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EFFECT OF *HIBISCUS SABDARIFFA* AQUEOUS EXTRACT (SOBO) ON LIVER MARKER ENZYMES IN PARACETAMOL-INDUCED HEPATOTOXICITY IN RATS

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SUMMARY
The hepatoregenerative properties of aqueous extract of *Hibiscus sabdariffa* in Paracetamol induced hepatotoxicity in rats was investigated. 30 adult albino Wistar rats (100-200g body weight) were used for the study. Rats in the control group (n =10) were fed normal rat chow, rats in group B (Paracetamol-treated group, n=10) were administered Paracetamol intraperitoneally at a dose of 750 mg/kg body weight while those in group C were administered Paracetamol 750 mg/ kg body weight + 10 ml/Kg body weight of aqueous leaf extract of *H. sabdariffa* orally for 4 weeks. All animals were allowed free access to clean drinking water. At the expiration of the treatment regime, serum levels of Aspartate transaminase (AST), Alanine aminotransferase (ALT), Alkaline phosphatase (ALP) were examined. AST in the Paracetamol + *H. sabdariffa*-treated group was significantly higher ($p< 0.05$) than the control and Paracetamol- treated groups respectively. ALT in the Paracetamol-treated group was significantly lower ($p< 0.05$) than the control and Paracetamol + *H. sabdariffa*-treated groups respectively. ALP in the Paracetamol treated group was significantly higher ($p< 0.05$) than the control group. It was however significantly lower ($p< 0.05$) than in the Paracetamol + *H. sabdariffa*-treated group. In conclusion, results of the study revealed that the aqueous extract administration of *H. sabdariffa* could not confer its hepatoregenerative property in rats challenged with Paracetamol-induced hepatotoxicity.

KEYWORDS: Paracetamol**,** *Hibiscus sabdariffa*, Alkaline phoshpatase, Aspartate aminotransferase, Alanine amino transferees.

INTRODUCTION

Hibiscus sabdariffa (Sobo) is native to Central and West Africa, but grows throughout many tropical areas. The major producing counties are Jamaica and Mexico (USDA NRCS, 2009; Ali *et al.* 2005; and Leung, 1980). Hibiscus has a long history of use in Africa and neighboring tropical countries for many conditions, including hypertension, liver diseases, cancer, constipation, and fever. The fleshy red calyx is used in the preparation of jams, jellies, drinks, and cold/ warm teas. The plant is also widely used in Egypt, Iran, Thailand, as well as in Western countries. Hibiscus flowers often are found as components of herbal tea mixtures (USDA NRCS, 2009; Ali *et al.* 2005; and Leung, 1980 Haruna, 1997; Abu-Tarboush *et al.* 1997). It is a major component of the popular herbal blend Red Zinger.

A large variety of compounds have been isolated from the hibiscus plant (Ali *et al.* 2005). Hibiscus flowers contain various polyphenols, including anthocyaninis, proanthocyanidins, flavonols, and other pigments Ali *et al.* (2005); Du and Francis (1973); Wilkinson *et al.* (1977); Sayago-Ayerdi *et al.* (2007). Oxalic, malic, citric, stearic, and tartaric acids have been identified and are, along with 15% to 28% of hibiscus or hibiscus acid.

Roselle (*H. sabdariffa*) seed oil contains more than 25 carbons, aldehydes, and alcohols (Abu-Tarboush *et al.* 1997; Ali *et al.* 2005). The oil is rich in gamma tocopherol (Mohamed *et al.* 2007). The seeds and flowers contain high amounts of protein and crude oil, ash, and carbohydrates. Abu-Tarboush *et al.* (1997) and Sayago-Ayerdi *et al.* (2007) had earlier reported the presence of high amounts of arginine, aspartic acid, and glutamic acid in the protein isolated from the hibiscus seed. Protective effects of the plant extracts on induced testicular and hepatic toxicity have been demonstrated in animals and are attributed to antioxidant action Wang *et al.* 2000; Amin and Hamza (2006); and Liu *et al.* 2006).
The liver is a large, chemically reactant pool of cells that have a high rate of metabolism, sharing substrates and

energy from one metabolic system to another, processing and synthezing multiple substances that are transported to other areas of the body, and performing myriad other metabolic functions (Guyton and Hall, 2011). The liver plays a central role in transforming and cleaning both endogenous and exogenous chemicals, and is susceptible to the toxicity from different agents (Zahra *et al*. 2012).

Drugs are known to be an important cause of liver injury (Bray et al. 2000). According to Ostapowiez *et al.* (2002) more than 900 drug toxins have been reported to cause liver injury even at therapeutic doses; they added that, liver injury is the common reason for drug withdrawal from the market. Drug-induced liver injury is a potential complication of nearly every medication since the liver is central to the metabolic disposition of virtually all drugs and foreign substances (Friedman *et al*. 2003). One of such drugs is Paracetamol (acetaminophen) known to cause liver problems (Laurie, 2000; Larrey, 2002; Aaron *et al.* 2009; Ansari *et al.* 2009; Aashish *et al*. 2012).

According to Agrawal and Khazaeni (2017) Paracetmol overdose is the common cause of acute liver damage. Acetaminophen toxicity is common primarily because the medication is so readily available and there is a perception that it is very safe. It is used in many products in combination with other preparations, especially with opiods and diphenhydramine. Unfortunately, many people are not aware that it is contained in these combinations (Athersuch *et al*. 2018; Jasani *et al*. 2018; Rajaram and Subramanian, 2018). It causes a multitude of interrelated biochemical reactions in liver cells producing multitude outcomes. Among these is covalent oxidation, lipid peroxidation, DNA fragmentation and deregulation of Ca^{2+} homeostasis each contributing to Paracetamol induced hepatoxicity (Jaeschke and Bajt, 2006). In modern day medicine, there are hardly any reliable drugs that protect the liver from damage and also help in the regeneration of the hepatic cell. However, traditional medicine has shown the protective effects of *H. sabdariffa L*. being used as antidote to poisonous chemicals and venomous mushrooms (Chifundra *et al*. 1994).

However, these metabolic disorders caused by Paracetamol at an overdose make it a suitable agent in researching about liver related problems using animal models. Hence this research, which sets out to investigate the hepatoregenerative effect of aqueous extract administration of *H. sabdariffa* in a Paracetamol-induced hepatotoxicity in rats.

MATERIALS AND METHODS

Plant materials

Fresh leaves of *H. sabdariffa* were purchased from Watt market in Calabar South, Cross River State, Nigeria. The leaves were authenticated by the Chief Botanist, Department of Botany, University of Calabar, Cross River State, Nigeria. 20 grams of the *H. Sabdariffa* was weighed and grounded in an electric mill to obtain particles less than 2 mm. It was used to make an infusion by adding 1 liter of clean water and allowed to stand for 48 hours. The solution was filtered using Whatman's No. 1 filter paper. The filtrate was stored in clean plastic containers and refrigerated. The extract was brought out of the refrigerator 2 hours to oral administration. The extraction was carried out according to the method of (Pi-Jen and Brain, 2002) with little modification.

Experimental animals

Approval for the study was obtained from the Animal Ethical Committee of the College of Medical Sciences. 30 Albino Wistar rats of both sexes weighing between 100-200g from the start of the experiment were used for this study. They were maintained in the animal facility of the Department of Physiology, University of Calabar, Nigeria, at a temperature of 30 ± 2^0 C and 12 h light/dark cycles. The rats were kept singly in improvised plastic metabolic cages with wire net covers. The rats were randomly assigned into three groups (A= control, B= Paracetamol-treated group and C= Paracetamol + aqueous leaf extract of *H. sabdariffa*-treated group). Each group consisted of ten rats. They were allowed free access to normal rat chow and clean drinking water. The Paracetamol-treated group received intraperitoneal inducement of Paracetamol (750 mg/kg body weight Hiroshini *et al.* (1987). The Paracetamol + extract of *H. sabdariffa* treated group received intraperitoneal inducement of Paracetamol (750 mg/kg body weight Hiroshini *et al*. (1987) and oral administration of aqueous leaf extract of *H. sabdariffa* (10 ml/kg body weight of rats) The animals in the control group were fed with only normal rodent chow and clean drinking water. Drug administration was done once daily for 3 weeks after which the blood samples were collected for analyses.

Collection of blood samples and measurement of liver enzymes

This was done using the method as described by Oloyede *et al*. (2008) and used by Umoren *et al*. (2015) with minor modifications. Venous blood samples were obtained from the jugular vein under thiopentone sodium anesthesia (Rolex Medica, GMBH, Germany), given at 60 mg/kg body weight. The blood samples were collected in test tubes and allowed to stand for 30 minutes to clot before being centrifuged at 300 x g for 10 minutes. The serum was extracted and stored at 4^0 C for subsequent analysis of ALP, ALT and AST enzyme levels. ALP level was analyzed according to the optimized standard method recommended by Deutsche Geseiischage for Klinissche Chemic (DGKC, 1972). Using P-nitrophenyl phosphate as substrate and measuring the phenol liberated by the enzyme. The AST and ALT levels were determined according to the method of (Reitman and Frankel, 1957). Enzyme activity was expressed in International Units per liter (IU/L). All analyses were checked for accuracy by concurrent analyses of AMES control sera for ALP, ALT and ASP.

Chemicals

P-nitrophenyl phosphate, P-nitrophenol, 4-aminoantipyrene and potassium ferric-cyanide and other commonly used chemicals were obtained from Randox laboratories (Dagenham, England). Other chemicals were D-L aspartic acid, ketoglutarate, D-L alanine, sodium pyruvate, 2, and 4— dinitrophenyl hydrazine was obtained from Sigma (Poole, England). All chemicals and reagents used in this study were of analytical grades.

Enzyme assays

Alkaline phosphatase (ALP) activity was determined using the method of Wright *et al.* (1972) and used by Umoren *et al*. (2015). Activities of Aspartate transaminase (AST) and Alanine transaminase (ALT) were determined based on the method described by Schmidt and Schmidt (1963), and used by Umoren *et al.* (2015) with minor modification.

Statistical Analysis

All results are presented as mean ± standard error of mean. The data were analyzed using a one-way analysis of variance (ANOVA) and $p<0.05$ was considered statistically significant.

RESULT

Serum Aspartate aminotransferase levels

The serum Aspartate aminotransferase levels in the control, Paracetamol-treated and Paracetamol + *H. sabdariffa*-treated groups are illustrated in Table 1. There was a significant increase ($p<0.05$) in serum AST level in the Paracetamol + *H. sabdariffa*-treated group as compared with the control and Paracetamol-treated groups respectively.

Table 1: Comparison of Aspartate aminotransferase (AST) levels in rats between the control, Paracetamol-treated and Paracetamol + H. sabdariffa-treated groups.

Group	AST (IU/L)
Control	13.00 ± 0.63
Paracetamol-treated	15.60 ± 0.60
Paracetamol + *H. sabdariffa*-treated	21.60 ± 1.40 ***

Values are mean ± SEM, n = 10, ***$p<0.05$ vs. control and Paracetamol-treated groups.

Serum Alanine aminotransferase levels

The serum Alanine aminotransferase levels in the control, Paracetamol-treated and Paracetamol + *H. sabdariffa*-treated groups are illustrated in Table 2. There was a significant decrease ($p<0.05$) in serum ALT level in the Paracetamol-treated group as compared with the control and Paracetamol + *H. sabdariffa*-treated groups respectively.

Table 2: Comparison of Alanine aminotransferase (ALT) levels in rats between the control, Paracetamol-treated and Paracetamol + H. sabdariffa-treated groups.

Group	ALT (IU/L)
Control	14.60 ± 0.24
Paracetamol	6.40 ± 0.24***
Paracetamol + *H. sabdariffa*	14.60 ± 0.24

Values are mean ± SEM, n = 10, ***$p<0.05$ vs. control and Paracetamol + *H. sabdariffa*-treated groups.

Serum Alkaline phosphatase levels

The serum Alkaline phophatase levels in the control, Paracetamol-treated and Paracetamol + *H. sabdariffa*-treated groups are illustrated in Table 3. There was a significant increase ($p<0.05$) in serum ALP level in the Paracetamol-treated group as compared to the control group and significantly lower ($p<0.05$) as compared to the Paracetamol + *H. sabdariffa*-treated group.

Table 3: Comparison of Alkaline phosphatase (ALP) levels in rats between the control, Paracetamol-treated and Paracetamol + H. sabdariffa-groups.

Group	ALP (IU/L)
Control	24.50 ± 0.37
Paracetamol-treated	50.40 ± 0.75***
Paracetamol + *H. sabdariffa*-ttreated	72.40 ± 1.23***

Values are mean ± SEM, n = 10, ***$p<0.05$ vs. control and Paracetamol + *H. sabdariffa*-treated groups.

DISCUSSION

Paracetamol also called *Acetaminophen* is a common analgesic and antipyretic agent which is safe at therapeutic doses but can produce life threatening hepatic and renal damages in man (Kurata *et al*. 1993; McJunkin *et al*. 1994), rats and mice (Mitchell *et al*. 1973; Kurata, *et al*. 1993) at toxic doses (Roberts and Morrow, 2001; Agrawal and Khazaeni, 2017). In the United States, *acetaminophen* toxicity has replaced viral hepatitis as the most common cause of acute liver failure (Lee, 2012).

Paracetamol toxicity can be influenced by various factors which include the dose and pattern of use, alcohol consumption, herbs and medication, age and genetic

factors, nutritional status and chronic liver disease (Schimdt *et al*. 2002; Liu *et al*. 2004).

This study investigated the role of *H. sabdariffa* aqueous extract on liver marker enzymes in Paracetamol-induced hepatoxicity in rats. (Wang *et al*. 2000; Liu *et al*. 2006; Amin and Hamza, 2006) had demonstrated the protective effects of the plant extracts on induced testicular and hepatic toxicity in animal and attributed the effect to its anti-oxidative property. Despite the hepatoprotective and antioxidative properties of *H. sabdariffa*, our result reveals liver damage following aqueous extract of *H. sabdariffa* administration in a Paracetamol-induced hepatotoxicity in rats. This finding is in consonant with the report of Yakubu *et al.* (2001) whose work had shown adverse effects on the liver due to adaptation of the liver to toxic assault from fresh synthesis of molecules in the plant extract.

The changes in liver enzyme profile following administration of aqueous extract of *H. sabdariffa* on Paracetamol induced hepatotoxicity in rats were measured. The levels of AST, ALT and ALP were elevated in the Paracetamol-treated group and Paracetamol + *H. sabdariffa*-treated group. Although the experimental methods used for the liver enzyme analysis have potential flaws related to product inhibition, they generally give adequate comparative results.

In the assessment of liver damage certain biomarkers of hepatotoxicity are measured and one of such biomarkers are enzyme levels such as AST and ALT because liver damage arising from necrosis or membrane damage normally releases the enzymes into circulation; therefore, measurement of these enzymes in serum gives an indication of the health status of the liver. High levels of AST indicate liver damage, which may be due to viral hepatitis, cardiac infarction and muscle injury. ALT catalyses the conversion of Alanine to pyruvate and glutamate, and is released in a similar manner. It is known that an increase in the enzymatic activity of ALT and AST in the serum directly reflects a major permeability or cell rupture, and thus a better parameter for detecting liver injury (Benjamin, 1978; Witter and Bohmwald, 1986). An increase in AST and ALT, a hepato-specific enzyme that is principally found in the cytoplasm in the rats following administration of a hepatotoxin is attributed to the increased release of enzymes from the damages liver parenchymal cell (Benjamin, 1978; Ringler and Dabich, 1979; Talwar, 1980).

From the result obtained, the serum AST level increased markedly in the Paracetamol-treated group when compared to the control group. This indicates liver toxicity. Surprisingly, the Paracetamol + *H. sabdariffa*-treated group also showed a marked increase in serum AST level when compared to control group as well as the group treated with Paracetamol only. This implies that the aqueous extract of *H. sabdariffa* potentiated toxicity of the liver. This result is however in contrast to the works of Joshua *et al.* (2017) who reported decreased level of serum AST, ALT, and ALP following aqueous extract of *H. sabdariffa* administration. It is opined that this contrast may be due to the differences in dosage administration. However, it should be noted that serum Amino transferase levels ALT and AST are two of the most useful measures of liver cell injury, although AST is less liver specific than is ALT level. Diseases that primarily affect hepatocytes, such as viral hepatitis, will cause disproportionate elevations of AST and ALT levels compared with the Alkaline phosphatase level.

Serum ALT level decreased significantly in the Paracetamol-treated rats when compared to the control and the Paracetamol + *H. sabdariffa*-treated groups respectively. It was however observed that ALT concentration increased markedly in the Paracetamol + *H. sabdariffa*-treated group. (Gopal and Rosen, 2000; Nyblom *et al*. 2004; Nyblom *et al*. 2006) had explained through their works that as chronic liver damage progresses, ALT activities decline and the ratio of AST to ALT gradually increases. This implies that when liver cirrhosis sets in, the AST level is often higher than the ALT level. This phenomenon might explain the decrease in ALT level in the Paracetamol treated group. It should be stated here that most causes of liver cell injury are associated with a greater increase in ALT than AST.

However, an AST to ALT ratio of 2:1 or greater is suggestive of alcoholic liver disease, particularly in the setting of an elevated gamma-glutamyl transferase (Moussavian *et al*. 1985). The AST to ALT ratio can also occasionally be elevated in a liver disease pattern in patients with non alcoholic steatohepatitis (WHO, 2018; NASH, 2016), and it is frequently elevated in an alcoholic liver disease pattern in patients with hepatitis C who have developed cirrhosis (Cirrhosis, 2014). In addition, patients with Wilson's disease or cirrhosis due to hepatitis may have an AST that is greater than ALT, though the ratio typically is not greater than two.

The serum ALP level increased significantly in the Paracetamol-treated group and the Paracetamol + *H. sabdariffa*-treated group when compared to the control group respectively. This also indicates increased liver toxicity. Serum Alkaline phosphatase comprises a heterogeneous group of enzymes. Hepatic Alkaline phosphatase is most densely represented near the canalicular membrane of the hepatocyte. According to Arvind and William (2017) diseases that predominantly affect hepatocyte secretion especially obstructive diseases, is usually accompanied by elevations in Alkaline phosphatase levels. Arvind and William (2017) also opined that Bile-duct obstruction, primary sclerosing cholangitis, and primary biliary cirrhosis are some of the reasons in which elevated Alkaline phosphatase levels are often predominant over transaminase level elevations. This may be attributed to the elevated serum level of ALP in rats used in this study.

In conclusion, results of the study indicate elevated serum levels of AST, ALT and ALP in Paracetamol-induced hepatotoxicity in rats. However, the liver marker enzymes level was on the increase following extract administration of *H. sabdariffa* in the Paracetamol-induced hepatoxicity. This was surprising despite the free radical scavenging property and the presence of natural antioxidants of the leaf extract. It therefore implies that following Paracetamol-induced hepatotoxicity, the hepatoregenerative property of *H. sabdariffa* extract may not be able to confer any protection on a challenged liver. Further investigation is hereby recommended to ascertain the mechanism of action of *H. sabdariffa* on hepatoprotection and possibly, the ameliorative safety dose level in humans.

Conflict of Interests

The authors have not declared any conflict of interests.

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