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ARTICLES

Full Length Research Paper

Anti-hemolytic and reduction of lipid peroxidation capacities of *Detarium microcarpum* Guill. and Perr. fruits

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This study was designed to investigate the ability of ethanol fruit extract from *Detarium microcarpum* to protect erythrocytes against hemolysis and lipid peroxidation. To achieve this objective, hemolytic, anti-hemolytic and lipid peroxidation from cell membrane assays were used. Hemolytic and anti-hemolytic activities (regarding H_2O_2 induced hemolysis) were assessed by determination of free haemoglobin at 540 nm. Inhibition of lipid peroxidation was measured at 532 nm, using thiobarbituric acid reaction on sodium nitroprusside and ferric sulfate induced liposome peroxidation models. *D. microcarpum* fruit ethanol extract (100 µg/ml) did not exhibit any hemolytic effect but reduces significantly hemolysis from human and rat erythrocyte with inhibitory percentages more than 50 and 75%, respectively. Furthermore, the extract caused a significant decrease in both ferric sulfate and sodium nitroprusside inducing lipid peroxidation in each rat tissue liposomes investigated. *D. microcarpum* fruit ethanol extract **protects** erythrocytes against hemolysis and lipid peroxidation, probably due to its antioxidant potential. Therefore, animal tissues disorders caused by cell membrane lipid damage could be potentially managed/prevented by dietary intake of *D. microcarpum* fruit pulp.

Key words: *Detarium microcarpum*, anti-hemolytic activity, inhibition of lipid peroxidation

INTRODUCTION

A positive link has been established between fruit consumption and the reduction of chronic diseases (Sumathy et al., 2013). Antioxidant compounds from fruits have been thoroughly studied in this focus, owing to their beneficial effects on human health (Seifried et al., 2007; Rauchovà et al., 2012). Antioxidant molecules protect tissues against oxidative stress and related diseases (Badmus et al., 2013) through several mechanisms, including the elimination of free radicals, the protection and regeneration of other dietary antioxidants (e.g. vitamin E) and the chelation of pro-oxidant metals (Lima et al., 2014).

Detarium microcarpum Guill. and Perr. (Caesalpiniaceae) is a well-known wild edible fruit specie

growing in Saharan and sub-Saharan countries. Its fruits are traditionally consumed as food source and for medicinal purpose (Wahedi et al., 2013). Hence, *D. microcarpum* is used in the treatment of diverse diseases, notably syphilis, dysentery, diarrhea, bronchitis, pneumonia, sore throat, malaria, leprosy and meningitis (Akah et al., 2012) while the fruits are used to treat skin infections, meningitis and malaria (Bamisaye et al., 2014). Fruit pulp extract of *D. microcarpum* showed an inhibition of the growth of the plant pathogenic fungus, *Cladosporium cucumerinum* and of the enzyme acetylcholinesterase, implicated in Alzheimer's disease (Cavin et al., 2006).

Studies on the nutritional values and the antioxidant properties of *D. microcarpum* fruit pulp are well documented. A remarkable nutritional value with important content of protein, vitamins, carbohydrates and mineral nutrients have been reported (Abreu et al., 2002; Bamisaye et al., 2014; Mariod et al., 2009; Oibiokpa et al., 2014). High content of flavonoid and polyphenols have been also reported along with a strong antioxidant capacity (Lamien-Meda et al., 2008). Additionally, *D. microcarpum* fruit pulp is benefitial to food digestibility and body weight while hematological parameters (red and white cells) are ameliorated (Obun et al., 2011; Wahedi and David, 2013).

The present investigation focused on the capacity of the fruit pulp from *D. microcarpum* to protect erythrocytes against hemolysis and lipid peroxidation damage mediated by pro-oxidant.

MATERIALS AND METHODS

Plant material collection and extraction

Fresh fruits from *D. microcarpum* Guill. and Perr. (Caesalpiniaceae) were harvested in January 2013 at Gampela (25 km, east of Ouagadougou, Burkina Faso). Botanical identity was assessed by Professor Jeanne MILLOGO-RASOLODIMBY from Laboratoire de Biologie et écologie végétale (Université Ouaga I Pr Joseph KI-ZERBO, Burkina Faso) and a voucher specimen (CI: 15928) deposited in the herbarium of the University Ouaga I Pr Joseph KI-ZERBO. Fresh fruits were washed with distilled water and pulp manually scraped prior soaking in ethanol (24 h, 25°C room temperature, continuous stirring). Extract was filtrated, concentrated to dryness in a vacuum evaporator and stored at 4°C until further investigations.

Experimental animals

Three-month-old male Wistar rats, weighing between 190 and 250 g were provided by the animal housing facility of Research Institute of Health Science (Burkina Faso). They were maintained at approximately 25°C, on a 12 h light/12 h dark cycle, fed with standard laboratory food and water *ad libitum*. Animals handling procedures strictly conformed to the Guide for the Care and Use of Laboratory Animals (National Institutes of Health, publication number 85-25, revised 1996) and approved by our institution, regarding the internationally accepted standard ethical guideline for laboratory animal use and care as described in the European

Committee Guidelines (EEC, 1986).

Chemicals and reagents

Gallic acid, sodium nitroprusside, thiobarbituric acid (TBA), trichloroacetic acid (TCA), sodium phosphate dibasic (Na_2HPO_4), diethyl ether and sodium phosphate monobasic (NaH_2PO_4) were purchased from Sigma-Aldrich (St. Louis, MO, USA). Hydrogen peroxide, ascorbic acid, ferric sulfate and sodium chloride were supplied by Labosi (Paris, France). Ketamine and ethanol were purchased from Prolabo (Paris, France).

Anti-hemolytic activity

Blood collection

Human blood sample was provided by the National Center for Blood Transfusion, Ouagadougou, Burkina Faso. Peripheral blood sample obtained from a healthy volunteer (28 years of age that did not smoke, drink, or use chronic medication) was collected by venipuncture using a top Vacutainer® (BD Diagnostics, Plymouth, UK). Blood collection was in accordance with the declaration of Helsinki (as revised in 64th WMA General Assembly, Fortaleza, Brazil, October 2013). Blood collected in heparinized tubes was store at 4°C for erythrocytes suspension preparation. Rat blood was collected in heparinized tube by heart puncture. Animals were previously anesthetized with ketamine by intraperitoneal injection (60 mg/kg of b.w) before blood collection.

In vitro hemolytic and anti-hemolytic assays

The anti-hemolytic activity of fruit pulp extract was examined *in vitro* as previously described (Sasikumar et al., 2012). Erythrocytes were separated by centrifugation (1500 G, 10 min, 4°C) and washed with isotonic sodium phosphate buffer (pH 7.4) until the supernatant was colorless. Erythrocytes suspensions (2 ml, 2% in isotonic sodium phosphate buffer pH 7.4) were mixed to 0.5 ml of test samples (1 mg/ml in sodium phosphate buffer), 2 ml of sodium phosphate buffer and incubated (37°C, 5 min). Mixtures were subsequently incubated (37°C, 2 h) without or with 0.5 ml of H_2O_2 (100 mM in buffer) for hemolytic and anti-hemolytic investigations, respectively. After centrifugation (1500 G, 5 min and 4°C) supernatants were collected and the extent of hemolysis determined by recording the absorbance at 540 nm, corresponding to hemoglobin liberation. Results were expressed as inhibitory percentage (%) of erythrocytes hemolysis. Ascorbic acid and gallic acid were used as positive controls.

In vitro reduction of lipid peroxidation from cell membrane

Liposomes preparation

Rats were decapitated under mild diethyl ether anesthesia and pancreas, heart, brain, kidney and liver were rapidly dissected, placed on ice and weighed. Each tissue was subsequently homogenized in cold phosphate buffer saline (1/10, w/v). Homogenates were centrifuged (3000 G, 10 min and 4°C) and pellet discarded. Low-speed supernatants containing mainly water, proteins and lipids (cholesterol, galactolipids individual phospholipids and ganglioside) were kept as liposome preparations for thiobarbituric acid assays.
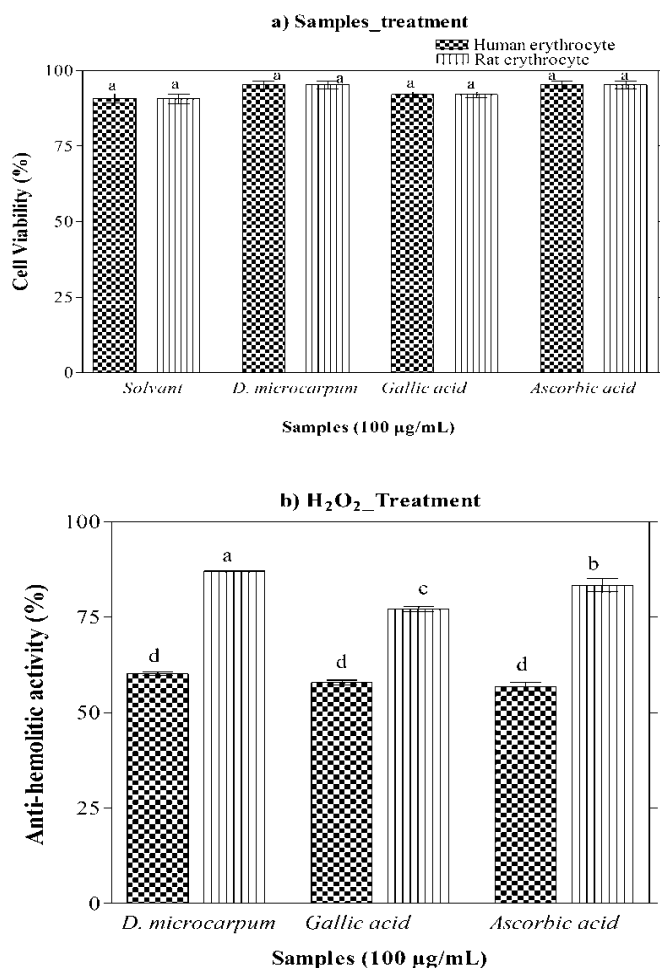
a) Samples_treatment

b) H₂O₂_Treatment

Figure 1. Hemollitic (a) and antihemolitic activity of fruit ethanol extract from *D. microcarpum* on hydrogen peroxide induced human and rat erythrocytes hemolysis (b). Data are expressed as the mean ± SD of triplicate independent experiments (n=3). Data with different superscrit letters (one-way ANOVA followed by Newman-Keuls post-test) are significantly different (P<0.05).

Thiobarbituric acid reactions

Liposomes were used to evaluate *in vitro,* the reduction effect of lipid peroxidation on Fe^{2+} induced-lipid peroxidation (Su et al., 2009) and sodium nitroprusside induced-lipid peroxidation (Akomolafe et al., 2012). Reaction mixture, containing test sample (100 µl, 1 mg/mL), hydrogen peroxide (100 µl and 10 mM), liposome preparation (700 µl) and ferric sulfate (100 µl, 10 mM) or sodium nitroprusside (100 µl, 50 mM), was incubated (37°C and 1 h). After incubation, trichloroacetic acid (1 ml, 15 %) and thiobarbituric acid (1 ml, 1%) were added to the mixture and boiled (100°C, 15 min). Mixture was centrifuged (3000 G and 10 min) and absorbance of supernatant measured at 532 nm. Results were expressed as inhibitory percentage (%) of lipid peroxidation. Ascorbic acid and gallic acid were used as positive controls.

Statistical analysis

Experiments were performed in triplicate and data presented as mean value ± standard deviation. GraphPad software (Graph Pad

Software Inc. San Diego, CA, USA) was used for statistical analyses. The one-way ANOVA for repeated measures followed by Newman-Keuls post-test was used to verify the impact of treatments on erythrocytes hemolysis and lipid peroxidation. P <0.05 was considered as being significant.

RESULTS AND DISCUSSION

The ability of ethanol fruit extract from *D. microcarpum* to protect the integrity of cell membrane against oxidative damage was assessed using inhibitions of hemolytic and lipid peroxidation assays.

To investigate the effect of *D. microcarpum*, fruit pulp on cell membrane integrity (cytotoxicity), erythrocytes were treated with vehicle (control) or fruit extract (100 µg/mL) and erythrocytes hemolysis was measured (Figure 1a). It was found that *D. microcarpum* fruit pulp ethanol extract did not affect significantly (p>0.05) the integrity of cell membrane when hemoglobin liberation was compared with control. These results suggest that *D. microcarpum* fruit pulp ethanol extract (100 µg/mL) did not exhibit a cytotoxic effect on human and rat erythrocytes *in vitro*.

To assess the anti-hemolytic activity of *D. microcarpum* fruit pulp, human and rat erythrocyte were incubated with hydrogen peroxide after fruit pulp extract application (Figure 1b). It was found that fruit pulp extract exercise some protective activities on rat and human erythrocytes against the hemolytic effects of hydrogen peroxide. Moreover, rat erythrocytes were more protected by extract than human erythrocytes. Interestingly, *D. microcarpum* fruit extract exhibited statically similar anti hemolytic activity than the standard compounds in the human erythrocytes model (p>0.05) while it was more potent in protecting rat erythrocytes against hemolysis (p<0.05).

Inhibition of lipid peroxidation by fruit pulp extract on liposomes from rat organs using ferric sulfate and sodium nitroprusside induced-lipid peroxidation models were demonstrated (Figure 2). It was found that fruit pulp extract at 100 µg/mL reduce more than 60% lipid peroxidation of tissues homogenate in both iron ion and sodium nitroprusside induced lipid peroxidation assays. Regarding Figure 2a, lipid peroxidation of heart, kidney and brain liposomes (separately) induced by iron ion were more inhibited by fruit pulp extract than liver and pancreas liposomes. Similar results were observed with standard compounds. When sodium nitroprusside was used as toxin (Figure 2b), lipid peroxidation of brain, liver and pancreas liposomes were less inhibited by extract than heart and kidney liposomes. Similar results were also observed with gallic acid and ascorbic acid, used as positive control. Furthermore, extract showed some similar lipid peroxidation inhibition (p>0.05) than standards compounds. In general, heart liposome was the most protected against both iron ion and sodium nitroprusside induced lipid peroxidation and liver liposome was the list protected. Moreover, all tissues liposomes were more protected by extract against iron
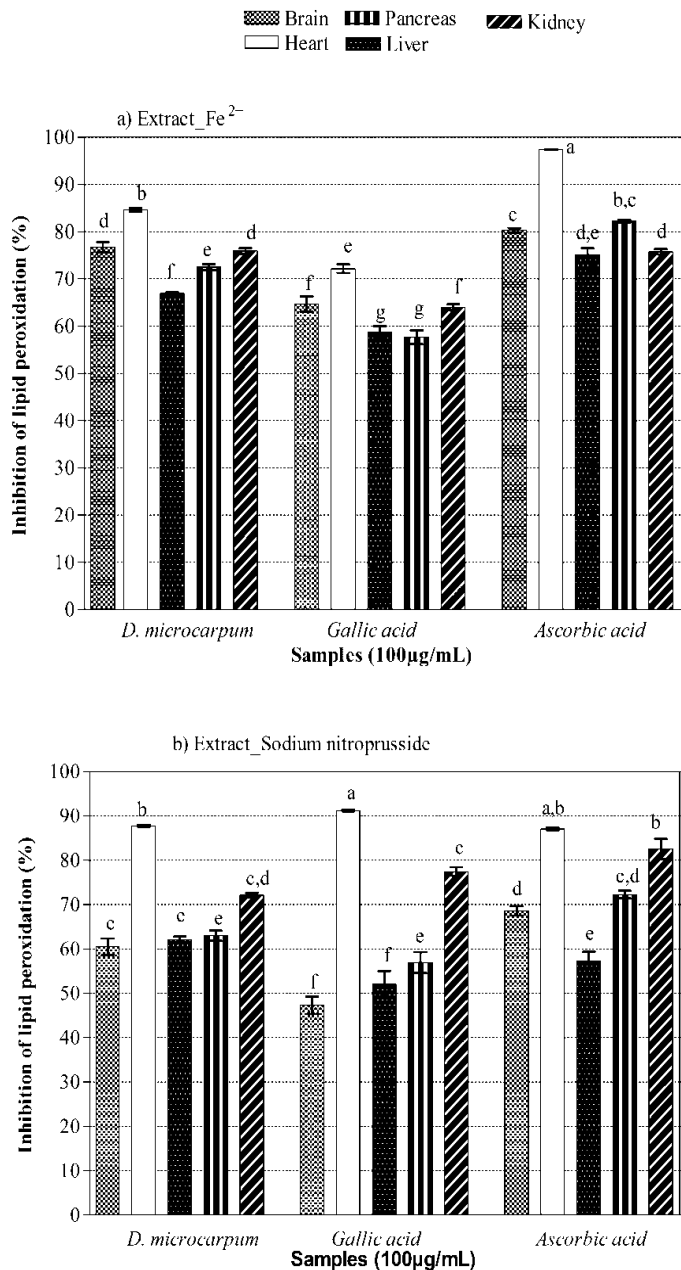
Figure 2. Protective effects of fruit ethanol extract of *D. microcarpum* on ferric sulfate (a) and sodium nitroprusside (b) induced lipid peroxidation in rat tissues homogenate. Data are expressed as the mean ± SD of triplicate independent experiments (n=3). Different letters in each toxin applicated assay represent significant differences using analysis of variance followed by Newman-Keuls post-test (*p* values < 0.05 were considered as significant).

ion induced lipid peroxidation than sodium nitroprusside. Together, these finding suggest that the capacities of fruit pulp ethanol extract from *D. microcarpum* to protect bio membrane from lipid peroxidation depend on the nature of pro-oxidant and the source of liposomes.

This study data demonstrated that fruit ethanol extract from *D. microcarpum* was an interesting radical fighter and seems benefitial in preventing cell membrane against oxidative damage. In the *in vitro* iron ion induced erythrocyte hemolysis model, hydroxyl radical is formed by hydrogen peroxide via Fenton reaction. This radical initiate the lipid peroxidation of bio membrane by electrophile attack, and the propagation of this peroxidation is conducted by alkoxyl and peroxyl radicals engendered, leading to haemoglobin liberation. Inhibition of hydrogen peroxide induced erythrocyte hemolysis by extract could be explained by the properties of extract to scavenge directly hydrogen peroxide by turning it into water molecule (James and Alewo, 2014) or to inhibit hydroxyl radicals formation via Fenton reaction, impeaching lipid peroxidation initiation (Su et al., 2009). Fruit extract could also stop lipid peroxidation propagation by alkoxyl and peroxyl radical scavenging. Verstraeten et al. (2004) reported in liposomes stability study, that flavonoids such as flavanols and related procyanidins can prevent the ferrous iron-mediated increase in membrane permeability. Together, these finding supported the hypothesis that these flavonoids can interact with the polar head group of lipids and consequently limit the incorporation of certain deleterious molecules that could affect membrane integrity and function. These finding suggested that the antihemolitic property of *D. microcarpum* fruit may be due in part to it richest in flavonoids content (Lamien-Meda et al., 2008). Rat erythrocytes were more protected than human erythrocytes against hydrogen peroxide toxicity according with previous studies (Pekiner, 2002). This author reported that membrane of rat erythrocyte was richer in lecithin contents than human erythrocyte, but the last one contained most rate of sphingomyelin. These finding suggested that sphingomyelin is more sensible to lipid peroxidation than lecithin. Regarding rat erythrocyte hemolysis test, extract showed higher erythrocyte protection than ascorbic acid. This finding suggested that ascorbic acid in certain conditions can exercise adverse effects on cell membrane stability. So, ascorbic acid in the presence of transition metals can react in turn to form radical, leading to cell membrane lipid peroxidation contributing to attenuate its membrane protective effect.

In iron ion and sodium nitroprusside induced liposomes lipid peroxidation model, iron ion can initiate liposomes lipid peroxidation by a direct one electron transfer on an unsaturated site in lipid carbon skeleton. Iron ion can also cause indirectly lipid peroxidation by hydroxyl radical formation via Fenton reaction and also accelerates peroxidation by decomposing lipid hydroperoxides into peroxyl and alkoxyl radicals that can themselves abstract hydrogen and perpetuate the chain reaction of lipid peroxidation (Ojo et al., 2014). In the *in vitro* sodium nitroprusside induced lipid peroxidation model, the hydrolysis of sodium nitroprusside in physiological middle releases Fe^{2+} causing lipid peroxidation via Fenton

Reaction and the concomitant formation of oxide nitric radical can react together with other reactive oxygen species amplifying lipid peroxidation by secondary radical species formation (Akomolafe et al., 2012). The protected activity of extract against these two pro-oxidants could be explained by the properties of extract to chelate iron ion or to trap radical species formed by these pro-oxidants. Previously, demonstrated radicals scavenging effect of fruits from *D. microcarpum* could justify its lipid peroxidation inhibition properties (Lamien-Meda et al., 2008). Extract could also stop the propagation of lipid peroxidation by alkoxyl and peroxyl radicals' neutralization. Extract inhibited more lipid peroxidation induced by iron ion than sodium nitroprusside (Figure 2a and b). This finding may be due to the probable synergic effect of Fe^{2+} and nitric oxide released from the hydrolysis of sodium nitroprusside on lipid peroxidation mediation (Khan, 2014). The highest sensibility of liver and brain to these pro-oxidants toxicity may be due to the presence of abundant polyunsaturated fatty acids in these organs. So, brain and liver contains higher amount of polyunsaturated fatty than heart, pancreas and kidney justifying their vulnerability to lipid peroxidation (De et al., 2008). These two organs are equally, potential sources of reactive oxygen species with a considerable reduction of antioxydant level comparatively to the other organs exposing their liposomes to lipid peroxidation (De et al., 2008).

The use of many drugs is limited because of their cytotoxicity effects associated with cell membrane degradation and these actual results could encourage the dietary and medicinal intake of *D. microcarpum* fruit.

Conclusion

The ethanol extract of fruit pulp of *D. microcarpum* reduces significantly *in vitro*, lipid peroxidation of all liposomes mediated by iron ion and Sodium nitroprusside. It also reduces significantly the hydrogen peroxide-induced erythrocyte hemolysis. A part of the mechanisms through which the ethanol extractable phytochemicals in these fruits protect the testes from oxidative stress may be due to the presence of antioxidant compounds. The use of *D. microcarpum* as food additive could protect consumers against tissues chronic diseases associated with cell membranes destruction caused by free radicals mediated-lipid peroxidation.

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CONFLICT OF INTERESTS

The authors declare that there is no conflict of interest.

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Full Length Research Paper

In vitro evaluation of antiasthmatic activity of ethanol leaf extract of *Guiera senegalensis* j. F. GMEL (Combretaceae)

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Guiera senegalensis is a medicinal plant that is widely used in West Africa against many illnesses. In this work, the antiasthmatic properties of ethanol leaf extract of the *G. senegalensis* were evaluated by using various *in vitro* animal models. *In vitro* models like isolated guinea pig tracheal chain preparation, isolated guinea pig ileum and rabbit jejunum preparations were studied to evaluate possible mechanism by which extract shows relaxant activity. The preliminary phytochemical screening of the extract revealed the presence of alkaloids, anthraquinones, flavonoids, saponins and tannins. The study showed that the extract contained phytochemicals such as flavonoids, alkaloids, steroids, tannins and saponins. Isolated tracheal smooth muscles contractions induced by histamine was significantly ($p<0.01$) reduced by the extract, so also that induced by acetylcholine at concentrations of 4 and 8 mg/ml. The findings were similar to that of 0.4 µg/ml isoprenaline. Guinea pig and rabbit jejunum contractions induced by histamine and acetylcholine were significantly ($p<0.01$) reduced by the extract. These observed effects can be attributed to the presence of phytochemicals such as flavonoids, alkaloids and steroids in the extract. The results of these studies indicated ethanol extract of *G. senegalensis* possess antiasthmatic activity.

Key words: Antiasthmatic activity, bronchoconstriction, *Guiera senegalensis,* guinea pig ileum.

INTRODUCTION

Asthma is a respiratory disease characterized by recurrent episodes of wheezing, chest tightness, cough and difficulty breathing brought about by bronchial constriction, inflammation, and excessive mucus secretion due to bronchial hyperesponsiveness (GINA, 2014). Although advancement in the treatment of asthma is on the increase, a great number of individuals have been affected by asthma. It has been estimated that

about 300 million people in both developed and developing countries suffer asthma attacks worldwide (GINA, 2015). Global prevalence of asthma has been approximated to be 10% among children and 5% among adults population (Rathore et al., 2011). In Nigeria, asthmatic children has been reported to be between 5.1 and 14.3% (Musa and Aliyu, 2014) while estimate for adult Nigerians having asthma has been put to 10%

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(Desalu et al., 2013). Prevalence of asthma has been estimated to increase by 59% worldwide in 2025 (Masoli et al., 2004). This can be attributed to increased industrialization and urbanization.

Although, pharmaceutical drugs are the major treatments given, a large number of patients worldwide (especially in Africa and Asia) still patronize traditional medicines as an alternative (WHO, 2013). It has been reported that 75 to 90% of the rural populations worldwide still depend on herbal preparations as their main source of medicines (Shettima et al., 2013). Research in traditional medicine is also increasing worldwide and has been encouraged by the World Health Organization because of the large number of patronizers of such kind of treatment for their healthcare (WHO 2013). *Guiera senegalensis* is used as a medicinal plant by many cultures and researches carried out on the medicinal uses of the plant turn out to give good results (Jigam et al., 2011). The plant has been used traditionally to treat various diseases including asthma and its use in treatment of pulmonary problems has been documented by Dénou et al., (2016) and Saraswathy et al. (2014). The plant could therefore be considered important in medical interventions and its potential as anti-asthmatic be exploited. This may generate data that can be used to complement pharmaceutical drugs for the benefit of humanity. This study therefore, aimed at evaluating the potential of ethanol extract of *G.senegalensis* (ELEGS) as anti-asthmatic.

MATERIALS AND METHODS

Drugs and chemical

The following drugs and chemicals were used in the experiment: Acetylcholine chloride (Sigma Chemicals, USA), histamine diphosphate (Sigma Chemicals, USA), atropine sulphate (Shandong Shenglu Pharmaceutical Co., Ltd.), isoprenaline hydrochloride (Sigma-Aldrid), mepyramine, sodium hydrogen carbonate (BDH Chemicals Ltd Poole, England), sodium chloride (Johnson and Solomon Ltd. London, England), D-glucose (BDH Chemicals Ltd. Poole, England), calcium chloride (BDH Chemicals Ltd. Poole, England), sulfuric acid, ferric chloride, acetic anhydride and ethanol.

Experimental animals

Adult guinea pigs (weighing 400 to 450 g) and rabbits (weighing 3.0 to 3.5 kg) were obtained from the animal house of the Department of Pharmacology and Therapeutics, Faculty of Pharmaceutical Sciences, Ahmadu Bello University, Zaria. The animals were kept in propylene cages under favourable conditions prior to the study. Water and feed were provided *ad libitum* according to the ethical guidelines of Ahmadu Bello University for the care and use of animals in research.

Plant

Fresh leaves of *G. senegalensis* were collected from Tofa Local Government Area in Kano State of Nigeria. Taxonomic identification was done and voucher number (BUKHAN0032) was deposited at the herbarium of Biological Sciences Department, Bayero University, Kano.

Extraction

Leaves of the plant material were thoroughly washed with distilled water to remove dirt and soil and dried under the shade. The plant material was then pulverized into fine powder and the powder macerated in 70% ethanol for 48 h. The resultant extract was filtered and then through Whatman® Filter Paper No.1, 185 mm. The filtrate was concentrated in an oven at 40°C.

Preliminary phytochemicals screening

Phytochemicals screening of the ethanol leaf extract of *G. senegalensis* (ELEGS) were carried out to determine the presence of secondary metabolites such as alkaloids, steroids and flavonoids as described by Tiwari et al. (2011).

Isolated guinea pig trachea preparation

Guinea pig of either sex weighing 400 to 450 g were starved overnight but allowed free access to water. The animals were then sacrificed and the entire tracheae was dissected out and removed and cut into individual rings. Twelve rings were tied together and mounted in 25 ml organ bath containing Krebs physiological solution. The tissue was maintained at 37°C under tension of 1 g and constantly aerated with carbogen (Vogel and Vogel, 2002). The tissue was allowed to equilibrate during which the bath solution was replaced every 10 min. After the equilibrium period, the action of the extract alone was evaluated; three graded concentrations were selected and used for the study. Contraction was then induced by adding acetylcholine or histamine separately. When the contraction reached maximum (initial spasm), the concentration was recorded and after 5 min the standard drug (isoprenaline 0.4 µg/ml) was added (Kulkarni, 2005). Thereafter, the test extract (2, 4 and 8 mg/ml) was added serially in the presences of histamine and then acetylcholine. The tissues were observed for bronchodilation.

Isolated guinea pig ilea preparations

Guinea pig of either sex weighing 400 to 450 g were starved overnight but allowed free access to water. The animals were then sacrificed and then dissected. The intestine was removed and the distal portion was cut into pieces 2 to 3 cm long. Mesentery was removed and tyrode solution passed through the pieces of the tissue until the effluent was clear. The tissue was then mounted in 25 ml organ bath containing Tyrode physiological solution. The tissue was maintained at 37°C under tension of 1 g and constantly aerated (Vogel and Vogel, 2002). At the end of the equilibration period, increasing concentrations of histamine and the extract were added separately. The concentration of histamine that produced the submaximal response was then added. Five minutes after, the standard drug (mepramine 0.04 µg/ml) was added. The tissue responses were recorded. The tissue was then rinsed and allowed to equilibrate after which the test extracts (2, 4 and 8 mg/ml) was added serially in the presence of histamine. The tissues were observed for bronchodilation using microdynanometer.

In another set up of isolated guinea pig ileum preparation, effects of the acetylcholine and acetylcholine in presence of atropine, and graded concentrations of the extract were studied as described by Kulkarni (2005). Responses of the tissues were recorded.

Table 1. Phytochemical constituentsof ethanol leaf extract of *Guiera senegalensis*.

Constituent	Inference
Alkaloids	+
Anthraquinones	+
Cardiac glycosides	-
Flavonoids	+
Phlabotanins	-
Saponins	+
Steroids	+
Tannins	+
Terpenoids	-

(+) present; (-) absent.

Isolated rabbit jejunum preparations

According to the method of Amos et al. (2000), overnight fasted rabbits (2.0 to 2.5 kg) were sacrificed and dissected. Segments of the rabbit jejunum of about 2 to 3 cm long were removed, washed with Tyrode solution and mounted in 25 ml organ bath and maintained at 37°C. The tissue was allowed to equilibrate and then, the effect of the various concentrations of the extract alone on the tissue was determined. After a washout period, various concentrations of the acetylcholine alone were added and the concentration that produced submaximal response determined. Using the concentration of acetylcholine that produced submaximal response, the procedure was repeated in the presences of atropine and then increasing concentrations of the extract. In another set up, the procedure was repeated using propranolol and isoprenaline in place of acetylcholine and atropine respectively.

Membrane stabilization assay

Bovine red blood cells (RBC) were obtained from slaughter house and made into 5% suspension. To 1 ml of the RBC suspension, 0.1 ml of 50 mM H_2O_2 was added and incubated at 37°C to initiate hemolysis. After 30 min, 4 ml distilled water was added to the mixture and centrifuged at 1000 rpm for 10 min and absorbance of the supernatant was then taken at 540 nm. This procedure was repeated in the presence of the various concentrations of the extract and percentage inhibition of hemolysis was calculated (Su et al., 2009).

Data analysis

The data obtained were expressed as Mean ± standard error of mean (SEM) or percentages protection. Data were analyzed using one way analysis of variance (ANOVA), followed by Dunnett's post-hoc tests. Values of $p < 0.05$ were considered statistically significant. Results were presented in tables and charts.

RESULTS

Extraction

Maceration of the *G. senegalensis* leaf in 70% ethanol

yields 19.2% dark coloured extract.

Phytochemical screening

Preliminary qualitative phytochemical screening of ELEGS indicated the presence of alkaloids, flavonoids, saponins, steroids and tannins (Table 1). Similar study on the leaf extract conducted by Akuodor et al. (2013) also found these phytochemicals with the exception of anthraquinones.

Effects of ethanol leaf extract of *G. senegalensis* on isolated guinea pig trachea

The extract alone produced neither contraction nor relaxation. However, histamine (0.6 µg/ml) induced-contraction was dose-dependently inhibited by the extract. The effect of the extract at 4 and 8 mg/ml was statistically significant ($p < 0.01$) and was comparable to that of 0.4 µg/ml isoprenaline (Figure 1). The extract also significantly ($p < 0.05$) inhibited acetylcholine (0.4 µg/ml) induced-contraction as compared to control, but lower than isoprenaline 0.4 µg/ml (Figure 2).

Effect of ethanol leaf extract of *G. senegalensis* on isolated guinea pig ileum

Graded doses of the extract produced dose-dependent relaxation of isolated guinea pig ileum. Responses were all significant ($p < 0.05$) compared to the baseline. Maximum relaxation was obtained at 3.2 mg/ml (Figure 3). On challenging the extract to histamine-induced contraction, significant and dose-dependent inhibition was observed. The extract at concentration of 1.6 mg/ml resulted to higher inhibition ($p < 0.001$) than 0.2 µg/ml mepyramine (Figure 4). Similar result was obtained on acetylcholine-induced contraction (Figure 5).
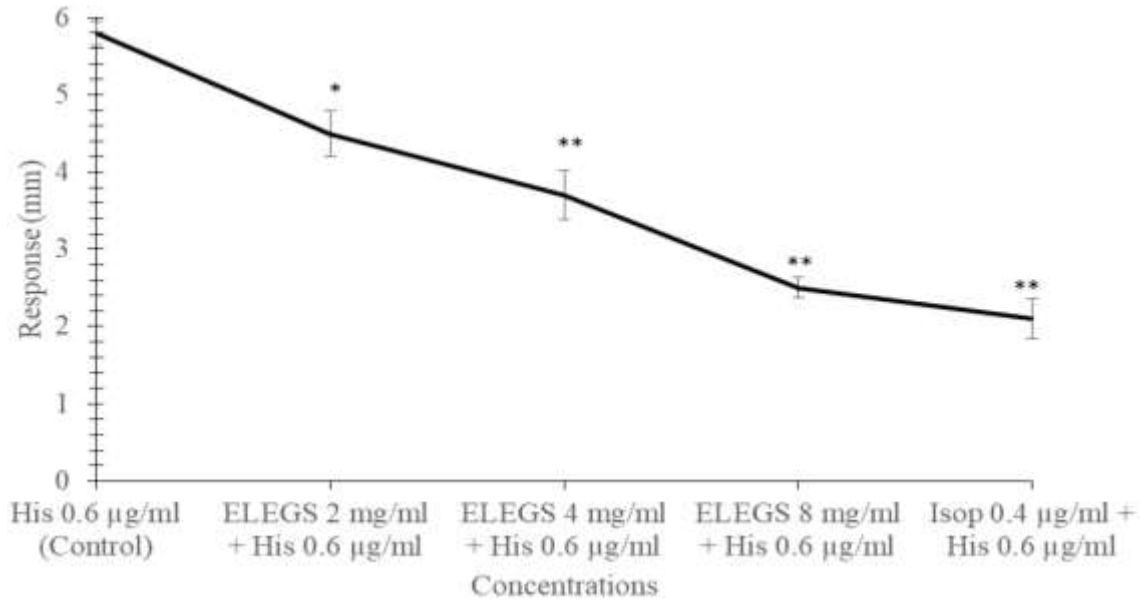
Figure 1. Effect of Ethanol Leaf Extract of *Guierasenegalensis*on Histamine Induced Contractions of Guinea Pig Trachea. Values represents mean ± SEM (n=3). Data analysis was performed by ANOVA followed by Dunnett's post-hock test to determine significance $(p<0.05)^*$ $(p<0.01)^{**}$. His: Histamine; Iso: Isoprenaline; ELEGS: Ethanol leaf extract of *Guiera senegalensis*.

Figure 2. Effect of Ethanol Leaf Extract of *Guierasenegalensis*on Acetylcholine Induced Contraction of Guinea Pig Trachea. Values are expressed as mean ± standard error of the mean (n=3). Statistical significances $(p<0.05)^*$ $(p<0.01)^{**}$ were determine by Dunnett's test after ANOVA. Ach: Acetylcholine; Iso + Isoprenaline; ELEGS: ethanol leaf extract of *Guiera senegalensis*.

Effect of ethanol leaf extract of *G. senegalensis* on rabbit jejunum

Dose-dependent inhibition of the spontaneous basal contractions of isolated rabbit jejunum was observed when ELEGS was used alone. The contraction was almost abolished at 6.4 mg/ml (Figure 6). The contractile responses induced by acetylcholine (0.04 µg/ml) were significantly $(p<0.01)$ inhibited by graded doses of ELEGS in a dose-dependent manner. Atropine (0. 04

Figure 3. Effect of ethanol leaf extract of *Guiera senegalensis* on isolated guinea pig ileum. Values are expressed as mean ± standard error of mean (n = 3). Data analysis was performed by ANOVA followed by Dunnett's test to determine significance (p<0.05)*, (p<0.01)**. ELEGS: Ethanol leaf extract of *Guiera senegalensis*
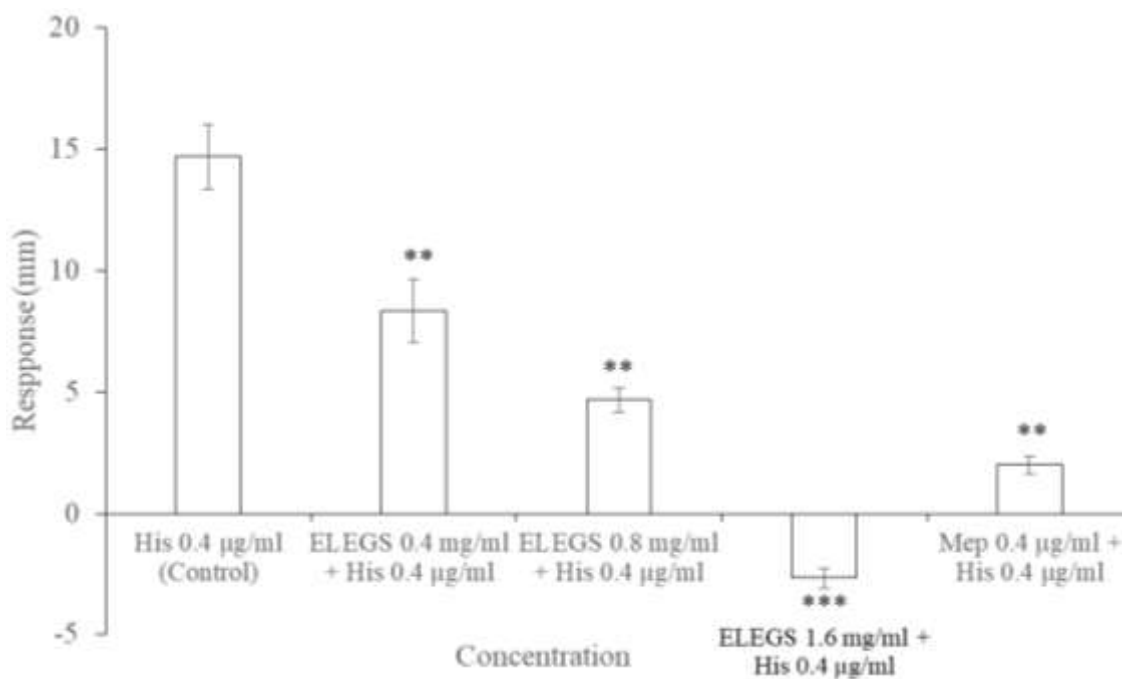
Figure 4. Effect of ethanol leaf extract of *Guiera senegalensis*on histamine induced contraction of guinea pig ileum. Values are expressed as mean ± standard error of the mean (n=3). Statistical analysis was performed by ANOVA. Dunnet's post-hock was used to determine statistical significance (p<0.01)**, (p<0.001)***. His: Histamine; Mep: mepyramine; ELEGS: ethanol leaf extract of *Guiera senegalensis.*
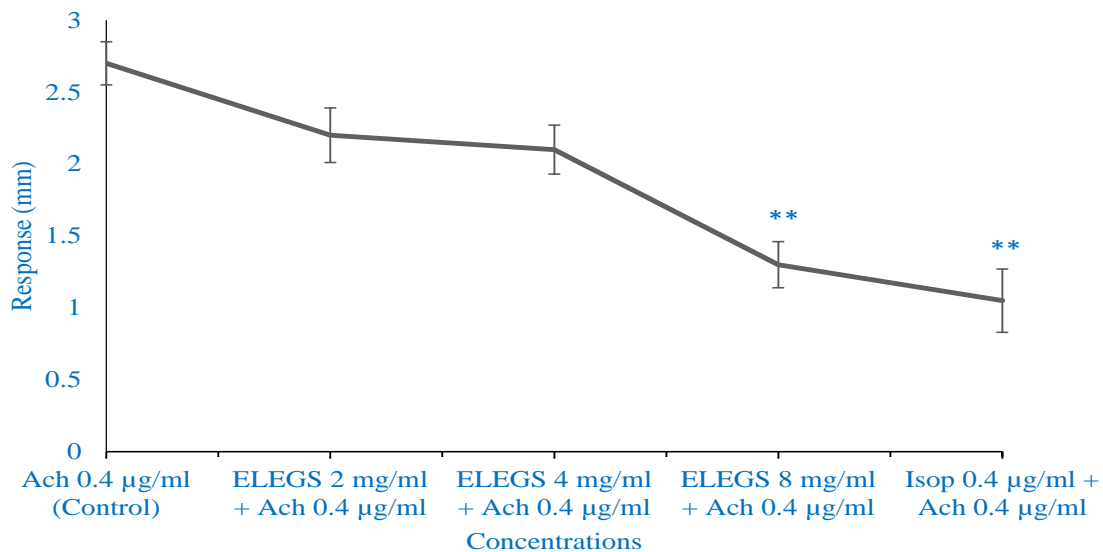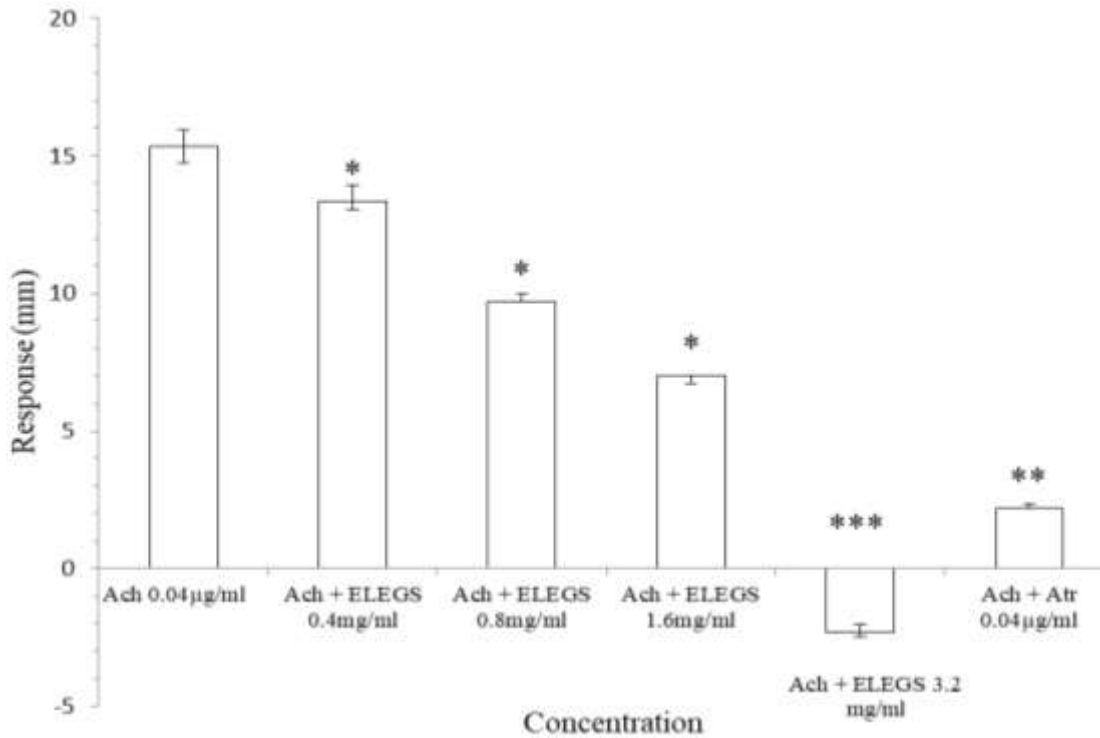
Figure 5. Effect of ethanol leaf extract of *Guiera senegalensis*on acetylcholine induced contraction of guinea pig ileum. Values are expressed as mean ± standard error of the mean (n = 3). ANOVA was used for data analysis followed by Dunnett's post-hock to test for significance (p<0.05)* (p<0.01)** (p<0.001)***. Ach: Acetylcholine; Atr: atropine; ELEGS: ethanol leaf extract of *Guiera senegalensis.*
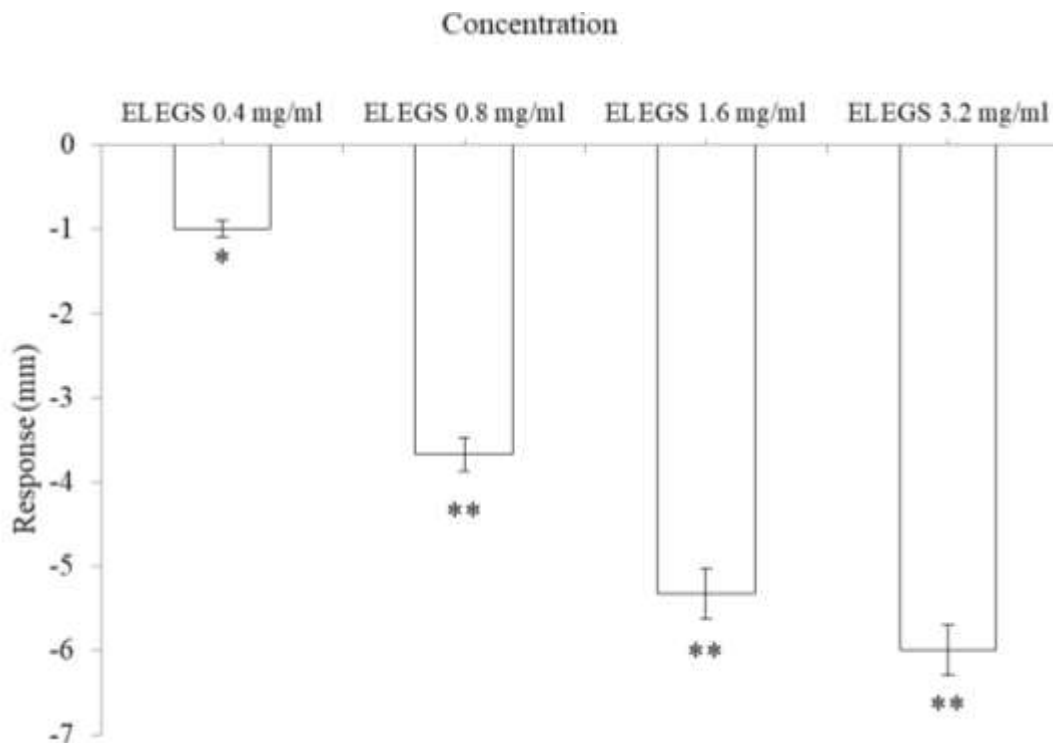

Figure 6. Effect of ethanol leaf extract of *Guiera senegalensis* on isolated rabbit jejunum. Values are expressed as mean ± standard error of mean (n = 3). Data analysis was performed by ANOVA while significances (p<0.05)* (p<0.01)** were determined using Dunnett's post-hock test. Key: ELEGS: Ethanol leaf extract of *Guiera senegalensis.*

Table 2. Effects of ELEGS on acetylcholine induced contraction of rabbit jejunum.

Treatment	Response (mm)
Ach 0.04 µg/ml (Control)	65.00 ± 4.5
Ach + ELEGS 0.8 mg/ml	47.33 ± 3.52**
Ach + ELEGS 1.6 mg/ml	38.33 ± 3.33**
Ach + ELEGS 3.2 mg/ml	30.66 ± 0.88**
Ach + ELEGS 6.4 mg/ml	11.33 ± 0.33***
Ach + Atropine 0.04 µg/ml	8.40 ± 0.70***

Values are expressed as mean ± standard error of mean (n=3). Data analysis was performed by ANOVA followed by Dunnett's test to obtain significance ($p<0.01$)**, ($p<0.001$)***. Ach: Acetylcholine; ELEGS: ethanol leaf extract of *Guiera senegalensis.*
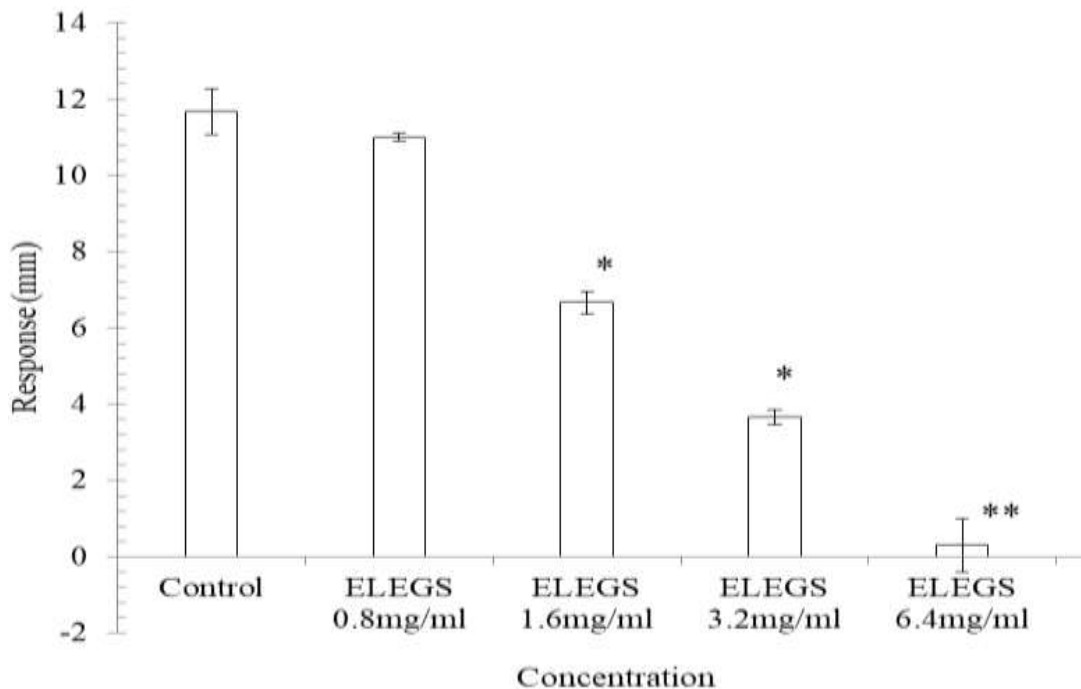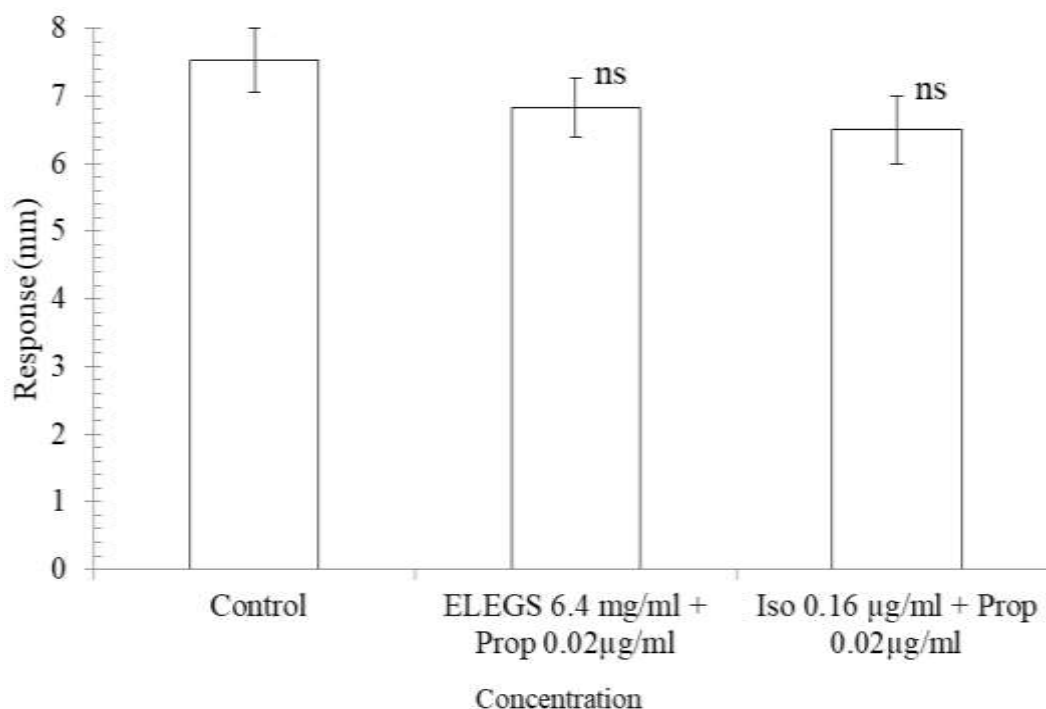

Figure 7. Effect of propranolol and ethanol leaf extract of *Guiera senegalensis* on rabbit jejunum. Values are expressed as mean ± standard error of mean (n = 3). Data analysis was performed by ANOVA followed by dunnett's post-hock test. Iso: Isoprenaline; Prop: propranolol; ELEGS: ethanol leaf extract of *Guiera senegalensis*; ns: not significant ($p>0.05$).

µg/ml) also significantly ($p<0.001$) inhibited the contractile response induced by acetylcholine (Table 2).

Effect of ethanol leaf extract of *G. senegalensis* and propranolol on rabbit jejunum

Responses obtained from rabbit jejunum after addition of ELEGS (6.4 mg/ml) to baths pretreated with propranolol were not significantly different from the control. Similar effect was observed with 0.16 µg isoprenaline (Figure 7).

Membrane stabilization effect of ethanol leaf extract of *G. senegalensis*

The membrane stabilizing effect of ELEGS was assessed using erythrocytes hemolysis inhibition assay. Maximum protection was observed at ELEGS concentration 1 mg/ml compared to the control (Table 3).

DISCUSSION

Bronchial constriction in asthma is primarily mediated by

Table 3. Effect of ethanol leaf extract of *Guiera senegalensis* on erythrocytes membranes stability.

ELEGS (mg/ml)	Absorbance	% Inhibition
Control	0.3640 ± 0.032	-
0.1	0.3131 ± 0.013	13
0.2	0.2630 ± 0.022*	28
1.0	0.2247 ± 0.019**	38
2.0	0.3030 ± 0.014	16

Values are expressed as mean ± Standard Error of Mean (n=3). Data analysis was performed by ANOVA followed by Dunnetts post-hock test to determine the level of significance $(p<0.05)$*, $(p<0.01)$** existing between each value compared to the control. Absorbance was read at 540 nm. ELEGS: Ethanol leaf extract of *Guiera senegalensis*

histamine released from degranulated mast cells and by acetylcholine which cause contractions of tracheal smooth muscles and increased mucus secretion. Inflammation of the bronchi also sets due to lipid mediators from arachidonic acid pathway generated from membrane phospholipids (Gosh, 1984). Clinical interventions so far aim at maintaining normal airway muscles tone by the use of bronchodilators and anti-inflammatory agents to reverse bronchoconstriction. Therefore, substance that can reverse bronchoconstrictions will possibly relieve symptoms of asthma. For this, the antihistaminic as well as the antimuscarinic activities of the plant extract were investigated using animals' isolated tissues. Smooth muscles are rich in many kinds of receptors with different localization specifications. Airway smooth muscles are rich in histamine, acetylcholine, and β_2 adrenergic receptors. Distribution of these receptors and their responses to agonists and antagonists is similar in both humans and guinea pigs. Therefore, guinea pigs are used in contrast to rats and mice.

In the present study, *in vitro* anti-asthmatic effect of the ethanol leaf extract of *Guiera senegalensis* (ELEGS) was evaluated. In isolated trachea guinea pig tracheal chain preparations, ELEGS was also found to possess antihistaminic and antimuscarinic effects. Although it did not exhibit any effect when used alone, the extract at concentrations of 8 mg/ml was able to significantly $(p<0.01)$ inhibit contractions induced by both spasmogens (histamine and acetylcholine) on the tracheal chains. The action of the extract was found to be similar to that of the standard drug (isoprenaline).

Histamine and muscarinic receptors are the dominant receptors in guinea pig ileum and this makes it a good tissue for studies involving these spasmogens. ELEGS exhibited smooth muscle relaxing effect on guinea pigs ileal strips and was able to significantly inhibit contractions induced by histamine $(p<0.01$ to $0.001)$ and acetylcholine $(p<0.05$ to $0.01)$ in a dose-dependent manner. Atropine (antimuscarinic) was used against acetylcholine while mepyramine (antihistamine) was used against histamine for comparison with the extract. These pharmacological antagonists significantly $(p<0.01)$ inhibited the ileal induced contractions but to a lesser effect than 3.2 mg/ml of the extract. At the 3.2 mg/ml, ELEGS completely abolished the contractions-induced by both histamine and acetylcholine and resulted to relaxation of the tissues.

On isolated rabbit jejunum, the extract when given alone was able to significantly $(p<0.05$ to $0.01)$ reduced the basal spontaneous contractions as well as contractions induced by acetylcholine $(p<0.01$ to $0.001)$ in dose-dependent pattern. Similar result was observed with atropine. These indicate that ELEGS has a nonspecific spasmolytic activity on smooth muscles that may probably be due to β-adrenergic stimulatory (Martin et al., 1994), histamine H1 (Popa et al., 1984) and/or muscarinic inhibition (Linden et al., 1993). However, result obtained from rabbit jejunum after addition of ELEGS up to 6.4 mg/ml to baths pretreated with propranolol was not significantly different from the control. Similar effect was observed with 0.16 µg isoprenaline. Propranolol is a nonselective β adrenergic receptor blocker that binds to all β receptors and inhibits their activity. This suggests that, ELEGS might be acting on the same receptors resulting in smooth muscle relaxation by activation of β_2 adrenoceptors.

Allergic asthma is a chronic inflammatory process occurring due to exposure to allergen resulting in the activation of T-lymphocyte with subsequent release of inflammatory mediators. Immuno-modulating agents are useful in the treatment of asthma by inhibiting the antigen-antibody (AG-AB) reaction and thereby inhibiting the release of inflammatory mediators. Membrane stabilization assay was used to study the possible inhibition of lipid mediators of inflammation in asthma. Percent inhibition of erythrocytes hemolysis was calculated and the ELEGS at all concentrations used was able to protect the membranes of erythrocytes from possible damage. Maximum effective concentration was

observed at 1 mg/ml.

Drugs effective in asthma are mostly steroidal in nature. ELEGS contained steroid, alkaloids, flavonoids, saponins, anthraquinones and tannins. These secondary metabolites have been known to possess various biological activities including antibacterial, antioxidant, anticancer, spasmolytic and anti-inflammatory effects (Parmar et al., 1999). Flavonoids have been known to inhibit the release of several mediators of inflammations such as prostaglandins, histamine, serotonin and bradykinins by inhibiting the biosynthetic pathways of inflammatory mediators (Macauder, 1986). Steroids are known for their maintenance of membrane integrity by inhibiting lipoxygenase and cyclo-oxygenase pathways and are used for their anti-inflammatory properties (Speroni et al., 2005). So antiasthmatic activity showed by *G. senegalensis* might be because of these chemical moieties.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interest.

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