. Ojewole and Odebiyi (1980) also showed that tetramethylpyrazine, isolated from *J. podagrica*, had a hypotensive effect in anaesthetized rats.

Peripheral vascular resistance mainly maintains the control of blood pressure and the major contributor is the vascular tone of several arterial beds (Santos et al., 2007). In order to verify if the hypotensive response from fractions could be due to a possible vasoconstriction reduction and consequent decrease in the peripheral vascular resistance, we performed *in vitro* experiments using rings from the isolated rat superior mesenteric artery on the reactivity to NA. In these preparations, the AFJg and CFJg inhibited, in both, a competitive and a non-competitive manner which the contractile responses induced by this agonist (Figures 3a and b).

It is know that the maintenance of smooth muscle contraction is dependent on Ca^{2+} influx from extracellular space through voltage and/or receptor operated calcium channels (VOCCs and/or ROCCs, respectively) and releases Ca^{2+} from sarcoplasmatic reticule. The high K^+-induced contraction is inhibited by Ca^{2+} channel blockers or by removal of external Ca^{2+} and is, therefore, entirely dependent on Ca^{2+} influx (Santos et al., 2007; Dantas et al., 2014). In order to investigate if the fractions-caused vasoconstriction reduction could be due to the decrease in the Ca^{2+} influx, we performed a concentration–response curve to $CaCl_2$ in high K^+ solution before and after incubation with fractions. In these conditions, only the CFJg fraction was capable of antagonizing, in a competitive manner, the $CaCl_2$-induced contractions (Figure 5b).

These initial results were in agreement with the dates observed by several authors that showed that ethanolic extract and fractions of *J. gossypiifolia* L. antagonized, competitive and non-competitive manner, NA or $CaCl_2$-induced contractile responses, as well as others stimulus in vascular and non-vascular smooth muscle from normotensive rats (Abreu et al., 2003; Silva et al., 2011; Paes et al., 2012), no however, formed a complex with calcium ions (Abreu et al., 2002). Other studies also shows that compounds isolated from Jatropha genus plants inhibited the contractions induced by several agonist in heart muscle or vascular and non-vascular smooth muscle from rats, guinea pigs and rabbits (Ojewole and Odebiyi, 1981; Calixto and Sant'ana, 1987; Trebien et al., 1988). Therefore, as reported by Chan et al. (2000), nifedipine, a L-type voltage-operated Ca^{2+} channel blocker, also inhibited the concentration–response curve to $CaCl_2$. The initial results in this work suggested that the hypotensive response induced by ethanolic extract and fractions of *J. gossypiifolia* L. may be due to a direct action on the peripheral vascular resistance.

On these data, we seek to investigate if the hypotensive mechanism demonstrated by EEJg and its fractions in NWR could alter the elevation blood pressure in SHR animals, one of the most studied models in

pathophysiology of essential hypertension (Fazan Júnior et al., 2001). SHRs show higher circulating levels of angiotensin II (Ang II), impaired vasorelaxation as well as increases in oxidative stress and inflammation, compared to normotensive rats (Jahandideh et al., 2014).

More studies indicate that renin-angiotensin-system, especially its main active agent Ang II, is critically important in control of blood pressure and regulation vascular responses in SHR. It demonstrated that (1) lower angiotensin AT_2 receptors (AT_2R) mRNA and protein expression can contribute to vasoconstriction in untreated SHR; (2) acute and chronic AT_1-blockade restored AT_2R expression and its vasodilator function in mesenteric resistance arteries and contributes to blood pressure control in SHR treated (You et al., 2005; Badzynska et al., 2014). Vasodilation induced by AT_2R stimulation has been associated with NO production by endothelial cells and cGMP production by smooth muscle cells (Hannan et al., 2004).

In mesenteric resistance arteries from SHRs rats, the endothelium dysfunction too has been demonstrated in several studies. The ACh-induced endothelium-dependent vasodilator response, presumably NO, was significantly attenuated, and verifies less ACh-induced intracellular cGMP content in mesenteric arterial tree, when compared with normotensive rats. However, the concentration-response curves to NO donor sodium nitroprussiade were similar in preparations from SHR and normotensive rats, indicating equal responsiveness of vascular smooth muscle cells to NO (Watt and Thurston, 1989; Rizzoni et al., 1994; Liu et al., 2002).

A activation of the sympathetic nervous system is believed to be the main mechanism that increases the peripheral vascular resistance (Fazan Júnior et al., 2001), causing a level of pressure regarded as spontaneous hypertension from the 7th week of life in SHR (Chamiot-Clerc et al., 2001). In this work, we studied the action of EEJg in SHR from the age of 8 week, which showed initial MAP and HR equal to 144 mmHg and 388 bpm, respectively, confirming high BP. We examined the effects of the EEJg on MAP and on the HR and the vascular reactivity of SHR animals. Chronic treatment with the EEJg (100 mg/kg/bw) for 8 weeks did not alter MAP or HR. In the *in vitro* tests using rings of superior mesenteric artery, the EEJg and fractions did not change the contractile responses to NA, suggesting that its substances possibly did not act through the mechanisms ACh-induced endothelium-derived relaxing or some AT_1R-blockade drug.

Taken together, the results indicate the presence of substances with hypotensive activity in the plant *J. gossypiifolia* L. that can act directly on the adrenergic receptors and/or decrease the mobilization of calcium ions in normotensive animals, but did not present effects in pathophysiology factors from spontaneously hypertensive rats. However, further experiments are necessary to clearly elucidate all action mechanism.

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Conflict of interest

The authors have declared that there are no conflicts of interest.

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Full Length Research Paper

Study on drug utilization pattern of antihypertensive medications on out-patients and inpatients in a tertiary care teaching hospital: A cross sectional Study

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This study aimed to evaluate drug utilization of anti-hypertensive medications in a tertiary care teaching hospital. In this study, a cross sectional study was conducted in hypertensive who visited General Medicine department in a tertiary care teaching hospital, during the period of August, 2013 to August, 2014. 1407 new prescriptions were analyzed according IP/OP numbers of rational drug utilization by using WHO Core drug use indicator and WHO ATC/DDD metric systems. A total of 10,638 drugs were prescribed. Of this, (20.4%) antihypertensive, (45.21%) angiotensin converting enzyme (ACE) inhibitors, 24.0% beta blockers, 17.7% calcium channel blockers, 11.4% diuretics, 1.5% angiotensin II receptor antagonist, 7.56 ± 2.76 mean number of drugs prescribed/prescription, 34.9% free drugs were prescribed from Tamil Nadu Standard Treatment Guidelines (TNSTG), 65.05% were prescribed from Rajah Muthaih Medical College Hospital Drug List (RMMCH). 14.9% antibiotics, 46.76% Injections, mean 13.8 min consultation and mean 147.48 seconds dispensation, both conceived as good patient care, 89.1% drugs actually dispensed, 87.1% drugs adequately labeled, 38.3% subjects had adequate knowledge on frequency of taking medicines. A total (n=82) subjects were admitted in the internal ward and average length of hospital stay was found to be 10.33 ± 6.66 and overall antihypertensive drug consumption was found to be 122.07 DDD/100 Bed-days. The present study finding showed, ACE inhibitors were most frequently prescribed and amlodipine was highest consumed drug in the internal ward. Many of the prescriptions were observed rationally, even though further more improvement is needed in drug prescribing practices for hypertensive study population.

Key words: Anatomic therapeutic chemical (ATC), core drug use indicators, defined daily dose (DDD), drug utilization, hypertension.

INTRODUCTION

The World Health Organization (WHO) 2013 has estimated that high blood pressure (BP) is a major public health issue and causes one in every eight deaths, hypertension being the third leading silent killer in the world. Globally, cardiovascular diseases accounts for approximately 17 billion deaths a year, complications of

hypertension account for 9.4 million deaths worldwide every year. Hypertension is responsible for at least 45% of deaths due to heart disease and 51% of deaths due to stroke (World Health Organization (WHO), 2013). In India, the situation is more alarming as hypertension attributes for nearly 10% of all deaths. Prevalence of hypertension in India is reported to vary from 10 to 30.9%. The average prevalence of hypertension in India is 25% in urban and 10% in rural inhabitants. Cardiovascular diseases are projected to cause 4.6 million deaths in India by 2020 (Mahmood et al., 2011). It is estimated that the worldwide prevalence of hypertension would increase from 26.4% in 2000 to 29.2% in 2025. Anti-hypertensive pharmacotherapy effectively reduces hypertension-related morbidity and mortality (Rachana et al., 2014).

Some important elements of drug use patterns are accuracy, appropriateness of dose, route of administration, dosage schedule, dosage form, subject medical history, if any allergic reaction previously with the prescribed drug, drug over utilization, drug under-utilization by the subject, adverse effect from current medications, drug disease interactions, and irrational therapeutics (Seiyadu and Parimalakrishnan, 2008). Irrational use of drugs is receiving medication inappropriately to their medical necessities for inadequate period of time, wrong dose intake and administering self medicament without physician advice. Drug utilization evaluation studies is one of the important measuring tool for measuring prescribing practices in health facility, distinguishing areas for betterment and developing drug prescribing practices, promote rational prescribing practices, reduce morbidity and mortality and decrease the economic burden in their cost of illness.

Drug utilization studies, which evaluate and analyze (Fowad et al., 2012) the medical, social and economic outcomes of the drug therapy are more meaningful, and observe the prescribing attitude of physicians with the aim to provide drug rationally. Drug utilization research is an essential part of pharmacoepidemiology as it describes the extent, nature and determinants of drug exposure (Ushadevi et al., 2013). Drug utilization data is required for analyzing annual drug acquisition cost, drug supply to the subjects, drugs over or under utilization, drug pricing cost, cost consumption analysis and use. The anatomical therapeutic chemical (ATC) and defined daily dose (DDD) methodologies are most important tool for measuring drug use, various drug therapy and comparing anti-hypertensive drug cost consumption and improve the drug use practices in the health facilities/ region (WHO, 2012).

World Health Organization (WHO) ATC/DDD system is the compilation of the anatomical therapeutic chemical (ATC) classification system and the defined daily dose (DDD). The DDD metric along with the ATC classification form a powerful technical tool used for analyzing patterns of drug utilization and the quality of drug use and health outcomes and also for measuring number of defined daily doses per thousand patient days (DDD/1000 patient days) and 100 patient days (DDD/100 patient days). Advantages of DDD methodology are that they can measure drug exposure, inexpensive, easy to use and allows integration with other databases (WHO, 2010).

WHO core drug use indicators is a tool that measures (indicators), and can describe the drug use situation in a country/region/health facility. The indicators can serve as simple supervisory tools to detect problems in performance of individual providers or health facilities. The drug use indicators can be used as "first line measures". The indicators of prescribing practices measure the performance of health care providers in several key dimensions related to the appropriate use of the drugs (World Health Organization (WHO), 1993). The purpose of this study is to find out drug utilization pattern of anti-hypertensive medications therapy on outpatients and inpatients at general medicine department in a Tertiary Care Teaching Hospital.

Aims and objectives

The present study aims to evaluate on drug utilization pattern of anti-hypertensive medications therapy using WHO recommended core drug use indicators like: (a) Prescribing indicators; (b) Patient care indicators and (c) Facility indicators. Also to evaluate the total consumption or utilization of antihypertensive drug in hypertensive subjects using ATC/DDD metric system at general medicine ward in a tertiary care teaching hospital.

MATERIALS AND METHODS

Study setting

A cross sectional drug utilization study was conducted in the subjects by the Department of General Medicine in outpatient department (OPD) and inpatient department (IPD) and total 1200 beds available with 29 wards established in a tertiary care teaching hospital located in rural Chidambaram, Tamil Nadu, South India.

Study design

The cross sectional, quantitative and observational study was

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adopted.

Study duration and period

The study was conducted for a period of 13 months (August, 2013 to August, 2014) in a tertiary care teaching hospital.

Sampling unit

Sampling unit was systematic random sampling method and patient encounters taking place at the inpatient and outpatient health facility for the treatment of acute, chronic and severe illness on hypertension. A total number of study samples were 1407 new prescriptions prescribed for 201 hypertensive subjects. The size of the sample consists of hypertensive subjects with multiple concomitant diseases who are enrolled and accomplished in these present study requirements as stated in the inclusion and exclusion study criteria.

Ethical considerations

Prepared standard research protocol, subject's proforma and informed consent form (Tamil/English) was approved by the Institutional Human Ethical Committee of Annamalai University (Approval letter No.: M18/RMMC/2013).

Study criteria

Inclusion criteria

1. Subjects who are newly diagnosed and established history of hypertension.
2. Subjects who are prescribed with one or more antihypertensive drug.
3. Subjects who are ≥ 35 years as well as both the genders.
4. The major co-morbidity disease of diabetes mellitus, hypertensive heart diseases, hypertensive retinopathy, diabetic foot ulcer, acute pulmonary edema, myocardial infarction and coronary artery disease associated with all stages on hypertension.
5. Subjects who are willing to participate and submitted informed consent form.

Exclusion criteria

1. Subjects with significant hepatic and renal diseases.
2. Pregnant women.
3. Subjects who had psychiatric illness, who were chronically ill-looking.
4. Subjects who were not treated with antihypertensive drug.
5. Subjects who were unable to give proper information for data collection form and not providing consent form.

Surveillance instruments

1. Standard socio demographic data collection form – age, substance abusers, number of days hospitalized, number of known/unknown case on hypertension, therapy duration, therapy duration with diabetic, major complications and other concomitant diseases.
2. Subjects informed consent form.
3. Prescribing Indicator form, patient care Indicator form and facility care indicator form.
4. Tamil Nadu Standard Treatment Guidelines (TNSTG) Year 2013 to 2014 effective from 04.03.2013.
5. Rajah Muthiah Medical College Hospital (RMMCH) Drug List.
6. WHO core drug use indicator guidelines.
7. WHO anatomical therapeutic classification (ATC)/defined daily dose (DDD) metric system.
8. WHO collaborating centre for drug statistics methodology by name or ATC Code.

Present study – Definitions

1. A drug utilization study is defined as research on "the marketing, distribution, prescription and use of drugs in a society, with special emphasis on the resulting medical, social and economic consequences (Ushadevi et al., 2013)".
2. The World Health Organization defined daily dose (DDD) is a widely applied international metric that transforms the physical quantities of drugs (capsules, vials, inhalers, etc.) into a standard unit of measure (WHO, 2003).
3. The WHO ATC/DDD standard system is a useful tool for comparing data on drug use at the international, national and/or local levels (World Health Organization, 2001).
4. The defined daily dose is a technical, fixed unit of measure, defined as the assumed average maintenance dose per day for a drug used for its main indication in adults (WHO, 2012).

Data compendium

In this present study, subjects were interviewed with face to face interaction based on inclusion and exclusion criteria. Total 1407 new prescriptions were collected according to subjects' case sheets IP/OP numbers by clinical pharmacist. In subject's case sheets, anti-hypertensive medications were written and prescribed by qualified medical personnel and post graduate doctors.

A standard subject socio demographic and clinical features data collection form was prepared and the characteristic like age, gender, subject IP/OP number, diagnosis, subject present/past medical history, number of days hospitalized, knowledge on hypertension, therapy duration for hypertension, therapy duration for other major complications like coronary artery diseases (CAD), myocardial infarction (MI), dilated cardio myopathy (DCM) with hypertensive heart diseases, and diabetic complications like Type II diabetes mellitus, diabetic foot ulcer, diabetic retinopathy, and other concomitant diseases like hypertensive retinopathy, CVA hemiplegia, unstable angina, tuberculosis, and hypertensive nephropathy was included, and the individual data were collected from individual subject's case sheets, then data was analyzed first manually and entered in Microsoft excel 2007.

To evaluate drug utilization pattern of anti-hypertensive medications in a tertiary care teaching hospital, WHO core drug use indicators and WHO ATC/DDD metric system were used. The assessment of prescribing pattern for rationality of drug therapy in hypertensive study population based on WHO core drug use indicators included the prescribing indicators, patient care indicators, and facility indicators. The individual subject's case sheets data were analyzed, performed, gathered and enrolled manually in ordinary prescriber indicator form and then data was analyzed to find out the number of drugs prescribed, percentage of generic name, percentage of antibiotics, percentage of injections, percentage of prescribed drugs analyzed from Tamil Nadu Standard Treatment Guidelines (TNSTG) and Rajah Muthiah Medical College Hospital (RMMCH) drug list.

The assessment of patient care was observed by the indicators of average consultation time, average dispensing time, and percentage of drugs actually dispensed, percentage of drugs adequately labeled, patients knowledge of correct dosage and facility care observed by the indicators of availability of TNSTG drug list and RMMCH drug list and percentage availability of anti-hypertensive drugs surveyed in a tertiary care. Frequencies of utilization of antihypertensive drugs were measured and grouped in 5 major categories: angiotensin converting enzyme inhibitor (ACEI) (enalapril and ramipril), β-blocker (atenolol, metoprolol, propranolol and nebivolol), diuretics (hydrochlorothiazide and furosemide (loop diuretics), calcium channel blockers (CCBs) (amlodipine and verapamil), angiotensin receptor II receptor antagonists (telmisartan). The prescribed antihypertensive drugs complied with TNSTG and RMMCH drug list. In addition, the utilization of different drug classes like anti platelet drugs (aspirin, clopidogrel), lipid lowering agent (atorvastatin), anti-diabetics drugs (insulin, metformin + glipizide, glimipride), non-steroidal anti-inflammatory drugs (paracetamol, ibuprofen, diclofenac sodium), antianxiety/anti-depressants (alprazolam, midazolam, diazepam), anti-coagulant agent (low molecular weight of heparin), anti-hypercholesterolemia agent (fenofibrate), anti-angina (isosorbide nitrate), vitamin B complex and other drugs like antacid, calcium tablets, anti-cold, anti-allergic, haematinic, stool softener agent were also analyzed, evaluated and recorded.

Measurements of drug utilization of anti-hypertensive medication were based on WHO ATC/DDD metric system. The average bed occupancy rate (statistical data) was obtained from Medical Record Departments (MRD). Every day/month bed occupancy index was calculated by MRD. Total number of units (anti-hypertensive drugs) administered during study period data was collected from a 24 h drug store. Informed consent form was collected from subjects before the starting day of the study.

Descriptions about some important core drug use indicators

1. Average consultation time is defined as sum of all consulting time and divided by the number of encounters. It is expressed in minutes.
2. Average dispensing time is defined as sum of all dispensing times and divided by the number of encounters. It is expressed in seconds.
3. Percentage of encounters with an antibiotic prescribed: Percentage was calculated by dividing the number of patient encounters during which an antibiotic is prescribed, by the total number of encounters surveyed, multiplied by 100.
4. Percentage of encounters with an injection prescribed: Percentage was calculated to measure the overall level use of commonly overused and costly forms of drug therapy. It was calculated by dividing the number of patient encounters in which an injection was prescribed by the total number of encounters surveyed, multiplied by 100.

Statistical analysis

This present study data was collected, analyzed and entered in the 'prescribing indicator form' manually and statistical tools like frequencies, averages/means, standard deviations and percentages were applied in this study.

Measurement of drug utilization of anti-hypertensive medication using WHO ATC/DDD metric system

1. Consumption of anti-hypertensive drugs in DDD in a general medicine ward on monthly basis (from August, 2013 to August, 2014).

2. Total consumption of anti-hypertensive drugs in DDD in a General Medicine Ward: Utilization of drugs was measured by the formula of ATC/DDD metric system (Joel et al., 2014; WHO, 2010):

Total inpatient days for a given period = (no. of beds per day × study period)

$$\text{Drug usage [DDD (Defined daily dose)]} = \frac{\text{Number of items used} \times \text{Amount of drug per item (mg)}}{\text{WHO recommended DDD of drug}}$$

$$\text{DDD/100 bed-days} = \frac{(No.of units\ administered\ during\ the\ study\ period\ (mg) \times 100)}{(DDD\ (mg) \times No.of days\ in\ study\ period \times No.of beds \times \boldsymbol{Bedoccupancy})}$$

$$\text{Bed occupancy rate} = \frac{\text{Total inpatient days for given period} \times 100}{\text{Available beds} \times \text{Number of days spent in study period}}$$

RESULTS

A total of 1407 prescriptions which have anti-hypertensive medications with other therapeutic medications prescribed for 201 hypertensive subjects who had multiple concomitant diseases were systematically randomly collected for this particular study. Data on subject's socio demographical characteristics and clinical features of hypertensive with other concomitant diseases is demonstrated in Table 1. Socio

Table 1. Socio demographic characteristics and clinical features of hypertensive with other concomitant diseases (Total number of subjects = 201).

Socio demographic characteristics	Frequency		
	n = 201 (%)	Male (%) n = 124	Female (%) n = 77
Mean age ± SD	-	62.6 ± 11.3	60.45 ± 10.5
Elderly	120(59.7)	77	43
Non-elderly	81(40.3)	47	34
Substance abusers			
Smoking, alcohol and caffeine	201	189(94.02)	12(5.97)
Tobacco, paan, caffeine with Betel nut addicts	201	124(61.69)	77(38.30)
Number of subjects hospitalized	82	48 (58.53)	34 (41.46)
≥ 10 days	63(76.82)	38(46.34)	25 (30.48)
≤ 10 days	19(23.17)	10(12.19)	9 (10.97)
Known case on hypertension			
Yes	77(38.30)	42	35
No	124(61.69)	58	66
Therapy duration of hypertension			
1-10 years	65(32.3)	58	7
10-20 years	55(27.4)	34(61.8)	21(38.2)
20-25 years	81(40.3)	68	13
Therapy duration of HTN with major complications			
CAD	81(40.3)	69(85.2)	12(14.8)
Myocardial Infarction	17(8.45)	9(53.0)	8(47.0)
DCM with Hypertensive heart diseases	07(3.5)	07	-
Diabetic complications			
Type II diabetes mellitus	55(27.4)	29(52.7)	26(47.3)
Diabetic foot ulcer	01	01	-
Diabetic retinopathy	01	01	-
Other concomitant diseases			
Hypertensive Retinopathy	01	01	0
CVA Hemiplegia	02	02	0
Unstable Angina	17(8.45)	14	03
Tuberculosis	05(2.5)	02	03
Hypertensive Nephropathy	01	01	0

demographical characteristics of 201 subjects' data's are demonstrated in Table 1. The median age of male subjects was 63 years (interquartile range 36 to 83 years). The median age of female subjects was 57 years (interquartile range 40 to 85 years). Substance abused was observed that both smokers and alcoholic addicts (94%) and 6% were non-smokers with non-alcoholics but caffeine addicts and 62% male subjects addicts with tobacco more than female subjects were used both tobacco and betel nuts (38%) as well as (60.5%) subjects had known case of hypertension and (39.5%) subjects no known case of hypertension. Regarding 'therapy duration' of hypertension it was observed that (n = 65) subjects had uncontrolled hypertension with irregular treatment. Emergency case admitted first in casualty then transferred to coronary cardiac unit (CCU) confirmed hypertension with major complications which was observed.

Data on classification of anti-hypertensive drugs and percentage analysis of overall drug utilization are demonstrated in Table 2. Data on some other drug classes were prescribed during the study period as shown in Table 3. Frequencies of utilization of antihypertensive drugs used as various drug therapy in hypertensive study population (n = 201) are shown in Table 4. Results of identification of the anti-hypertensive drugs for rationality of prescriptions in hypertensive with multiple concomitant diseases are shown in Table 5.

Table 2. Classification of anti-hypertensive drugs were used and overall drug prescribing pattern on hypertensive subjects with other concomitant diseases (n = 201).

Anti-hypertensive drug class used	Name of the drugs	Prescribed overall frequency of prescriptions (%)	Prescription (%)
ACE I	Enalapril and Ramipril	964	45.21
β-Blocker	Atenolol, metoprolol, propranolol, nebivolol	513	24.06
Diuretics	Hydrochlorothiazide and Furosemide	244	11.44
CCBs	Amlodipine and verapamil	379	17.77
Angiotensin II receptor antagonist	Telmisartan	32	1.5

Total number of anti-hypertensive drugs used were: 2132. ACEI: angiotensin converting enzyme inhibitor; CCBs: calcium channel blocker.

Table 3. Different drug classes were prescribed during the study period.

Drug Class	Frequency	% of Prescriptions
Anti-hypertensive	2132	20.04
Anti-platelet drugs	1441	13.54
Anti-diabetics	1215	11.42
Lipid lowering agent	1257	11.82
NSAIDs	528	4.97
Anti-anxiety/anti-depressants	448	4.22
Anti-coagulant agent	418	3.93
Anti-hypercholesterolemia	481	4.53
Thyroid hormones	022	0.20
Anti-angina drug	168	1.57
Vitamin B Complex	810	7.62
Other drugs	1718	16.14

Data on the assessment of drug prescribing pattern for rational drug use in the hospital using WHO prescribing indicators is shown in Table 6.

The assessment of patient care indicators

Total (n = 201) encounters (subjects) were taken as appointments in various consulting unit in a tertiary care teaching hospital. During the study period, (n = 119) cardiac out patients took appointment on Tuesday at every 15 days twice in a month, subjects assembled one by one in a cardiac outpatient consulting room (one general clinicians accompanied with one post graduate) with a consultation starting time at morning (8.00 a.m) and finishing time at 12.30 p.m., and (n = 28) encounters in a male and female intensive medical care unit (MIMCU and FIMCU) and (n = 54) encounters in a Coronary Care Unit (CCU) were admitted as inpatients (cardiac), consulted with one well qualified physician accompanied with five post graduates. General medicine unit is divided into four units (Medicine I, II, III and IV) and these units can follow-up both out patients and inpatients on every day and night ward rounds in respect of concerned days. The clinical Pharmacist observed consultation time and dispensing time in the morning shift for five respective concerned days in a week and prescribed drugs administered to the internal subject by nursing personnel in the ward itself. The dispensary counter was located nearby the cardiac medicine ward where the inpatients were admitted and for outpatients dispensary counter it was located just opposite to the cardiac OP consulting room, specially established for outpatients. So the subject does not wait for longer time to get the drugs at the dispensary counter. Percentage of actually dispensed drugs and percentage of drugs adequately labeled was recorded and enrolled in the 'patient care indicator form' analyzed and then evaluated.

The assessment of facility care Indicators

Availability of essential drug list or formulary

Total 3717 (34.93%) free drug components were prescribed. 2132 (20.04%) anti-hypertensive drugs +

Table 4. Frequencies of utilization of antihypertensive drugs used as various drug therapy in hypertensive study population (n = 201).

Various drug therapy	Anti-hypertensive drug class	Frequency	Percentage (%)
Single therapy	ACE I	676	31.7
	β-Blocker	104	4.87
	CCBs	234	10.97
	Diuretics	109	5.13
	Angiotensin receptor II antagonist	32	1.50
Total single therapy (A)		1155	54.17
Dual therapy	ACE I + β-Blocker	377	17.70
	ACEI + CCBs	185	8.67
	ACEI +Diuretics	102	4.78
	CCBs + β-Blocker	55	2.60
	CCBs + Diuretics	25	1.17
	Diuretics + β-Blocker	26	1.21
	ACE I+ Angiotensin II receptor antagonist	22	1.03
Total dual therapy (B)		792	37.16
Triple therapy	ACE I + Diuretics + β-Blocker	56	2.63
	Diuretics + CCBs + β-Blocker	47	2.20
	ACE I + Diuretics + CCBs	25	1.17
	ACE I + β-Blocker + CCBs	20	0.94
	CCBs + β-Blocker + Angiotensin II receptor antagonist	20	0.94
	Diuretics + CCBs + β-Blocker	17	0.79
Total triple therapy(C)		185	8.67

ACEI: angiotensin converting enzyme inhibitor; CCBs: calcium channel blocker.

Table 5. Identification of the anti-hypertensive drugs for rationality of prescriptions in hypertensive with other concomitant diseases.

Identification of the antihypertensive drugs	Frequency
Total number of prescription analyzed	1407
Total frequency of drugs were utilized over this study	10,638
The mean number of drugs prescribed per prescription	7.56
Total number of free drug components (Hypertension + co morbidities) as per TNSTG	29
Mean number of free drugs per prescription	2.6
Percentage of free drug components prescribed	3717 (34.9%)
Percentage of paying drugs getting from Drug store	5767 (54.2%)
Percentage of subjects received single therapy	1155 (54.17%)
Percentage of frequently prescribed single therapy (ACE I)	31.7%
Percentage of subjects received dual therapy	792 (37.16%)
Percentage of frequently prescribed dual therapy (ACE I + β-Blocker)	17.7%
Number of subjects received triple therapy	185 (8.67%)
Percentage of frequently prescribed triple therapy (ACE I + Diuretics + β-Blocker)	2.63%
Distribution of anti-hypertensive drugs prescribed to males	1389(65.15%)
Distribution of anti-hypertensive drugs prescribed to females	743(34.84%)

1585 (14.89%) common drugs were complying with Tamil Nadu Standard Treatment Guidelines (TNSTG) effective from 04.03.2013 and 6921 (65.1%) prescribed and percentage drugs actually dispensed to the subjects 5767

Table 6. Drug prescribing pattern for rational drug use in a tertiary care teaching hospital using WHO prescribing indicators (n = 201 encounters).

Measured Prescribing Indicators	Total drugs/ encounters	Average percentage	Mean ± SD
Percentage of generic name of the medicines prescribed	3001	28.2	2.1 ± 1.6
Percentage of encounters with an antibiotic prescribed	330	14.9	0.2 ± 0.4
Percentage of encounters with an injection prescribed	1734	46.76	1.2 ± 1.3
Percentage of free drug components	3717	34.9	2.64 ± 1.3
Percentage of paid drug components	5767	54.2	4.92 ± 2.76

Table 7. Patient care and facility care indicators for rationalities of drug use in a tertiary care teaching hospital based on WHO prescribing indicators.

Measured patient care indicators	Average/Percentage	Mean	Standard deviation
Total number of drugs prescribed	10,638	7.56	2.76
Average consultation time (in minutes)	13.77	13.8	3.75
Average dispensing time (in seconds)	147.48	2.44	1.2
Percentage of drugs actually dispensed	9484 (89.15%)	6.73	2.46
Percentage of drugs adequately labeled	87.1%	6.57	2.40
Subjects' knowledge of correct dosage	38.30%	0.38	0.48
Measured facility care indicators			
Availability of EDL list			
Tamil Nadu Standard Treatment Guidelines (TNSTG)	34.93%	2.64	1.3
Rajah Muthaih Medical College Hospital (RMMCH) drug list	65.1%	4.98	2.42
Availability of key drugs	52.63%	0.52	2.46

(54.2%) drugs' complied with Rajah Muthaih Medical College Hospital (RMMCH) drug list.

The assessment of drug prescribing pattern

Among 1407 prescriptions analyzed for rational medication therapy: generic name (28.2%), Percentage of encounters with an antibiotic prescribed (14.9%), Percentage of encounters with an injection prescribed (46.76%), (34.9%) of free drug components prescribed as per Tamil Nadu Standard Treatment Guidelines (TNSTG) and (54.2%) prescribed as per Rajah Muthaih Medical College Hospital (RMMCH) Drug List of paid drug components, The most commonly prescribed antibiotics were as follows: Ciprofloxacin (3.6%) followed by Amikacin (1.1%), Amoxycillin (3.3%), Ofloxacin (4.9%) and piperacillin (2.0%) and more frequently prescribed injections were as follows: Ceftriaxone (28.2%), Insulin (8.2%), Piperacillin (2.0%), Amikacin (1.1%), and Low molecular weight of heparin (7.26%).

Availability of key (anti-hypertensive) drugs

We evaluated the availability of number of (52.63%) antihypertensive drugs surveyed in a tertiary care teaching hospital recommended for the treatment of hypertension based on WHO core drug use indicator. Patient care and facility care indicators based on WHO prescribing indicators are shown in Table 7. Results on patient care and facility care indicators for rationalities of drug use in a tertiary care teaching hospital based on WHO prescribing indicators are displayed in Table 7.

Measurement of drug utilization of anti-hypertensive medication using ATC/DDD metric system

Measurement of drug utilization of antihypertensive medications were observed as follows as: Angiotensin converting enzyme inhibitor (ACE I) Enalapril and Ramipril; beta blocking agents – metoprolol, atenolol, propranolol, nebivolol; calcium channel blockers –

Table 8. Monthly consumption of anti-hypertensive drugs in defined daily dose in a tertiary care teaching hospital (From August, 2013 to January, 2014).

Generic name available in study site/Class	Aug' 2013	Sep' 2013	Oct' 2013	Nov' 2013	Dec' 2013	Jan' 2014
Angiotensin converting enzyme inhibitor (ACE I)						
Ramipril	3.6	20	35	4.05	4	31
Enalapril	29.25	16.25	16.25	15.5	14.75	26.25
Beta blocking agents						
Metoprolol	4.61	4.55	0.75	0.25	3.58	20.13
Atenolol	1.5	3.2	3.5	2.0	1.5	2.9
Nebivolol	6.7	25.0	12.0	8.0	10.0	5.0
Propranolol	0.8	0.75	0.25	1.0	1.2	0.3
Calcium channel blockers (CCBs)						
Verapamil	1.5	2.2	1.3	1.1	1.9	2.2
Amlodipine	25.5	85.5	8.5	2.0	19.0	41.0
Angiotensin converting enzyme II inhibitor						
Telmisartan	8.0	2.0	2.0	3.0	4.3	1.9
Diuretics						
Furosemide	28.5	33.0	3.5	6.0	1.5	38.75
Hydrochlorthiazide	1.1	1.2	1.1	0.4	1.0	2.2

verapamil and amlodipine; angiotensin converting II enzyme inhibitor - telmisartan and diuretics. Furosemide and hydrochlorothiazide drugs were used during this period (August. 2013 to August, 2014) and monthly consumption of antihypertensive drugs was calculated in defined daily dose study data shown in Tables 8 to 10. During this study period (August, 2013 to August, 2014) the anti-hypertensive drug consumption data were collected and analyzed in general medicine ward. Total 82 subjects were randomly collected and enrolled in the study. Twenty eight (28) subjects were admitted in MIMCU and FIMCU, and 54 subjects were admitted in CCU. The number of beds available in the ward is 450 and the number of days spent was 397 days. The average bed occupancy rate during this study period was 0.6. Amlodipine medication was most frequently utilized with respect to the number of 32.55 DDD/100 bed days and then followed by Ramipril, with 24.70 DDD/100 bed days and enalapril, with19.55 DDD/100 bed days. The total consumption of anti-hypertensive drug was detected to be 122.07 DDD/100 bed-days. The impressed number of DDD assigned for each anti-hypertensive is given by the DDD/ATC WHO metric system as recorded in Table 11.

DISCUSSION

This present study is considered to be a good prescription based evaluation study and the study is used as one of the systematic way for rationality and assessment of drug utilization, aiming to measure the rationality which can reduce morbidity and mortality.

Age factor

The results of our study of age factor proposed that hypertension was more prevalent in male subjects (61.7%) than female subjects (38.3%).The mean age ± SD for male (elderly + non-elderly) was 62.6 ± 11.3 and female (elderly + non-elderly) was 60.45 ± 10.5. The course of study was confirmed with one previous Indian study (Jhaj et al., 2001) on hypertensive subjects who reported the number of male subjects as 51% and female subjects as 49% and another Indian study (Jainaf et al., 2014) revealed that the number of men was 52% and women was 48% on hypertensive subjects. Rachana et al. (2014) revealed that out of 300 prescriptions, hypertension was more prevalent in male subjects (55%) and female subjects (45%) which is confirmed with the present study on hypertensive subjects. In a confounding report (Lee et al., 1997) on hypertensive subjects, women (57%) and men (43%) suffered with hypertension in an overseas study conducted in Hong Kong.

Substance abused

The substance abused in this study population were

Table 9. Monthly consumption of anti-hypertensive drugs in Defined Daily Dose (DDD) in a Tertiary Care Teaching Hospital (From Feb' 2014 – Aug' 2014).

Generic/Brand name available in study site/Class	Feb' 2014	March 2014	April 2014	May 2014	June 2014	July 2014	August 2014
Angiotensin converting enzyme inhibitor (ACE I)							
Ramipril	132	50.5	31.4	17	40	2.25	25
Enalapril	24.25	29.0	20.75	27.25	10.5	22.0	22.75
Beta blocking agents							
Prolomet	12.06	2.59	5.4	4.9	1.41	0.68	2.44
Atenolol	1.4	2.2	2.1	1.3	1.4	1.1	3.89
Nebivolol	7.8	13.5	2.2	6.9	8.9	10.0	14.0
Propranolol	0.3	0.6	0.5	0.3	0.2	0.3	0.25
Calcium channel blockers (CCBs)							
Verapamil	1.1	1.2	1.8	1.9	1.2	0.8	1.0
Amlodipine	51.0	19.0	61.0	76.0	6.0	13.0	26.0
Angiotensin converting enzyme II inhibitor							
Telmisartan	1.0	0.6	0.5	1.1	2.1	0.7	1.8
Diuretics							
Furosamide	10.0	5.8	1.8	2.9	6.0	10.0	2.0
Hydrochlorthiazide	1.1	1.2	1.0	0.5	2.2	1.1	2.9

Table 10. Total consumption of anti-hypertensive drugs in defined daily dose (DDD) in the General medicine ward (From August, 2013 to August, 2014).

Generic/Brand name available in study site/Class	Total drug utilization in Defined Daily Dose (DDD)	Total number of defined daily dose in class wise
Angiotensin converting enzyme inhibitor (ACEI)		
Ramipril	395.8	670.55
Enalapril	274.75	
β-Blocker		
Prolomet	63.35	228.09
Atenolol	27.99	
Nebivolol	130.0	
Propranolol	6.75	
Calcium channel blockers		
Verapamil	19.2	452.0
Amlodipine	433.5	
Angiotensin converting enzyme ii inhibitor		
Telmisartan	29	29.0
Diuretics		
Furosemide	149.75	166.75
Hydrochlorthiazide	17.0	

cigarettes smoking, alcohol, caffeine, tobacco, betel leaf, betel nuts and paan. Substance abused results has shown that total number of smokers, alcohol with caffeine addicts in male subjects' was 94% and 6% female subjects were addicts with caffeine, and 62% male subjects were addicts with tobacco, paan and caffeine.

Table 11. Anti-Hypertensive drug consumption in general medicine wards using DDD/100 bed-days (From August, 2013 to August, 2014).

Classification of Anti-hypertensive drugs	ATC Code	WHO recommended DDD (mg)	DDD/100 bed days
Amlodipine	C08CA01	5	32.55
Ramipril	C09AA05	2.5	24.70
Enalapril	C09AA02	10	19.55
Furosemide	C03CA01	40	15.90
Nebivolol	C07AB12	5	8.85
Prolomet	CO7AB02	150	6.85
Telmisartan	C09CA07	40	4.44
Atenolol	C07AB03	75	3.20
Verapamil	C08DA01	240	3.85
Hydrochlorothiazide	C03AA03	25	1.85
Propranolol	C07AA05	160	0.33
Total anti-hypertensive drug consumption			122.07

About 38% female subjects were addicts with tobacco and betel leaf and nuts. The present study has shown that age factor and smoking, tobacco, betel leaf and alcohol not only increased risk of hypertension, even though the course can be authored with sedentary life style, unhealthy eating habits, excess sodium intake, excess salt intake, restless work and stress. This statement agreed with a previous Indian study (Tiwari et al., 2004) which reported 26% hypertensive age groups.

Known case on hypertension

On accountability of known case on hypertension, we had 42 (20.89%) male subjects and 35 (17.41%) female subjects while with no known case on hypertension with newly diagnosed, it was 58 (28.85%) male subjects and 66 (32.83%) female subjects. This study data was collected from subject's case sheet of subject present and past medical history.

Average length of hospital stay

A total of 82 subjects were admitted in the internal ward. 48 (58.54%) male and 34 (41.46 %) female subjects', who stayed more than 10 days, had severe uncontrolled hypertension with increased blood sugar level and dilated cardiac myopathy, and were kept on constant observation for their blood pressure and blood sugar level. These subjects' stay ranged from 10 to 17 days with reduced morbidity, and average length of hospital stays was 10.33 ± 6.66. Subjects who stayed less than 10 days observed that acute or chronic stage, newly diagnosed on hypertension with atherosclerotic disease and reduced morbidity during stayed in this study period and given appointment, counseled them to continuous proper pressure monitoring. This present study confirmed with one similar previous Indian study by Joel et al., 2014, who reported that the average length of hospital stay was 11.54 ± 7.57. The relationship between length of hospital stay and blood pressure monitoring needs to be inquired in further studies which would improve subject's quality of life and reduce their morbidity and mortality.

Overall Drug prescribing pattern

Fowad et al. (2008) study revealed that out of 192 hypertensive subjects, overall drug prescribing pattern is as follows: diuretics 42.2%, β adrenergic blocker 41.2%, calcium channel blocker 39.1%, angiotensin converting enzyme inhibitor 26% and angiotensin receptor II antagonist 23.4%, α 1-blocker (9.4%). On accountability of overall drug prescribing pattern of this present study, 201 hypertensive subjects had very less usage than that reported by Fowad Khurshid et al in 2012. But in this present study, angiotensin converting enzyme inhibitor (ACE I) drug class (Enalapril and Ramipril) had 45.21%, which was more frequently used than study that reported by Fowad Khurshid et al in 2012.

Single therapy (54.17%) Vs Multiple combinations therapy (45.83%)

Mono therapy was more frequently used than combination therapy (54.17% vs. 45.83%) and very least prescribed drugs rate was as follows: Angiotensin II receptor antagonist 32 (1.5%), angiotensin converting enzyme inhibitor (ACE I) + angiotensin II receptor antagonist 22 (1.03%) and diuretics + calcium channel

blockers + β-Blocker 17 (0.79%) proposed in this present study. Combination therapy drug utilization had very less usage when compared with the study carried out by Fowad et al. (2008) Monotherapy of angiotensin converting enzyme inhibitor (ACE I) 31.7% and angiotensin converting enzyme inhibitor (ACE I) + β-Blocker 251 (17.8%) were more frequently utilized than reported by Fowad et al. (2008) who revealed that Monotherapy 45.4% vs. Combination therapy 54.6%.

Assessment of drug prescribing pattern

The prescription analysis for rational drug therapy was done using WHO core drug use indicators. The mean number of drugs per prescription is computed to the measure of degree of poly pharmacy, the mean found in the present study (7.56 ± 2.76 drugs per prescription) was administered due to multiple concomitant diseases in our hypertensive study population and prescriptions containing 4 to 10 drugs were prescribed in 98% cases, and use of poly pharmacy can also extend risk of an adverse reactions, drug interactions, cost of illness and medication errors (Shankar et al., 2010). On similar study found in Central India by Vandana and Sanjaykumar (2012), average number of drugs was 7.5 per subjects, Western Nepal study reported by Shankar et al. (2010), mean number of drugs was 7.73 ± 4.24, and overseas study conducted in Tanzania (Rimoy et al., 2008); while in the present study, anti-hypertensive drugs contained 1.0 to 3.0 drugs per prescription which is compatible with the Tanzania studies by Rimoy et al. (2008) (2 to 3 drugs). In percentage of prescribed generic name (measure the trends of prescribing by generic name), in the present study, a result of (28.2%) out of 1407 prescriptions was very much lower than that reported by (Bhavesh et al. (2012) (100.0%), similar study found in Lucknow district studied by Kumari et al. (2008) in 2008 showed 27.1% low generic name, another previous study found in Ethiopia reported by Akshaya srikanth et al. (2013) in 2013 showed 94.3%, and percentage of antibiotics prescribed was 14.9% (ciprofloxacin 3.6%, amikacin 1.1%, amoxycillin (3.3%, ofloxacin (4.9%) and piperacillin (2.0%) was lower than the study carried out by Bhavesh et al. (2012) (46.17%) out of 1200 prescriptions and another study reported by Nihar et al. (2000) studied in Dr. Rajendra Prasad Centre for Ophthalmic Sciences (RPC) hospital. In the present study, percentage of injections prescribed (46.3%) was higher than the study carried out by Bhavesh et al. (2012) (0.17%), the most common drug prescribed was Vitamin B Complex (7.65%) which had less usage than the results revealed by Bhavesh et al. (2012) (Vitamin B Complex 11.03%) and 34.9% of free drug components and 54.2% paid drug components in a tertiary care teaching hospital, medical personnel prescribed and dispensed drugs according Tamil Nadu Standard Treatment Guidelines (TNSTG) effective from 04.03.2013 and Rajah Muthaih Medical College Hospital (RMMCH) drug list for the treatment on high blood pressure. These two guidelines were strongly accepted by health care professionals in a tertiary care teaching hospital.

The assessment of patient care Indicator

Percentage of drugs actually dispensed

About 89.15% of all drugs were actually dispensed (34.9% free drug components based on TNSTG + 54.2% paid drug components based on RMMCH drug list). This present study result showed a high frequency of drugs dispensed, when compared with overseas study in Ethiopia reported by Akshaya srikanth et al. (2013) (78% drugs actually dispensed), which is a possible alternative that the TNSTG and RMMCH drug list may manifest medical personnel prescribing medications to most of the hypertensive population thus reducing their morbidity and mortality. This phenomenon is defined by Pepe (1994) as a “consensus between the selection criterion and ‘culturally consolidated’ prescription practices”.

Average consultation time

With respect to the present study, results showed an average consultation time of 13.8 min, which is being classified as ‘excellent’ according to the limitations, with below 15 min as the WHO recommended standard. Though this consultation time is longer than the results revealed by Vania dos Santosa et al. (2004) (9.2 min) and overseas study which revealed that longest consultation time for Nigeria was 6.3 min, while another overseas study reported in Ethiopia by Akshaya Srikanth et al[20] was 4.13 min and shortest consultation time in Bangladesh was 54 s (Hogerzeil et al., 1993).

Average dispensing time

WHO recommended that the pharmacists spending time with subject should be at least 180 s dispensation with each subject. Hence, the duration of dispensation of 147.4 s (2.44 min) in the current study results is good, a proper dispensation and compatible with WHO recommendations, and this result compared with one overseas dispensation reported in the literature in Ethiopia studied by Akshaya srikanth et al. (2013) (4.13 min) and some shortest dispensation reported in the literature in Lopes et al. (1996) (17 s), Tanzania (77.8 s) (Rimoy et al., 2008), Campo Grande (Cunha et al., 2002)

(55 s), Nigeria (12.5 s), Vania dos Santosa et al. (2004) (18.4 s) and Bangladesh (23 s) (Hogerzeil et al., 1993).

Drugs adequately labeled

In this present study carried in a tertiary care teaching hospital at dispensary counter, all drugs were completely labeled, medicines packed with specified carton box with leaflets with 87.1% drugs adequately labeled and medicines prepackaged, with perfect and proper labeling. This present study result is compatible with the Ethiopia study by Akshaya srikanth et al. (2013) (86.4% prepackaging and labeling).

Subjects' adequately knowledge of correct dosage

Akshaya Srikanth et al (2013) study revealed that 77.4% of patient had adequate knowledge about all dosage schedules of the drugs. As a result of this current study subjects, 38.3% had knowledge than that reported by Akshaya et al. (2013). The current study population it was found that 61.7% subjects had inadequate knowledge about how to administer the drugs which has been prescribed. The reason for this was because most of the subjects were illiterate and older people, unable to travel from their home town, irregular treatment, long therapy duration, inability to read prescription due to age, elderly people were affected with mental problems and sedentary life style. So all these factors lead to very poor adequate knowledge on hypertension.

The assessment of facility Indicators

Availability of essential drug list or formulary

A total of 34.9% drugs is prescribed according Tamil Nadu Standard Treatment Guidelines (TNSTG) and mean ± SD number of drugs per prescription should be 2.64 ± 1.3, while 65.1% drugs is prescribed following Rajah Muthaih Medical College Hospital (RMMCH) drug list with mean ± SD per prescription as 4.92 ± 2.40 in a tertiary care teaching hospital.

Availability of key drugs

52.63% anti-hypertensive drugs were freely surveyed in this present study population in a tertiary care teaching hospital and the mean number of anti-hypertensive drugs contained 1.0 to 3.0 per prescription. This present study result is compatible with the Tanzania studies by Rimoy et al. (2008) (2 to 3 drugs).

Total consumption of anti-hypertensive drugs in DDD

The total antihypertensive drug consumption in General medicine wards was measured in impressed number of DDD/100 bed-days. In our study, class wise found that total antihypertensive consumption of (ACE I - Ramipril and Enalapril) (670.55 DDD) was highly utilized. But drug wise, high utilization was found in the general medicine wards having amlodipine with 32.55 DDD/100 bed days than other drugs which we analyzed in present study. A similar study report was found by Joel et al., (2014) (amlodipine 33 DDD/100 bed days) and another study by Jhaveri et al. (2014) postulated that amlodipine utilization in the wards was 29 DDD/100 bed-days. Current study found that total antihypertensive drug consumption in the General medicine wards were 122.07 DDD/100 bed-days.

Conclusion

ACE inhibitors were most frequently utilized and amlodipine was the highest consumed in the internal ward during this study. The clinical pharmacists can be effectively employed for rationality use of medication in hypertensive population on a routine basis. In the present study, cross sectional, observational study parameters was carried out and evaluated, and measured based on WHO core drug use indicator and ATC/WHO DDD metric system. Here, many of the prescriptions were rational, but further improvement is needed in drug prescribing practices and prescribers may contribute to the progress of rational prescribing drug practices in hypertensive study population. Patients too need to express their interest to know more about the drugs they have been prescribed, and this can promote a safe knowledge on their illness and special care, which would improve their quality of life. Further, the present study can lead to finding out the influence of prescribing practice on cost of burden in the subjects which will be carried out in a future research.

Conflicts of interest

Authors declare that there are no conflicts of interest.

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