

Full Length Research Paper

# The beneficial effects of extracts of *Hibiscus sabdariffa* calyces in alloxan-diabetic rats: Reduction of free-radical load and enhancement of antioxidant status

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This study evaluates the effects of aqueous and ethanolic extracts of *Hibiscus sabdariffa* calyces on the *in vitro* and *in vivo* antioxidant activities in alloxan induced diabetic rats. Both the aqueous and ethanolic extracts showed marked antioxidant activity in oleic acid systems of ferric thiocyanate and thiobarbituric acid (TBA). They also exhibited 1, 1-diphenyl-1-picrylhydrazine (DPPH) scavenging activity and reducing power in a concentration dependent manner. The *in vivo* study of the plant on the liver and kidney level of thiobarbituric acid reactive substances (TBARS), catalase, vitamin C and packed cell volume (PCV) showed that the extract exhibited significant reduction in the level of thiobarbituric acid reactive substance (TBARS) and increased the activity of catalase and concentration of vitamins C. The extract also exhibited a considerable increase in the level of PCV when compared with diabetic control group. Thus our study shows that *Hibiscus sabdariffa* exhibits *in vitro* and *in vivo* antioxidant action in alloxan induced diabetic rats.

**Key words:** *Hibiscus sabdariffa*, antioxidant, thiobarbituric acid (TBARS), catalase.

## INTRODUCTION

*Hibiscus sabdariffa* (Linn) (family Malvaceae) is an annual dicotyledonous herbaceous shrub popularly known as 'Gongora' in Hindi or 'Pulich Keera' in Tamil. This plant is well known in Asia and African country, in Nigeria is called 'Yakuwa' in Hausa, 'Ishapa' in Yoruba and 'Shah' in Higgi. In Gambia it is known as "Wanjo". The beverage made from the plant is called 'Zobo' in Nigeria and 'Karkade' in Egypt (Pin-Der et al., 1997). In the Ayurvedic literature in India, different parts of this plant have been recommended as a remedy for various ailments such as hypertension, pyrexia, liver disorders and as an antidote to poisoning chemicals (Andreas et al., 2000). Anthocyanins, flavonols and protocatechoic acid (PCA) along with other, phytochemicals have been identified as contributors to the observed medicinal effect of *Hibiscus sabdariffa* (Seca et al., 2001). The excess generation of reactive oxygen species (ROS) during various pathophysiological states can lead to alterations of the

cellular constituents resulting in diseased conditions (Sies, 1991). Much attention has been focused on the mechanism of oxidative damage in various diseased states including cardiac dysfunction, ischemia, aging, diabetes and atherosclerosis (Hansen, 1995; Berliner and Hienecke, 1996). Oxidative injury is considered as a major cause of myocardial injury during infarction, hypothermic preservation of donor heart, and during cardioplegia involving heart surgery (Keith, 1993; Southward and Belzer, 1995).

In recent years, there has been considerable interest in natural products with antioxidant property in human diet. One of the areas which attracted a great deal of attention is the possible use of antioxidant supplements in the prevention of disease caused by oxidative damage (Ong and Packer, 1992; Odeh and Cornish, 1995). Diabetic Mellitus has been known to be associated with complications of kidney, liver, neurons and blood vessels as a result of oxidative stress from increased free radical generation (Sanders et al., 2001). Compared to common antioxidant such as ascorbate, anthocyanins were found to be much more potent antioxidant (Wang et al., 1997). It is well documented that most medicinal plants are

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enriched with phenolic compounds and bioflavonoids that represent potent antioxidant (Shirwaikar et al., 2003). The hypoglycaemic and hypolipidaemic activities of *Hibiscus sabdariffa* has been reported (Sini et al., 2011). There are no available reports on the antioxidant action of *Hibiscus sabdariffa* calyces in diabetics. Therefore we undertook the present study to determine the effect of administration of extracts of *Hibiscus sabdariffa* on oxidative species and antioxidant reserves of diabetic rats.

## MATERIALS AND METHODS

### Plant material

*Hibiscus sabdariffa*: Fresh mature calyces were collected from Samaru district of Kaduna in Nigeria in June, 2007. The plant was identified at the Herbarium of the Department of Biological Sciences, Ahmadu, Bello University Zaria. A specimen voucher (No 1056) was deposited in the Department.

### Preparation of extract

The fresh calyces of *Hibiscus sabdariffa* were collected and sorted to remove the dead ones, wash without squeezing to remove debris and dust particles. Large quantities of the calyces were collected and shade dried for seven days. The dried calyces were milled using pestle and mortar to get a coarse powder used for the extraction.

### Animals

Thirty male Wister strain Albino rats (body weight 150 to 200 g) were purchased from the department of pharmacology Ahmadu Bello University Zaria for the studies. The animals were fed on a pellet diet (product of Pfizer Nigeria Ltd). The food was withheld 12 h before the experiment, but there was free access to water.

### Extraction of aqueous *Hibiscus sabdariffa*

A suspension of 300 g of the powdered calyx in 2.5L distilled water at 70°C was stirred mechanically for (3 h), after which it was filtered using filter paper and the filtrate concentrated at 40°C on water bath. The percentage yield was calculated.

### Extraction of the ethanolic *Hibiscus sabdariffa*

A suspension of 80 g of the powdered calyces in 500 ml ethanol was stirred mechanically overnight (12 h) at room temperature. It was then filtered using filter paper and the filtrate concentrated at 40°C on water bath. The percentage yield was determined. The crude extracts obtained were used for the assessment of the *in vitro* antioxidant activity of *Hibiscus sabdariffa*.

### *In vitro* antioxidant activity

#### Ferric thiocyanate (FTC) method

The ferric thiocyanate method of Mitusda et al. (1967) which was slightly modified by Kikuzaki and Nakatani (1993) was used. Ferric

thiocyanide method was used to determine the antioxidant activity of *Hibiscus sabdariffa* following decrease in the absorbance level as a mark of antioxidant activity of the extract and the standard drugs used.

#### Thiobarbituric acid (TBA) method

The method of Ottolenghi (1959) was used to determine the anti-oxidative values of the samples. The formation of malonaldehyde is the basis for the well-known TBA method used for evaluating the extent of lipid peroxidation. At low pH and high temperature (100 °C), malonaldehyde binds TBA to form a red complex that can be measured at 532 nm.

#### Reducing power

The reducing power of the ethanolic and aqueous extracts was measured according to the method of Oyaizu (1986). Various concentrations (0.10, 0.25, 0.50, 1.0 mg/ml) of the ethanolic and aqueous extracts (0.5 ml) were mixed with 2.5 ml of phosphate buffer (0.2 M, pH 6.6) and 2.5 ml of potassium ferricyanide (1%). The mixture was incubated at 50°C for 20 min trichloroacetic acid (10%, 2.5 ml) was added. The mixture was centrifuged at 650 g for 10 min the supernatant (2.5 ml) was mixed with 2.5 ml of distilled water and 0.5 ml of ferric chloride and the absorbance was measured at 700 nm. Higher absorbance of the reaction mixture indicated greater reducing power. The reducing power of the ethanolic and aqueous extracts was compared with that of ascorbic acid (0.2 mg/ml).

#### Scavenging effect on DPPH radical

The effect of ethanolic and aqueous extracts on diphenylpicrylhydrazyl (DPPH) radical was estimated according to the method of Hatano et al. (1988). The extracts 30µl of (0.1, 0.25, 0.5, and 1.0 mg/ml) concentrations were added to a methanolic solution (1 ml) of DPPH radical (final concentration of DPPH was 0.2 mM). The mixture was shaken vigorously and left to stand at room temperature for 30 min the absorbance of the resulting solution was measured spectrophotometrically at 517 nm.

#### Diabetic induction

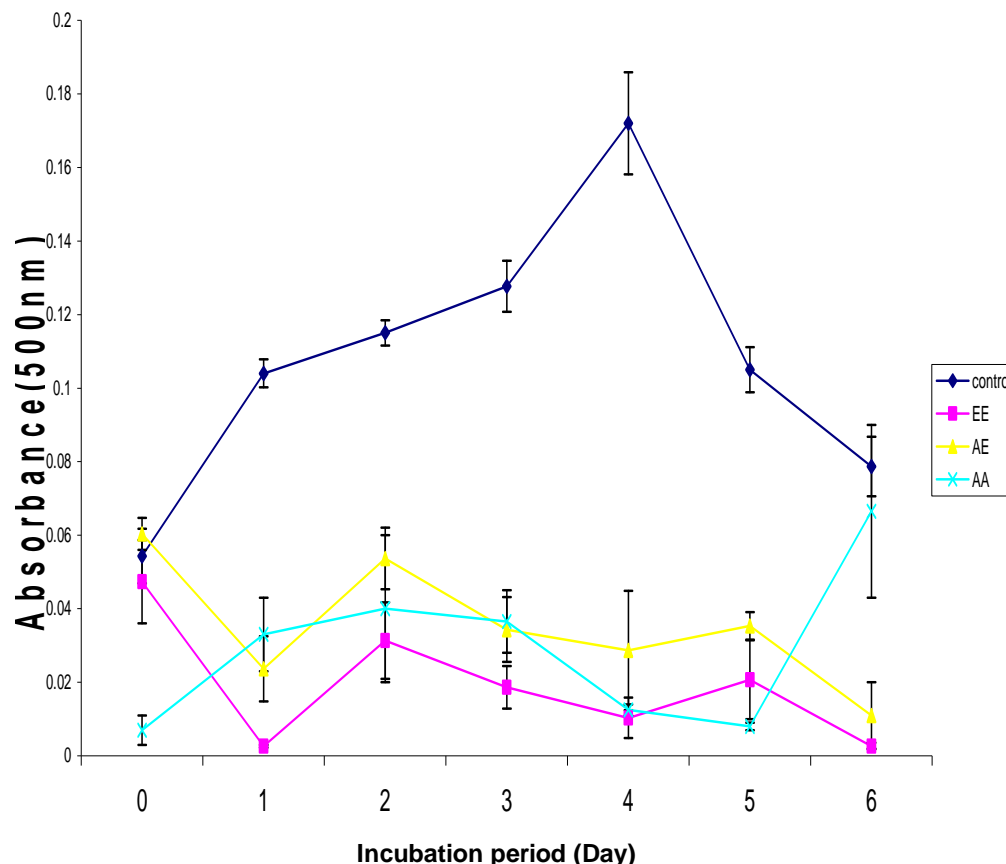
Diabetes was induced by subjecting the animals to 12 h fasting overnight, followed by a single injection of 150 mg/kg alloxan dissolved in normal saline (Damasceno, 2002) at the base of the tail. Fasting blood glucose concentration was determined after 72 h. Weekly blood glucose concentration was determined using a glucometer (ACCU CHECK Active, Roche, USA). Rats with fasting blood glucose of 140 mg/dl and above were used for the study (Sini et al., 2011).

#### Experimental design

Thirty male rats included for this study were divided into six groups each consist of five animal each, out of the six groups, four groups were made diabetic as describe above. Diabetic was confirmed by the determination of fasting blood glucose level as described above. Rats were divided into the following groups.

Group 1 – Normal control, rats given only distilled water as their drinking water labeled NC.

Group 2 – Normal control rats given 0.5 mg/ml of the aqueous



**Figure 1.** Profile of the changes in absorbance of sample at 0.02% concentration using FTC method. EE-ethanolic extract, AE-aqueous extract AA-ascorbic acid.

extract solution as their drinking water labeled: EC.

Group 3 – Diabetic control rats given only distilled water as their drinking water labeled: DC.

Group 4 – Diabetic rats given distilled water as their drinking water and administered daily with standard drug glibeclamide 0.08mg/kg labeled: DG

Group 5 – Diabetic rats given ascorbic acid 0.2mg/ml as their drinking water labeled: DA

Group 6 – Diabetic rats given 0.5 mg/ml of the aqueous extract as their drinking water labeled: DE.

Treatment was by oral administration. The weekly fasting blood glucose was determined as described above and PCV was determined using heparinized capillary tubes throughout the period of the experiment. After 5 weeks of the treatment, the animals were anaesthetized using chloroform and sacrificed, blood was collected in heparinised tubes and liver and kidney tissue in ice-cold container and used for various biochemical estimations.

#### Determination of tissue thiobarbituric acid reactive substance (TBARS), catalase and Vitamin C

Thiobarbituric acid reactive substance TBARS in tissues were estimated by the method of Fraga et al. (1988). The pink coloured chromogen formed by the reaction of 2-thiobarbituric acid with breakdown products of lipid peroxidation was measured at 532 nm absorbance. Catalase activity was assayed by the Method of Machley and Chance (1954) by quantifying the hydrogen peroxide after reacting with dichromate in acetic acid. The initial and final

absorbance was measured at 620 nm. Vitamin C was determined by the method of Roe et al. (1973).

#### Statistical analysis

The results obtained were statistically analyzed by analysis of variance ANOVA and students t test. All the results were expressed as mean±SEM from 5 rats in each group.

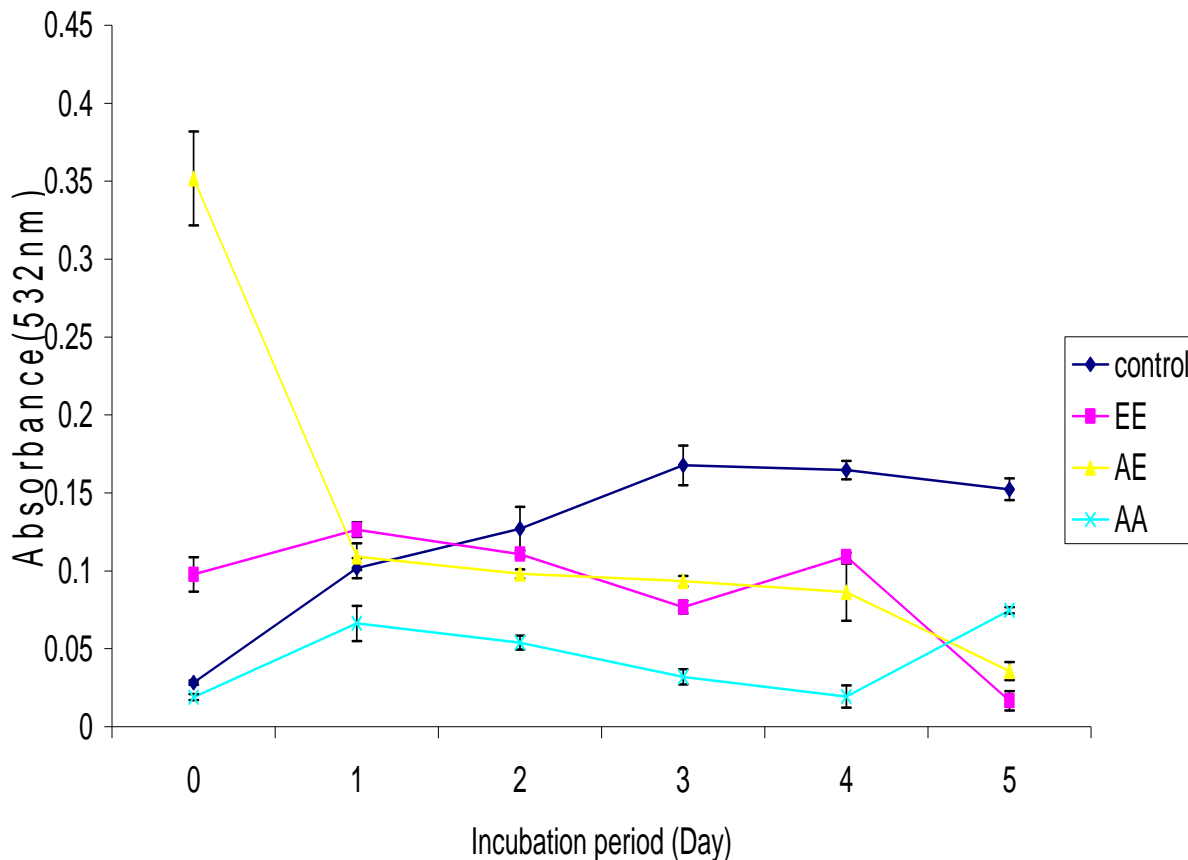
## RESULTS

### Percentage yield

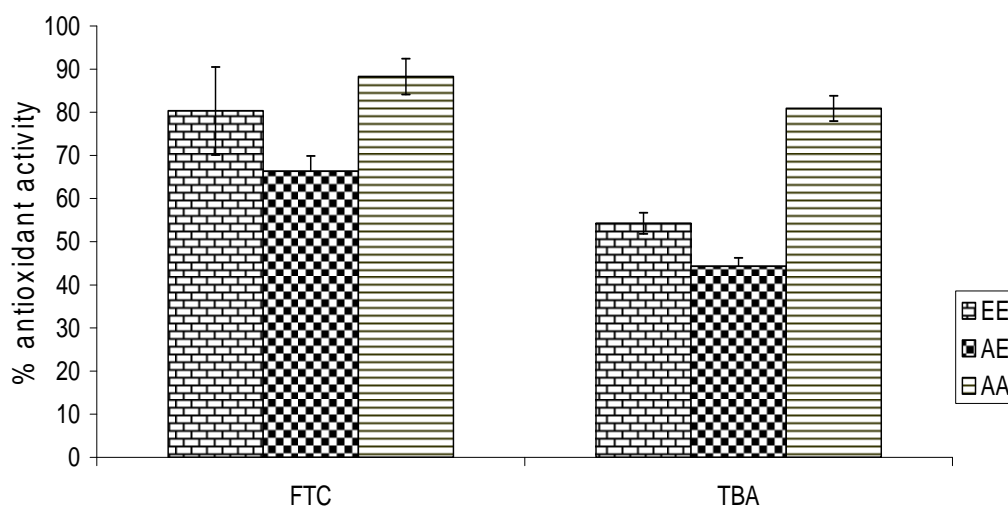
The percentage yield for the ethanolic and aqueous extract were 8.35 and 10.93%, respectively.

### *In vitro* antioxidant activities of the ethanolic and aqueous extracts of *Hibiscus sabdariffa*

The individual activities of samples by the FTC and TBA methods showed that ascorbic acid showed the least increase in absorbance value from day 1 – 4 using FTC method Figure 1 and day 1 to 3 using TBA method.



**Figure 2.** Profile of changes in absorbance values of samples at 0.02% concentration using TBA method. EE-ethanolic extract, AE-aqueous extract, AA-ascorbic acid.

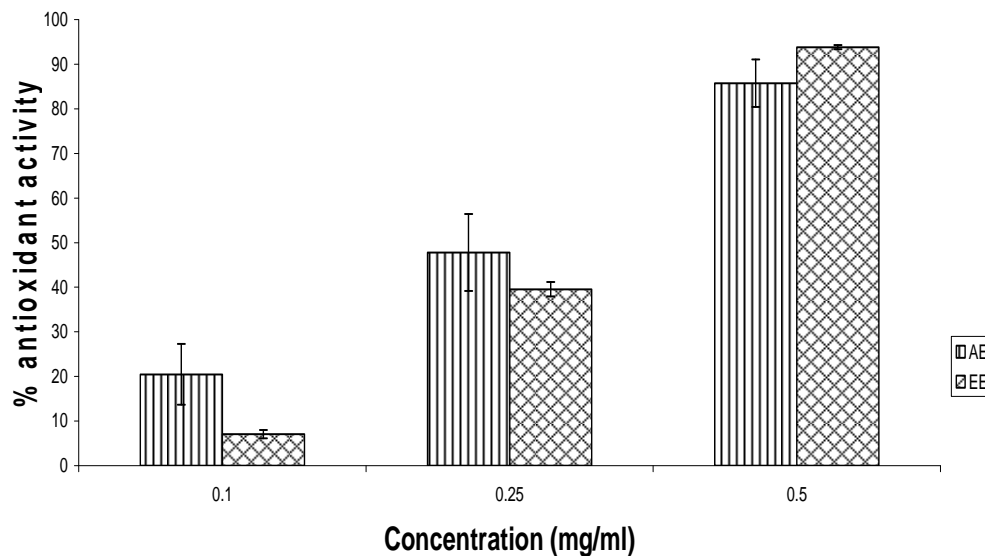


**Figure 3.** The total antioxidant activity of ethanolic extract and aqueous extracts of HS using FTC and TBA methods. FTC-ferric thiocyanite, TBA-thiobarbituric acid, EE-ethanolic extract, AE-aqueous extract, AA-ascorbic acid.

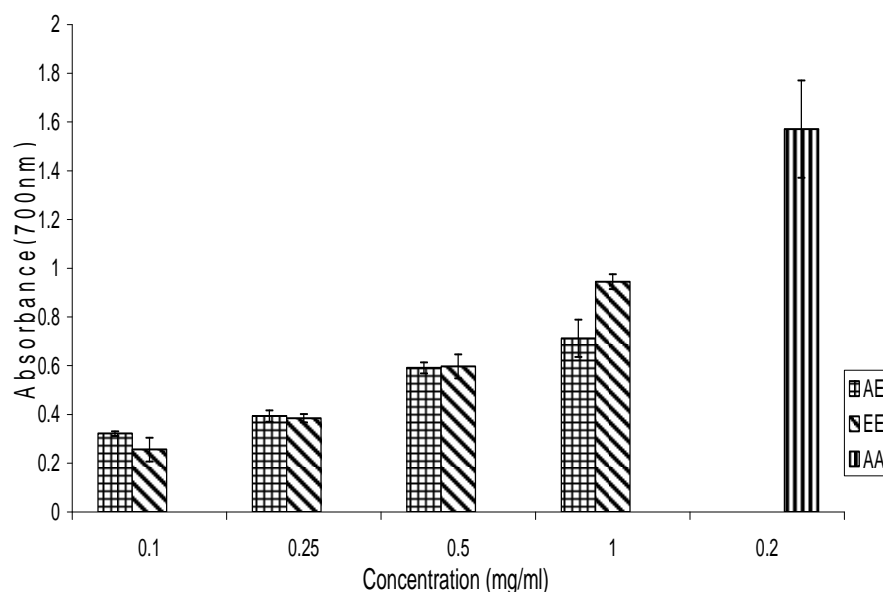
Figure 2, followed by the ethanolic extract and aqueous extract although no significant difference was observed between the two extracts. Following the FTC method, the

maximum absorbance was recorded at day 4 using FTC method and day 3 using TBA method.

Figure 3, showed the total antioxidant activity of the



**Figure 4.** Scavenging effect of aqueous and ethanolic extracts of HS on 1,1 diphenyl-2-picrylhydrazyl (DPPH) radical AE-aqueous extract, EE-ethanolic extract.



**Figure 5.** Reducing power (as absorbance at 700 nm) of aqueous and ethanolic extract of HS and ascorbic acid. AE-aqueous extract, EE- ethanolic extract, AA-ascorbic acid.

ethanolic and aqueous extracts of *Hibiscus sabdariffa* using FTC and TBA methods. A significant ( $P < 0.05$ ) differences between the total antioxidant activity of *Hibiscus sabdariffa* was observed when compared with ascorbic acid using TBA method with ascorbic acid exhibiting the highest antioxidant activity of 90%. There is no significant difference between the aqueous and ethanolic extract. Using the FTC method, there was no significant difference in the total antioxidant activity of ascorbic acid and the aqueous extract, also no significant difference was observed between the aqueous and

ethanolic extracts.

The DPPH radical scavenging activity of *Hibiscus sabdariffa* extract was shown in Figure 4. Both the aqueous and ethanolic extracts exhibited concentration dependent increase in antioxidant activity. There was no significant difference between the two extracts. Ascorbic acid exhibited 100% antioxidant activity at 0.2 mg/ml concentration.

The reducing power was determined following increase in absorbance Figure 5. The absorbance at 700 nm is directly proportional to the reducing power of the

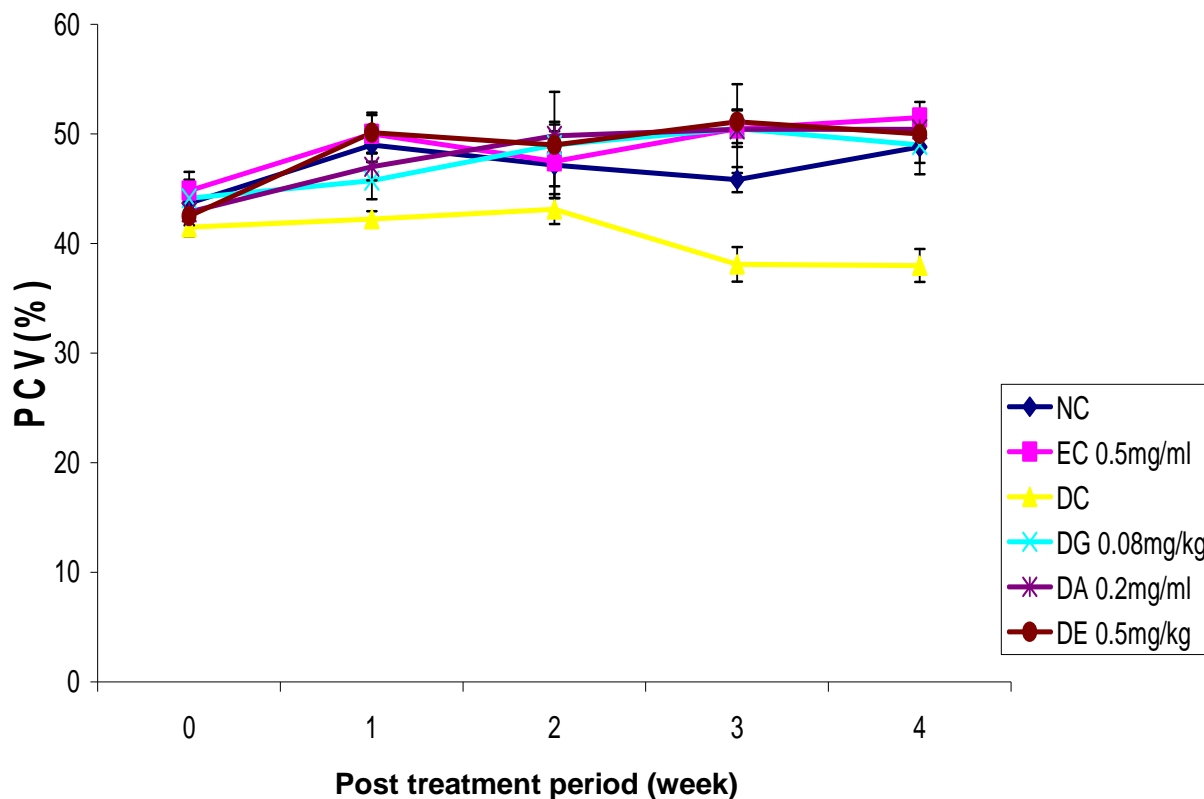


Figure 6. Profile of weekly PCV in all groups of rats.

Table 1. Mean  $\pm$  SEM of Percentage change in PCV.

Group n = 5	NC	EC 0.5 mg/ml	D C	DG 0.08 mg/kg	DA 0.2 mg/ml	DE 0.5 mg/ml
Change in PCV(%)	4.09 $\pm$ 1.43 <sup>a</sup>	2.07 $\pm$ 0.43 <sup>b</sup>	-4.95 $\pm$ 3.28 <sup>c</sup>	3.57 $\pm$ 2.24 <sup>ad</sup>	2.59 $\pm$ 0.70 <sup>d</sup>	2.17 $\pm$ 0.09 <sup>eb</sup>

constituents of the reaction mixture. The extracts exhibited concentration dependent increase in reducing power activity; ascorbic acid demonstrated the highest increased in reducing power. There was no significant difference in reducing power between the aqueous and ethanolic extract.

#### Hypoglycaemic and hypolipidaemic activities

The result of the hypoglycaemic and hypolipidaemic activities was reported in our previous findings (Sini et al., 2011), significant reduction ( $P < 0.05$ ) in hyperglycaemic and hyperlipidaemic activities in alloxan induced diabetic rats were recorded.

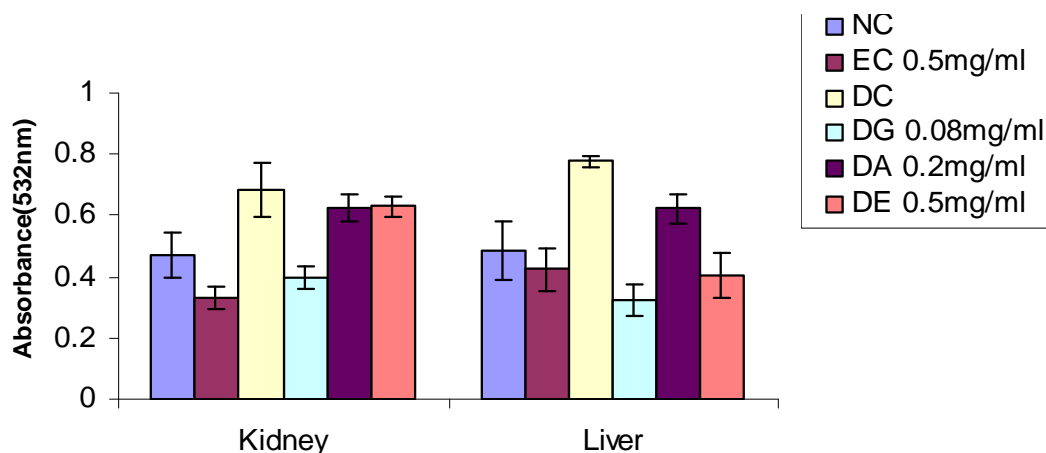
#### The change in PCV

The profile of weekly PCV is shown in Figure 6. At week 0, PCV level in all the animals were almost at the same

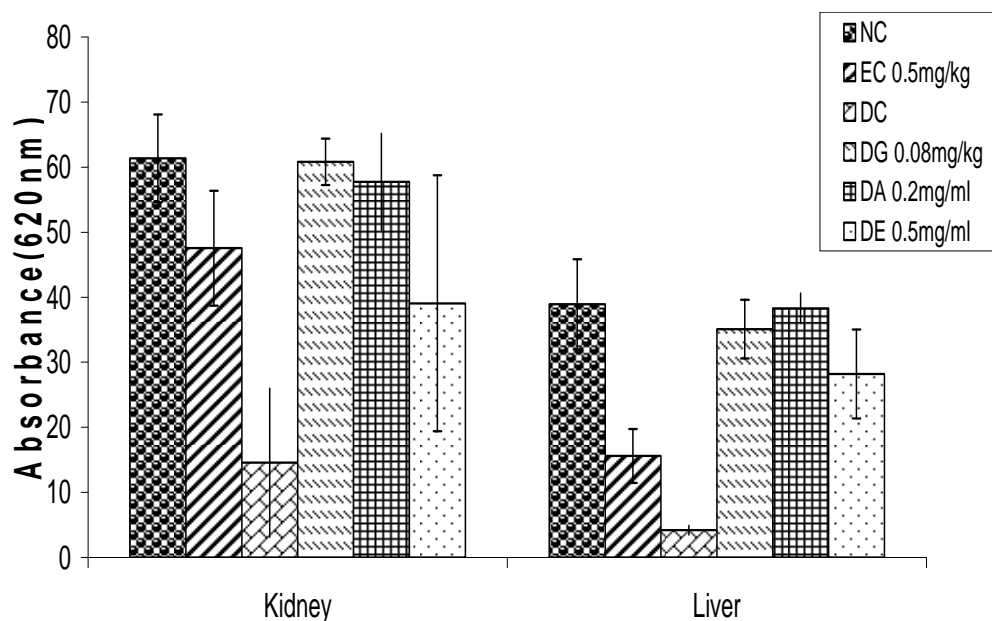
level. Consistent increase in PCV was recorded from the two control groups; normal control and the extract control group from week 0 to week 4. Decreased in PCV was recorded in all the diabetic group from week 0 to 2 with significant increased in the PCV level of extract, vitamin C and glibenclamide treated group from week 2 to 4 respectively. A consistent decreased in PCV level was recorded by diabetic control group from week 0 to 4. Table 1 show a significant decreased in the level of PCV by diabetic control group ( $P < 0.05$ ) when compared with normal control and diabetic treated with extract, standard drug glibenclamide and vitamin C.

#### Organ TBARS concentrations

The level of TBARS in the liver and kidney of the rats is shown in Figure 7. There was significant increase ( $P < 0.05$ ) in the level of TBARS in the kidney and liver of diabetic control rats. Treatment with *Hibiscus sabdariffa* Vitamin C and standard drug glibenclamide tended to



**Figure 7.** Mean level of TBARS (express as absorbance at 532 nm) in liver and kidney of all groups of rats.



**Figure 8.** Mean catalase activity ( $\mu\text{mole}/\text{min}$ ) in liver and kidney of all groups of rats.

decrease the TBARS level in liver and kidney of diabetic rats. Glibenclamide confer the greatest protection in rats kidney TBARS when compared with the extract and ascorbic acid while in the liver both the glibenclamide and extract had considerable protection in TBARS than ascorbic acid.

#### Organ catalase activity

The activity of catalase in liver and kidney of the rats is shown in Figure 8. There was significant reduction in the activity of catalase in the liver and kidney of the diabetic control group. Treatment of diabetic rat with *Hibiscus*

*sabdariffa*, glibenclamide and Vitamin C tended to increase the activity of catalase in the rats liver and kidney. In both kidney and liver, glibenclamide and ascorbic acid exhibited the highest increased in catalase activity compared with the extract.

#### Effect of *Hibiscus sabdariffa* on rats liver and kidney level of vitamin C

The levels of vitamin C in liver and kidney of normal and diabetic rats are presented in Table 2. The level of Vitamin C was decreased in diabetic control group. Treatment with *Hibiscus sabdariffa*, glibenclamide and

**Table 2.** Mean  $\pm$ SEM level of Vitamin C (mg/100g of wet tissue) in Liver and Kidney of all the groups of rats.

Group (n=5)	NC	EC 0.5 mg/ml	D C	DG 0.08 mg/ml	DA 0.2 mg/ml	DE 0.5 mg/ml
Liver	5.48 $\pm$ 0.48 <sup>a</sup>	6.94 $\pm$ 0.32 <sup>b</sup>	1.65 $\pm$ 0.23 <sup>c</sup>	4.80 $\pm$ 0.55 <sup>ab</sup>	6.33 $\pm$ 0.49 <sup>d</sup>	5.75 $\pm$ 0.51 <sup>ad</sup>
Kidney	0.46 $\pm$ 0.12 <sup>a</sup>	0.43 $\pm$ 0.06 <sup>a</sup>	0.14 $\pm$ 0.04 <sup>b</sup>	0.71 $\pm$ 0.06 <sup>cd</sup>	0.78 $\pm$ 0.10 <sup>d</sup>	0.41 $\pm$ 0.18 <sup>a</sup>

Values with different superscript along a row are significantly different (P<0.05)

Vitamin C showed a significant improvement in the level of Vitamin C (P<0.05). In both liver and kidney, ascorbic acid confers the highest increase in vitamin C when compared to the glibenclamide and extract treated groups.

## DISCUSSION

The result of this study shows that *Hibiscus sabdariffa* extract demonstrated a significant *in vitro* antioxidant and *in vivo* antioxidant activities in alloxan induced diabetic rats. Antioxidant are known to alleviate oxidative stress which is generally perceived as one of the major causes for the accumulation of mutations in the genome Susi et al. (2002). Antioxidants are believed to provide protection against cancer (Ames, 1983). Peroxide is gradually decomposed to lower molecular compounds during the oxidation process and these compounds are measured using FTC and TBA methods. Antiradical activity of antioxidant is determined using DPPH radical scavenging activity (Gulgun, 2004). The redox active antioxidants like ascorbate, glutathione, urate, flavonoids, tocopherols, carotenoids and hydroxycinnamic acid present in plants (Eastwood, 1999) may be the contributing factor for scavenging effect on DPPH. Yen and Duh (1993) reported that the reducing power of methanolic extract of peanut hulls containing high level of polyphenols was concomitant with the antioxidant activity. It appears that the reducing power activity observed in *Hibiscus sabdariffa* could be due to the increased polyphenol content (Andreas et al., 2000).

There was decreased in PCV in diabetic control group when compared to normal and diabetic treated group Figure 6. This may be as a result of deleterious effect of free radicals on haematocrit cell membrane phospholipids. Abstraction of electron from the phospholipids will render the cell leaking and thereby leads to cellular death. RBC membrane isolated from type 1 and 2 diabetic mellitus patients was much more susceptible to oxidation than the normal subject (Bowie et al., 1993).

The lipid peroxidation process has been implicated in a variety of disease conditions (Jain et al., 1994). The role of free radicals in etiopathogenesis of diabetic's mellitus is well known (Rauscher, et al., 2001). Sato et al. (1979) were the first to report the increased level of lipid peroxidation in the plasma of diabetic patients. An

increase the level of lipid peroxides in plasma is generally thought to be the consequences of increased production of and liberation into the circulation of tissue lipid peroxides due to pathological changes (Selvam and Anuradha, 1990). Previous study has revealed an increase lipid peroxidation in the plasma of diabetic rats (Prince et al., 1998). This study shows that, *Hibiscus sabdariffa* Ascorbic acid and glibenclamide lowered significantly the level of tissue TBARS when compared to diabetic control.

Decreased in the level of tissue catalase was observed in diabetic control groups (Figure 8). Catalase is an enzymic antioxidant widely distributed in all animal tissues and the highest activity is found in the red blood cells and liver. Catalase decomposes hydrogen peroxide and protects the tissue from highly reactive hydroxyl radicals (Chance et al., 1952). Therefore, a reduction in the activity of the enzyme may result in a number of deleterious effects due to the accumulation of hydrogen peroxide. Treatment with extract, standard drug glibenclamide and ascorbic acid significantly maintained the catalase activity close to normal.

The tissue level of vitamin C is shown on Table 2. A significant decrease in the level of Vitamin C was observed in diabetic control group. Vitamin C is an excellent hydrophilic antioxidant in plasma because it disappears faster than other antioxidant when plasma is exposed to reactive oxygen species (Frie et al., 1986). Oral administration of *Hibiscus sabdariffa* extract and standard drugs glibenclamide and ascorbic acid increased the level of vitamin C close to normal.

## Conclusion

Thus, the results of our study shows that *Hibiscus sabdariffa* possesses an *in vitro* and *in vivo* antioxidant activity which could exert beneficial action against pathological alteration caused by the presence of free radicals in alloxan diabetic rats.

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