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Effects of aqueous extract of *Hibiscus sabdariffa* L. calyces on liver marker enzymes of phenobarbitone-induced adult wistar albino rats

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ABSTRACT

Hibiscus sabdariffa is an annual dicotyledonous herbaceous shrub plant popularly known as "zobo" in Nigeria which is an indigenous edible medicinal plant used in Ayurvedic medicine in India, China and Thailand. The purpose of this studies is to investigate the effect of Hibiscus sabdariffa leaf extract on liver marker enzymes such as AST (aspartate aminotransferase), ALT (alanine aminotransferase) and ALP (alkaline phosphatase), for its hepatoprotective effect in phenobarbitone-induced rats. Phenobarbitone treated rats showed a significant increase ($P<0.05$) in the levels of circulatory AST, ALT and ALP. These changes were significantly decreased ($P<0.05$) in rats treated with HSEt and phenobarbitone. These results indicate that HSEt offers hepatoprotection by influencing the levels of liver markers in phenobarbitone-induced rats and this could be due to its free radical scavenging property and the presence of natural antioxidants.

Keywords: Haematology; Phenobarbitone; *Hibiscus sabdariffa*.

INTRODUCTION

Phenobarbitone is a commonly prescribed sedative and hypnotic. It is mainly used for its anti-epileptic properties. Its common side effects are ataxia, polyurea, polydipsia, polyphagia, and it may depress both the respiratory drive and the mechanism responsible for the rhythmic character of respiration (Branum *et al*., 1998). In addition to these side effects, Phenobarbitone stimulates the division of liver cells (Albert *et al*., 1998). And causes increases in serum alkaline phosphatase (ALP), aspartate aminotransferase (AST) and alanine aminotransferase (ALT) (Chauvet *et al*., 1995). Phenobarbital (PB) is a major drug in the treatment of canine, feline and

human epilepsy and can significantly reduce the severity of seizures. PB raises the threshold for seizure discharge and inhibits the initiation, diffusion, and spread of discharge from the neural focus. Drug is rapidly and completely absorbed after oral doses and clinically effective 12 to 24 h after oral administration. PB is metabolized by liver and extracted by the kidney (Barragry, 1994; Foster *et al*., 2000; Gieger *et al*., 2000).

Common side effects of PB are ataxia, sedation, polyuria, polydipsia, polyphagia, and it may depress both the respiratory drive and the mechanisms responsible for the rhythmic character of respiration (Hobbs, 1996; Booth, 1998; Branum *et al*., 1998). In addition to these side effects, PB stimulates the division of liver cells (Alberts *et al*., 1998) and causes increases in serum alkaline phosphatase (ALP), aspartate aminotransferase (AST) and gamma glutamyl transferase (GGT) activities (Aiges *et al*., 1980; Luoma *et al*., 1980; Verma and Haidukewych, 1994; Chauvet *et al*., 1995). ALP, AST and GGT enzymes are accepted as indicators of hepatotoxicity (Boyd, 1982; Isogai *et al*., 1994; Rosenthal, 1997). In addition, AST activity alterations were mainly due to PB (Verma and Haidukewych, 1994). Many studies reported that PB caused increases in serum AST (Fortman and Witte, 1985; Haidukewych and John, 1986; Kitchin and Brown, 1987) and ALP activities (Chauvet *et al*., 1995; Filardi *et al*., 2000; Foster *et al*., 2000).

There is a need for the search for appropriate protective agents against Phenobarbitone induction. This can be focused on plants used in traditional medicine because of their/herbal/medicinal potencies which are provided by natural products that may offer better treatment than currently used drugs.

Hibiscus sabdariffa L. (roselle); a member of family Malvaceae; is cultivated in Egypt for multipurpose uses. It is well known in Egypt with the name of “Karkadeh”, its purplish sepals (calyx and epicalyx) are the most important economic parts of the plant which is used in food (Jam and Jelly) and cosmetic industries as a source of natural colouring agent (El-Meleigy, 1989). Kalt *et al*. (1992) reported that the pigments (anthocyanin) which are responsible primarily for red colour were delphinidin 3 - glucoside and cyanidin- 3 glucoside.

Also, it has a favorable effect on the functions of stomach. It possesses a high intestinal antiseptic action and can be used to resist various infections of intestinal diseases (Owolabi *et al*., 1995). Obiefuna *et al*. (1994) added that *Hibiscus sabdariffa* flowers can be used to relax the pain muscles of the uterus and intestine. The extract also proved experimentally to have highly antibacterial properties. Tanaka *et al*. (1993) also stated that protocatechuic acid (a simple phenolic compound) detected in *Hibiscus sabdariffa* could be used to fight pyrexia and liver disorders. Also, it has been demonstrated that this compound is an effective agent in reducing the carcinogenic action of diethylnitrosamine in the liver (Raifa *et al*., 2005).

In traditional medicine, this plant has good features useful in several applications, such as antidotes to poisonous chemicals (acids, alkali, pesticides) and venomous mushrooms (Chifundera *et al*., 1994). Previous phytochemical investigations of this plant show the presence of phenolic compounds, anthocyanins, flavonols, protocatechuic acid (PCA), etc. (Seca *et al*., 2000, 2001). It is a well-documented fact that most medicinal plants are enriched with phenolic compounds and bioflavonoids that have excellent antioxidant properties (Shirwaikar *et al*., 2003).

In folk medicine, the calyx extracts are used for the treatment of several complaints, including high BP, liver diseases and fever. In view of its reported nutritional and pharmacological properties and relative safety, *H. sabdariffa* and compounds isolated from it could be a source of therapeutically useful products (Ali *et al*., 2005; Hirunpanich *et al*., 2005). Chen *et al*. (2003) showed that ST extracts reduce triglyceride, cholesterol, low-density lipoprotein cholesterol (LDLc) and LDLc/HDLc in hyperlipidaemic rats.

Anthocyanins were also found to have many times more activity than common antioxidants such as ascorbate (Wang *et al*., 1997). Previous reports show that alcoholic extract of HS flowers and calyses revealed marked nitric oxide scavenging activity (Obiefuna *et al*., 1993; Adegunloye *et al*., 1996; Wang *et al*., 2000; Odigie *et al*., 2003; Amin and Hamza, 2005).

Hibiscus sabdariffa is well known in Nigeria and is commonly used to make jellies, jams and beverages. In the Ayurvedic literature of India, different parts of this plant have been recommended as a remedy for various ailments like hypertension, pyrexia and liver disorders (Ali *et al*., 2005). Phytochemical analysis of this plant shows phenolic compounds, anthocyanins, flavinols and protocatechuic acid (Ali *et al*., 2005), which have excellent antioxidant properties (Shirwaiker *et al*., 2003). However, there are various phytochemical constituent and diverse medicinal activities attributed to this plant and no biochemical studies have been carried out to shed light on the role of *Hibiscus sabdariffa* on the level of liver marker enzyme in phenobarbitone–induced rat. With this in mind, this present study investigates the influence of *Hibiscus sabdariffa* extract on liver marker enzymes of phenobarbitone-induced adult Wister albino rats.

However, there are various studies on the phytochemical constituents and diverse medicinal activities attributed to this plant and no biochemical studies have been carried out to shed light on the role of *Hibiscus sabdariffa* on the levels of liver marker enzymes in phenobarbitone-induced experimental rats. In the light of the above, the present study was undertaken to investigate the effect of *Hibiscus sabdariffa* leaf extract on the liver marker enzymes such as (aspartate aminotransferase (AST), alanine transaminase (ALT) and alkaline phosphatase (ALP) in phenobarbitone-treated rats and control rats.

EXPERIMENTAL SECTION

2.1 Plant Material

The leaves of *H.sabdariffa* (Zobo leaf) were bought from Ogige Market Nsukka and was identified by Mr. A. Ozioko of the Department of Botany, University of Nigeria, Nsukka.

2.2 Preparation of Plant Extract

The leaves of *H. sabdariffa* were collected and dried under temperature (25^{O}C to 35^{O}C) for 3 weeks. After which the leaves were grounded into coarse form with milling machine and the weight was taken as 330.5 g. The coarse form was then macerated in hot water of 100^{O}C, which was contained in a clean pot. The system was left to stand for 24 hours after which the extractive was filtered out with the help of a clean white filter cloth and funnel. The resulting aqueous extract was concentrated till a slurry form was obtained under 65^{O}C in a water bath. A known

weight of the dry extract was determined and it was established that 2 g of the extract is equal to 1ml of the stock.

2.3 Animals

The experimental animals used for this study were adult Wister albino rats within the range of 79.0 g to 269.0 g body weight. The rats were obtained from the Animal House of Department of Zoology, Faculty of Biological Sciences, University of Nigeria, Nsukka.

2.4 Inducant (Phenobarbitone)

A quantity of 500g of phenobarbitone was dissolved in 50ml of normal saline to give a concentration of 10mg/ml. The dose range was taken to be 40mg/kg – 60mg/kg. But to achieve sedation 40mg/kg was used.

2.5 Preparation of Red Blood Cell Diluting Fluid

The diluting fluid was prepared by mixing 10 ml of formalin (40% formaldehyde) with 1000 ml of 31.3 g/l Trisodium citrate solution. The fluid was filtered and the filtrate was stored in a glass bottle at room temperature. The fluid was used as the diluent of the red blood cells.

2.6 Experimental Design

Forty two (42) adult Wistar rats were divided into seven (7) groups and housed in separate cages made up of plastic with metal wire cover. The rats were six (6) in each group and were kept in the animal house of Department of Home Science and Nutrition (HSN), University of Nigeria, Nsukka and were left for acclimatization for seven (7) days. The route of administration of the inducant and extract was via intraperitoneal (i.p.). The rats were all fed with normal rat diet and tap water *ad libitum*. GROUP I represented the NORMAL CONTROL fed with normal rat diet and water. 0.5 ml/kg body weight (b.w.) of distilled was administered to them. GROUP II represented the POSITIVE CONTROL which was injected via IP with a single dose (40 mg/kg b.w.) of the PB. GROUP III represented the group injected intraperitoneally with a single dose (0.5 ml/kg b.w.) of the extract of *H. sabdariffa* extract (HSEt). GROUP IV represented the group injected via intraperitoneally with a single dose (0.5 ml/kg b.w.) of extract plus a single dose of PB (40 mg/kg b.w.). GROUP V represented the group administered via intraperitoneally single dose (1.0 ml/kg) of extract plus a single dose (40 mg/kg b.w.) of the PB. GROUP VI represented the group administered a single dose (1.5 ml/kg) of extract plus a single dose (40 mg/kg b.w.) of the PB. GROUP VII represented the group also administered a single dose (2.0 mg/kg) of the extract plus a single dose (40 mg/kg b.w.) of the PB. In general, the rate of administration of extract of *H. sabdariffa* was twice per week while that of the PB was once a week. The samples (whole blood) for experiment were collected on different occasions and was put into an anticoagulant bottle.

1st collection (7th day): This was done on the seventh day, two rats from each group were sacrificed and their blood samples used immediately for biochemical analysis.

2nd collection (14th day): On this day, two rats from each group were also sacrificed and their blood samples used for analysis.

3rd collection (21st day): This was the final day, also two rats remaining in each group were sacrificed and used (blood samples) for biochemical analysis.

2.7 Biochemical Assays

The body weights of the animals were determined and recorded on each appropriate day before administration of the extract and inducer. Determination of basic liver function enzymes' {aspartate aminotransferase (AST), alanine aminotransferase (ALT) and alkaline phosphatase (ALP)} activities were assayed using commercially available diagnostic assay kits (Randox).

2.8 Statistical Analysis

The results were analysed for statistical significance by One-Way ANOVA and Posthoc test; the data was expressed as Means ± SEM, with $P<0.05$ considered as significantly different.

RESULTS

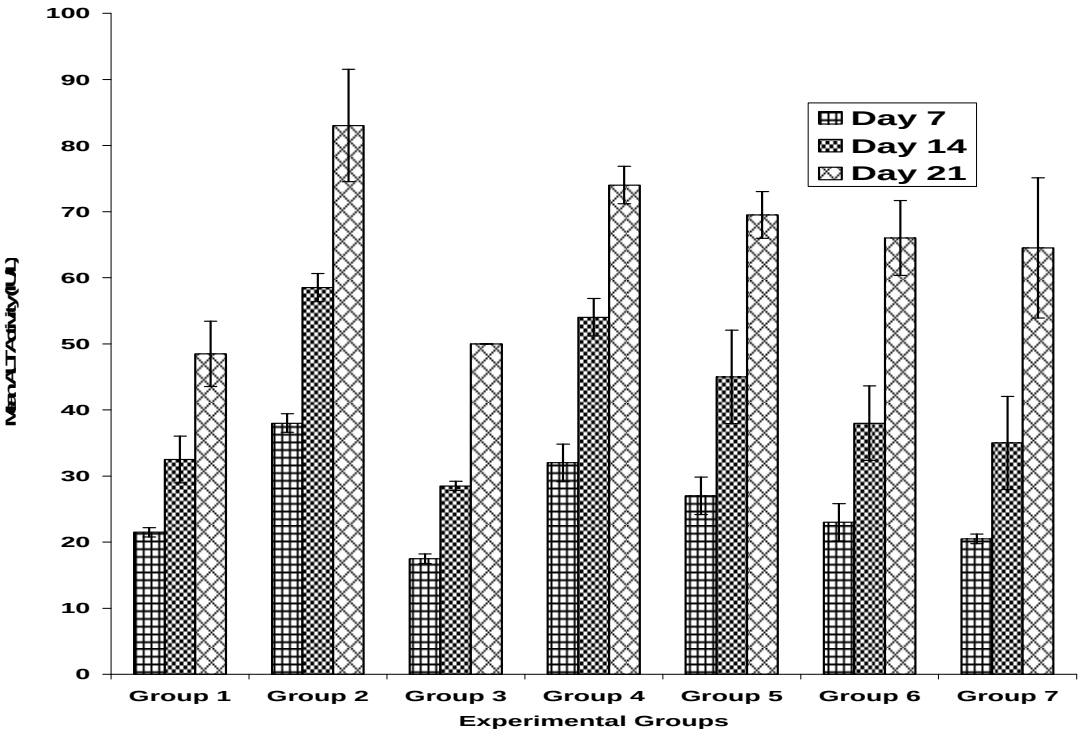

Fig. 1: Effect of *H. Sabdariffa* Extract and Phenobarbitone on the Alanine aminotransferase (ALT) Activities.

Group 1=Normal Control
Group 2=40 mg/kg Phenobarbitone
Group 3=0.5 ml/kg Extract
Group 4=0.5 ml/kg Extract + Phenobarbitone
Group 5=1 ml/kg Extract + Phenobarbitone
Group 6=40 mg/kg Phenobarbitone + 1.5 ml/kg Extract
Group 7=40 mg/kg Phenobarbitone + 2 ml/kg Extract.

Effect of *H. sabdariffa* Extract on the Alanine aminotransferage (ALT) Activity

The result presented in Fig. 1 shows that on the Day 7, the activity of ALT increased significantly ($P<0.05$) in group 2 (animals administered 40mg/kg PB when compared to group 6 (administered 40mg/kg PB + 1.5 ml/kg HSEt) group 5 (administered 40mg/kg PB + 1ml/kg HSEt) and group 7 (administered 40mg/kg PB + 2ml/kg HSEt). The level of ALT activity for

group 1 (Normal Control) was a bit higher than group 3 (administered 0.5 ml/kg HSEt) which had the lowest AST activity. On the Day 14, ALT activity in group 6 (administered 40mg/kg PB + 1.5ml /kg HSEt) is low when compound to group 2 (administered 40mg/kg PB). For Day 21. ALT activity decreased significantly (P<0.05) in group 3 (administered 0.5 ml/kg HSEt) and group 1 (Normal Control) when compared to group 2 (administered 40mg/kg PB) and group 4 (administered 40mg /kg PB + 0.5 mg /kg HSEt). From the figure below, it shows that the ALT level on Day 7 increased significantly (P<0.05) on day 14 and 21, with day 21 having the highest ALT activity as shown in group 2 (administered 40mg/kg PB).

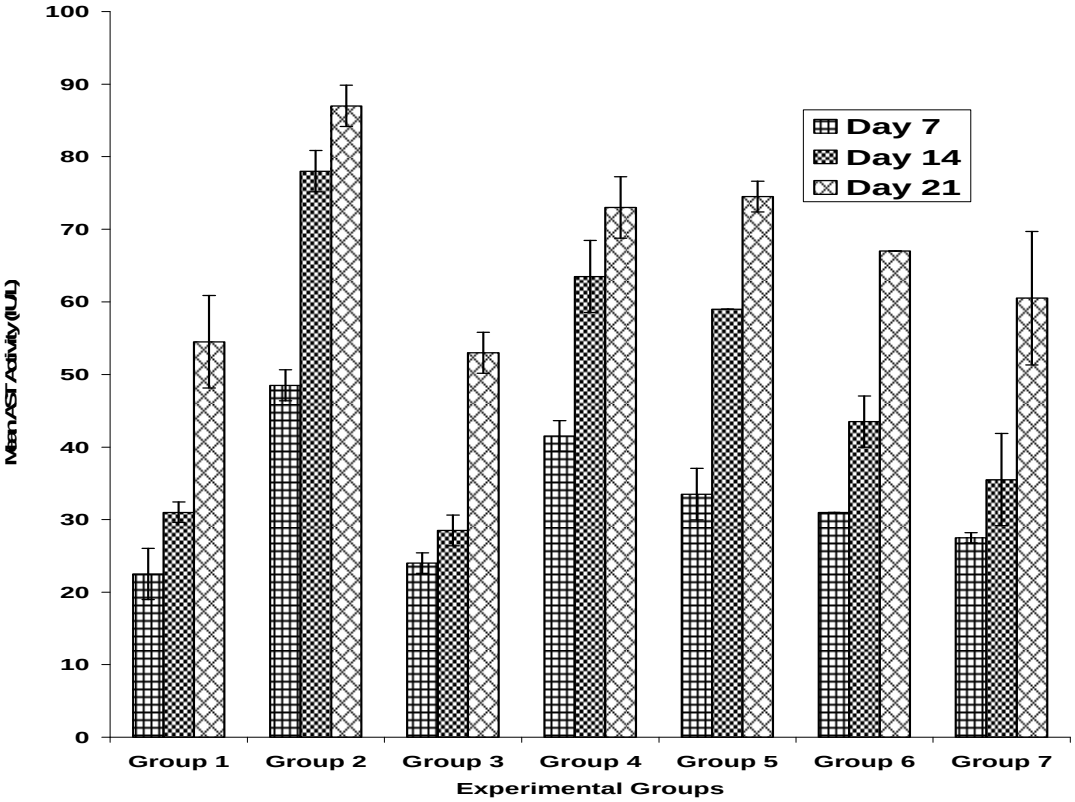

Fig. 2: Effect of *H. Sabdariffa* Extract and Phenobarbitone on the Aspartate aminotransferase (AST) Activities.

Group 1=Normal Control **Group 2=40 mg/kg Phenobarbitone**
Group 3=0.5 ml/kg Extract **Group 4=0.5 ml/kg Extract + Phenobarbitone**
Group 5=1 ml/kg Extract + Phenobarbitone **Group 6=40 mg/kg Phenobarbitone + 1.5 ml/kg Extract**
Group 7=40 mg/kg Phenobarbitone + 2 ml/kg Extract.

Effect of *H. Sabdariffa* Extract on the Alkaline phosphatase (ALP)

ALP being one of the liver marker enzymes showed a significant increase (P<0.05) on Days 7, 14 and 21 as shown in group 2 (administered 40mg/kg PB only). But on Day 21, the increase became significantly (P<0.05) higher than that of Days 14 and 7, while Day 14 is significantly higher (P<0.05) than Day 7 in the ALP activity. On day 7, ALP activity is significantly lower (P<0.05) than in group 1 (Normal Control) when compared to group 2 (administered 40mg/kg PB only). This is also obtained on Days 21 and 14.

Effect of *H. Sabdariffa* extract on the aspartate aminotransferase (AST) activity

In Fig. 2, AST activity on day 7 is decreased significantly (P<0.05) in group 7 (administered 40 ml/kg PB + 2ml/kg HSEt) when compared to group 4 (administered 40mg/kg PB + 0.5ml/kg HSEt). But the level of AST activity is considered the highest in group 2 (administered 40mg/kg PB only). Day 14 has a significantly decreased (P<0.05) AST activity in group 3 (administered 0.5 ml/kg HSEt) when compared to group 5 (administered 40mg/kg PB+ 1ml/kg HSEt) and group 4 (administered 40mg/kg PB + 0.5 ml/kg HSEt) with significantly increased AST activity (P<0.05). AST activity on the final day (Day 21) is significantly decreased (P<0.05) in groups 7 (administered 40ml/kg PB + 2ml/kg HSEt) when compared to group 5 (administered 40ml/kg PB + Iml/kg HSEt) and group 2 (administered 40mg/kg PB). In general, AST activity on Day 21 is higher than that on Days 14 and 7 as seen in group 4, (administered 40mg/kg PB + 0.5 ml/kg HSEt). In group 7, (administered 40mg/kg PB + 2ml/kg HSEt), the level of AST activity on day 7 is significantly lower (P<0.05) than Days 14 and 21.

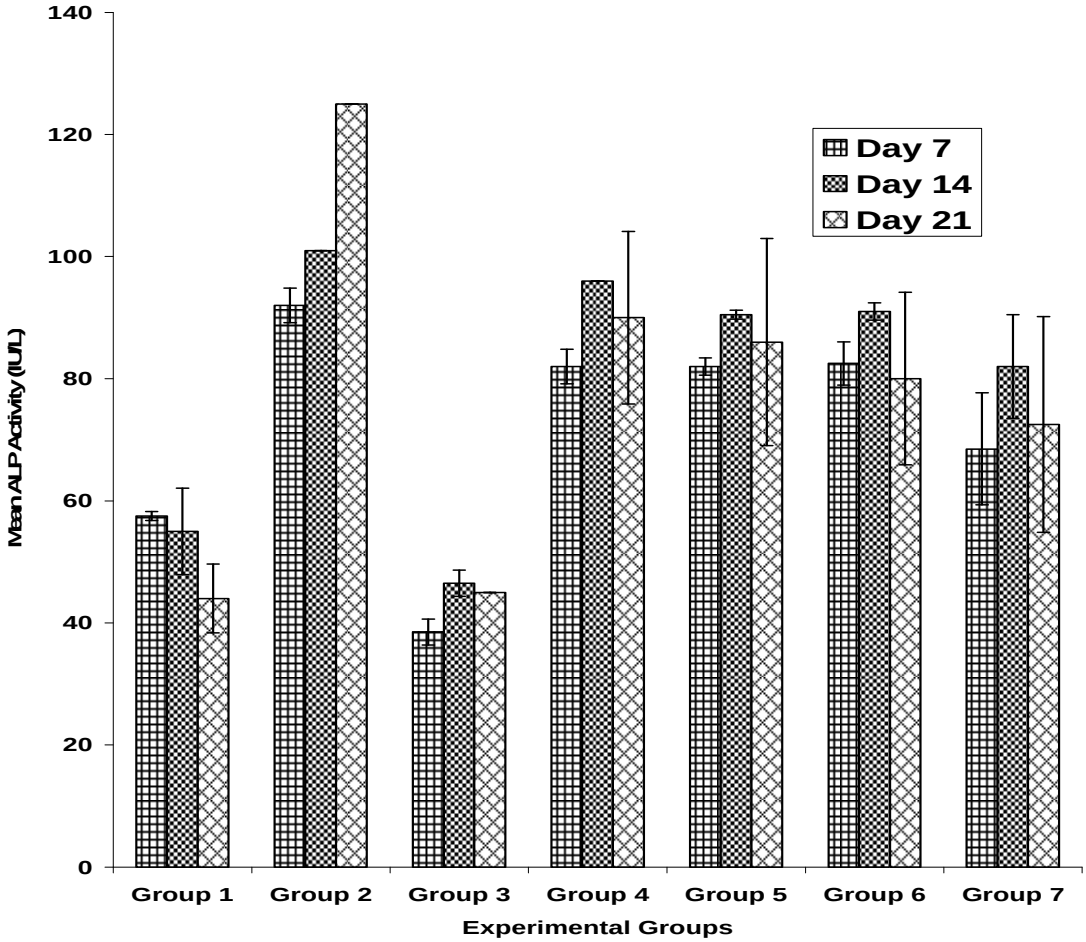

Fig. 3: Effect of *H. Sabdariffa* Extract and Phenobarbitone on the Alkaline phosphatase (ALP) Activities.

Group 1=Normal Control **Group 2=40 mg/kg Phenobarbitone**
Group 3=0.5 ml/kg Extract **Group 4=0.5 ml/kg Extract + Phenobarbitone**
Group 5=1 ml/kg Extract + Phenobarbitone **Group 6=40 mg/kg Phenobarbitone + 1.5 ml/kg Extract**
Group 7=40 mg/kg Phenobarbitone + 2 ml/kg Extract.

DISCUSSION

This study examined the role of *H.sabdariffa* (Zobo) extract on liver marker enzymes in phenobarbitone-induced rats. PB is metabolized in the liver and excreted by kidney (Gierger *et al*., 2000). Its common side effects are ataxia. sedation, polyuria, polydispsia, polyphagia and it may depress both the respiratory derive and the mechanisms responsible for the rhythmic character of respiration (Branum *et al*., 1998). In addition to these side effects, PB stimulates the division of liver cells (Alberts *et al*., 1998) and causes increases in serum alkaline phosphate (ALP), aspartate aminotransferase (AST) and Alanine aminotransferase (ALT) (Chauvet *et al*., 1995).ALP, AST and ALP enzymes are accepted as indicators of hepatotoxicity (Rosenthal, 1997). In this study, on day 7 in as shown in Figs 1, 2 and 3, there was decreased level of liver marker enzymes in group 2 (administered 40mg/kg PB only) when compared to group 2 on Days 14 and 21, with Day 21 having the highest activity of these liver enzymes.

On Day 7, Figs 1, 2 and 3 it show that the activity of AST, ALT and ALP decreased significantly ($P<0.05$) in group 7 (administered 40mg/kg PB + 2ml/kg HSEt) when compared to group 2 ((administered 40mg/kg PB only). This decrease suggests that the administration of HSEt has a protective effect on the level of circulatory liver marker enzymes and hence, liver damage. The same can be said of the effect of HSEt on liver marker enzymes' activities and liver damage on Days 14 and 21. This findings is consistent of Tseng *et al*. (1996), Duh and Yed (1997) and Tsai *et al*. (2002) who showed that rats treated with HSEt alone showed no significant differences ($P>0.05$) in levels of ammonia, urea, TBARS, HP, AST, ALT and ALP when compared with control rats.

Hibiscus sabdariffa is known to contain a number of bioflavonoids such as anthocyanins, glycosides, PCA, hydroxycitric acid, etc. (Lewis and Neelakantan 1965, Osman *et al*., 1975). Phytochemicals such as anthocyanin, flavonols, glycosides, etc. are well known potent free radical scavengers and also it was reported that the calyses extract of *Hibiscus sabdariffa* tend to reverse the change in lipid peroxidation activity, indicating decreased lipid peroxidation and damage to cells and tissues. *H. sabdariffa* is a good source of antioxidants (Tseng *et al*., 1996; Duh and Yed, 1997; Tsai *et al*., 2002). Hence, the possible mechanism by which the HSEt exerts a hepatoprotective effect in a hyperammonemic condition could be attributed to the presence of natural antioxidants and its free radical scavenging properties. The exact mechanism has to be still investigated and isolation of the active constituents is required.

Significant difference ($P<0.05$) was observed in the level of liver enzyme markers in the group administered phenobarbitone as compared with the normal control and other experimental test groups in a duration-dependent manner. The animals administered phenobarbitone in the Day-21 group had significant increase ($P<0.05$) in the level of the liver enzyme markers as compared with those of Day-7 and Day-14 respectively. This may be attributed to the long-term effect of the drug (phenobarbitone) in those experimental animals. In addition, AST activity alterations were mainly due to PB (Verma and Haidukewych, 1994). Many studies reported that PB caused increases in serum AST (Fortman and Witte, 1985; Haidukewych and John, 1986; Kitchin and Brown, 1987) and ALP activities (Chauvet *et al*., 1995; Filardi *et al*., 2000; Foster *et al*., 2000).

Previous reports show that alcoholic extract of HS flowers and calyses revealed marked nitric oxide scavenging activity (Obiefuna *et al*., 1993; Adegunloye *et al*., 1996; Wang *et al*., 2000; Odigie *et al*., 2003; Amin and Hamza, 2005).

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