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Effects of Ethanolic Extract of Hibiscus Sabdariffa Calyxes and 2, 4- Dinitrophenylhydrazine on Liver Function Parameters of Rats

Augustine Olusegun Olusola

Department of Biochemistry, Adekunle Ajasin University, Akungba Akoko, Ondo State, Nigeria.

***Corresponding Authors:** Augustine Olusegun Olusola, Department of Biochemistry, Adekunle Ajasin University, Akungba Akoko, Ondo State, Nigeria.*

ABSTRACT

This study aims at evaluating the effects of Hibiscus sabdariffa and 2, 4-Dinitrophenylhydrazine (2, 4-DNPH) on the liver function parameters of rats. 20 rats were evenly distributed into 4 groups and treatment lasted for a week. The animals in groups one (1) and four (4) were given water only while groups two (2) and three (3) rats received H. sabdariffa alone. At the end of the 6th day, the animals were fasted overnight and on the 7th day, 28mg per kg body weight of DNPH was administered to each animal in groups 3 and 4 and after 7 hours they were sacrificed under diethyl ether anesthesia. Serum and liver samples were taken and prepared for biochemical assays to determine their ALT, AST, LDH, BIL, GGT, ALP levels. Decrease in the levels of these markers in the liver of the rats treated with DNPH alone relative to their respective controls, signifying that DNPH potentiates cytotoxicity in the liver. H. sabdariffa showed hepatoprotective properties which counteracted the toxicity effect of DNPH when the rats in the group treated with H. sabdariffa prior to the treatment of DNPH were compared to the rats treated with DNPH alone. In view of the foregoing it can be established that ethanolic Hibiscus sabdariffa extract has hepatoprotective properties by reducing the effect which DNPH has on liver.

Keywords: *Hibiscus sabdariffa, Ethanolic Extract, 2, 4-dinitrophenylhydrazine, Liver Function Parameters, hepatoprotective.*

INTRODUCTION

Hibiscus sabdariffa Linn (Roselle) is also known locally as asamsusur, asampaya or Ribena in Malaysia, and closely resembles cran-berries in flavour. It is also called karkade in Switzerland and in Arab-speaking countries. Roselle is used in jams, jellies, sauces and wines. The young leaves and tender stem are eaten raw in salads and chutney. They are also added to curries and some Malaysian dishes as seasoning. The seeds are somewhat bitter but, in Africa, they are ground into meal for human food due to their high protein content. They have also been roasted to use as a substitute for coffee (Morton, 1987). In northern Nigeria, roselle seeds are fermented in the presence of some spices to prepare a food known as Mungza Ntusa (Balami, 1998). Its seeds also contain a substantial amount of oil that resembles cotton seed oil (Mohammed, *et al.*, 2007). Many medicinal applications of this plant have been developed around the world. For example, in China it is used to treat hypertension, pyrexia, liver damage and leukaemia due to its high content of protocatechuic acid (Tseng *et al.*, 2000). Studies by Muhammad and Shakib (1995) have shown that roselle can prevent cancer, lower blood pressure and improve the digestive system in humans. Its calyx extract has also been used as an effective treatment for patients with kidney stones due to its uricosuric effect (Prasongwatana, *et al.*, 2008). In addition to having the above mentioned activities, roselle extract can also per- form as an antioxidant. For example, it protects against low density lipoprotein (LDL) - oxidation and has hypolipidemic effects in vivo (Hirunpanich *et al.*, 2006). In some instances, it is also used to preserve food. Up until now, most of the scientific study has only focused on the calyces of roselle. Very few data on antioxidant activity of extracts obtained from other parts of the roselle plant are available. Thus, the aim of this work was to evaluate the antioxidant activity of extracts from calyxes, leaves, seeds and stems of roselle plants, and compare these to a commercial synthetic antioxidant, butylatedhydroxytoulene (BHT). In addition, the anti- oxidant activity of roselle seeds was also

tested in a whole food sys- tem by determining effects of roselle on lipid oxidation in cooked beef stored at 4 C for up to 14 days.

Indications exist that red extract of the dried flower of *Hibiscus sabdariffa* is protective against oxidative stress in rat primary hepatocyte (Tseng *et al.*, 1997; Ologundudu and Obi 2005) and hence possesses antioxidant principles.

The aim of this research was to evaluate the effect of the ethanolic extract of *Hibiscus sabdariffa* antioxidant on 2, 4-diniotro-pehnylhydrazine on liver function markers in Rat.

MATERIALS AND METHODS

Materials

Reagents

2, 4- dinitrophenylhydrazine, Sodium chloride (NaCl), Diethyl ether, Trichloroacetic acid (TCA), Ethyl acetate, [BDH Chemical LTD, Poole England], Absolute ethanol, Formic acid, Hydrochloric Acid (HCl), [WN Laboratories USA], 2-Thiobarbituric acid (TBA), [Koch-light laboratories, Ltd Colebrook Bucks, England], Tris, [Lab Tech. Chemicals].

Methods

Preparation of the Ethanolic Extraction

Dried flower of *Hibiscus sabdariffa* were purchased at the central market of Ikare-Akoko, Ondo state, Nigeria. The extraction process was carried out adopting the method described by Ologundudu and Obi (2005). 100g of the dried flower (*Hibiscus sabdariffa*) minus the seed were packed into a thimble of a soxhlet apparatus, while one litre absolute ethanol was put in soxhlet still pot; this was heat at ethanol boiling point (73^0C) for 24hours and the extract was siphon back to the still pot, to obtain the red coloured extract, the filtrate. The solvent was evaporated in a rotary evaporator and a viscous mass was obtained as residue. This was then reconstituted in 10% aqueous ethanol, put into a bottle, sealed and left at 4^0C until required.

Animal Treatment and Toxicant Administration

Pathogen-free rats (Rattus norvegicus) were obtained from a breeder in Ibadan. The animals were housed in galvanized rat cages and acclimatized on guinea grower mash (product on Bindel feeds and flour mills, BFFM Ltd, Ewu, Nigeria) for two weeks in the laboratory with 12 hours light and darkness cycle. Thereafter, they were evenly distributed into eight experimental groups of three rats each. All the animals drank water *ad libitum* throughout the duration of the experiment.

Group I (Control): received 2.5 ml/kg body weight distilled water twice daily by gavage for 7 days.

Group II: animals received 100 mg/kg body weight of the ethanolic extract of *H. sabdariffa* for 7 days.

Group III: animals received 100 mg/kg weight of the ethanolic extract of *H. sabdariffa* for 7 days + 28 mg/kg body weight of 2, 4-dinitrophenylhydrazine at the end of the 7^{th} day after overnight fasting.

Group IV: treated with 28 mg/kg body weight of 2, 4 – dinitrophenylhydrazine after overnight fasting.

Collection and Preparation of Serum and Liver Samples

At the end of the treatment entirely, the animals were sacrificed under diethyl ether anaesthesia and blood was collected by cardiac puncture into properly labelled heparin bottles. The bottles were placed in ice cold water. Other set of blood samples were collected into non-heparinized bottles for serum. The plasma and serum subsequently obtained by centrifugation at 3500rpm for 15 minutes. Some were used for analysis immediately while others were stored in the refrigerator at -20^0C pending analysis but usually not exceeding 48hours.

The liver of each rats was quickly excised homogenized in ice-cold saline (1:4wt/vol) and centrifuged at 3,500rpm for 15 minutes to remove the debris. The supernatant was recovered and used for analysis or stored pending use but not exceeding 48hours.

Biochemical Assays

Assay kits for AST, ALT, ALP, LDH, Creatinine, Urea and total protein were products of Randox Laboratory Ltd., Ardmore, Diamond Road, Crumlin, Co. Arlim, United Kingdom. They were estimated using a commercial diagnostic kit.

Statistical Analysis

The data obtained were subjected to one-way statistical analysis of variance (ANOVA) using the SAS software (SAS Inst. Inc. 1999). Treatment means were compared using the

Duncan procedure of the same software. The significance level was set at P<0.05.

RESULTS

The results obtained from the experimental analysis of the various tissues of test animals for the effects of ethanolic extract of *H. sabdariffa* on 2, 4 dinitrophenylhydrazine induced changes on liver makers are presented numerically in their respective tables. Values obtained are expressed as mean in all tables and are put into bar chart.

The effect of the ethanolic extract of *Hibiscus sabdariffa* on 2, 4 dinitrophenylhydrazine induced changes on aspartate aminotransferase (AST) levels in serum and liver are shown in Figure 1, the results indicate that there is no significant difference between the control group, ethanolic extract treated group and ethanolic extract treated with DNPH which is group three in the liver, moreover there is no significant difference between the control group and the *H. sabdariffa* ethanolic treated group in the serum meanwhile there is significant difference in the ethanolic extract treated with DNPH group compared to groups treated with DNPH only. *H. sabdariffa* showed counter balance effect to DNPH effect in liver as there is significant difference between the ethanolic extract group treated with DNPH and groups treated with DNPH only.

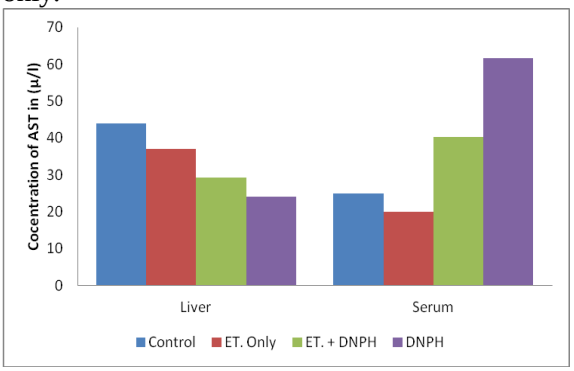

Figure1. *Effects of ethanolic extract of Hibiscus sabdariffa on 2, 4 dinitrophenylhydrazine induced changes in (AST) concentration in serum and liver.*

The effect of the ethanolic extract of *Hibiscus sabdariffa* on 2, 4 dinitrophenylhydrazine induced changes on alanine aminotransferase (ALT) levels in liver and serum which is shown in figure 2, the results indicate that DNPH has effect on ALT levels in both liver and serum, there is no significant difference between the control group and *H. sabdariffa* ethanolic extract treated groups in the liver and serum. There is no much difference between ethanolic extract treated with DNPH group compared to groups treated with DNPH only. That takes the toxicant only has the highest level of the marker in the serum, which indicate that the toxicant causes liver damage.

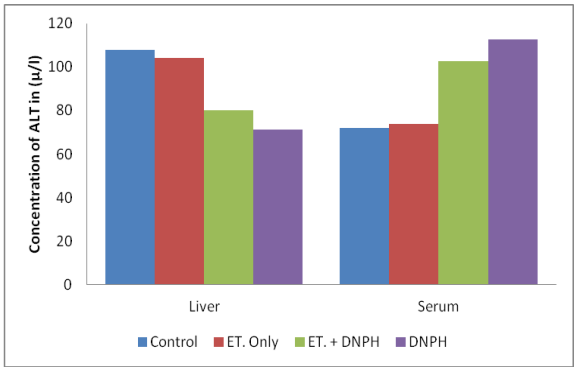

Figure2. *Effects of ethanolic extract of Hibiscus sabdariffa on 2, 4 dinitrophenylhydrazine induced changes in (ALT) concentration in serum and liver.*

The effect of the ethanolic extract of *Hibiscus sabdariffa* on 2, 4 dinitrophenylhydrazine induced changes on lactate dehydrogenase (LDH) levels in serum and liver and is shown in figure 3, the results indicate that DNPH reduces the LDH levels in liver thereby increasing the maker in serum, there is no significant difference between ethanolic extract treated with DNPH which is group three compared to groups treated with DNPH only.

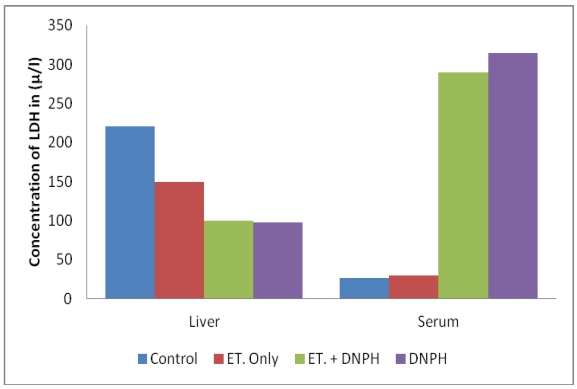

Figure3. *Effects of ethanolic extract of Hibiscus sabdariffa on 2, 4 dinitrophenylhydrazine induced changes in (LDH) concentration in serum and liver.*

The effect of the ethanolic extract of *Hibiscus sabdariffa* on 2, 4 dinitrophenylhydrazine induced changes on alkaline phosphatase (ALP) level s in liver and serum are shown in figure 4. The results indicate that there is no significant difference in control group and ethanolic extract treated group. This result indicates DNPH proliferate the membrane for the maker which causes high increase of the marker in the serum.

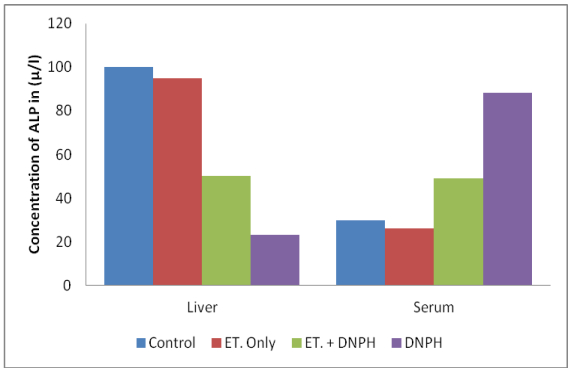

Figure4. *Effects of ethanolic extract of Hibiscus sabdariffa on 2, 4 dinitrophenylhydrazine induced changes in (ALP) concentration in serum and liver.*

The effect of the ethanolic extract of *Hibiscus sabdariffa* on 2, 4 dinitrophenylhydrazine induced changes on gamma glutamine transferase (GGT) levels in serum and liver and are shown in figure 5, The results indicate that there is no significant difference between control group and ethanolic extract treated group only, *H. sabdariffa* counter balance the effect the toxicant (DNPH) on the liver by reducing the GGT levels in the serum by the group administered with ethanolic extract and DNPH.

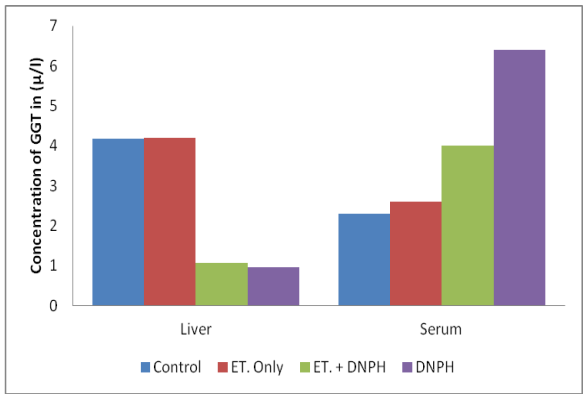

Figure5. *Effects of ethanolic extract of Hibiscus sabdariffa on 2, 4 dinitrophenylhydrazine induced changes in (GGT) concentration in serum and liver.*

The effect of the ethanolic extract of *Hibiscus sabdariffa* on 2, 4 dinitrophenylhydrazine induced changes on total bilirubin (T. BIL) levels in serum and liver and are shown in figure 6. The results indicate that group treated with DNPH only, causes increase in total bilirubin levels in serum, while the groups treated with ethanolic extract with the toxicant showed extreme low level of the marker compared with the group that took only the toxicant. This result indicates that *H. sabdariffa* possesses properties that inhibit the action of DNPH and therefore protect the liver

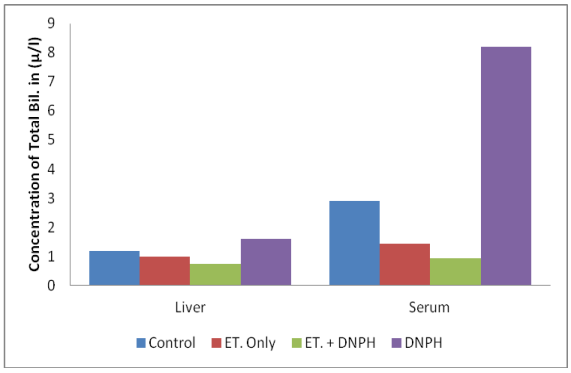

Figure6. *Effects of ethanolic extract of Hibiscus sabdariffa on 2, 4 dinitrophenylhydrazine induced changes in total bilirubin concentration in serum and liver.*

The effect of the ethanolic extract of *Hibiscus sabdariffa* on 2, 4 dinitrophenylhydrazine induced changes on direct bilirubin (D. BIL) levels in serum and liver and are shown in figure 7, the results indicate that group treated with DNPH only reduces enzyme activity levels of the marker in liver comparing to an increase enzyme activity in serum, this shows that DNPH causes liver damage which causes the rise in Total Bilirubin activity in the serum. *H. sabdariffa* minimize the increase in enzyme activity of the marker.

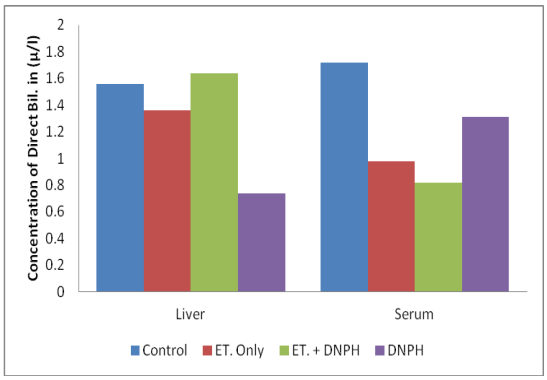

Figure7. *Effects of ethanolic extract of Hibiscus sabdariffa on 2, 4 dinitrophenylhydrazine induced changes in (Bil.) concentration in serum and liver.*

DISCUSSION

In this study, the level of aspartate amino-transferase (AST), alanine aminotransferase (ALT) lactate dehydrogenase (LDH), alkaline phosphatase (ALP), gamma glutamyl transferase (GGT) and bilirubin (BIL), which are all markers used in determining or for diagnosing liver damage caused by 2,4 dinitrophenylhydrazine and protective effect of *Hibiscus sabdariffa* against the damaging effect of the DNPH. In correlation with previous similar studies such as Bolton (1935) and Maduka *et al.*, (2003), the results obtained in

this work show that DNPH depleted the level of the respective afore mentioned parameters and the ethanolic extract of *H. Sabdariffa* was able to protect the animals from liver damage.

Pre-treatment of rats with 100g of the *H. sabdariffa* ethanolic extract once a day per kg body weight for a week before the administration of the toxicant (DNPH) and for additional a day for the administration of the toxicant, significantly reduced the toxic effect on the liver damage which the toxicant normally caused. The results presented in form of bar chat in chapter three of this write up showed that *H. sabdariffa* ethanolic extract was able to suppress the liver parameters depleting action of the toxicant in the liver.

The extract displayed a strong hepatoprotective effect as it significantly reduced DNPH-induced hepatotoxicity in rats, as judged from the serum activities of ALT, AST, and ALP in chapter three of this work fig. 1, 3 and 4. These results suggest that the ethanolic extract of dried flower of *Hibiscus sabdariffa L*. possesses antioxidant and hepatoprotective on DNPH which causes damage in rat's liver. AST is an enzyme that is present in high quantities in the cytoplasm and mitochondria of liver, also present in the heart, skeletal muscle, kidney and brain. ALT is a hepato-specific enzyme that is principally found in the cytoplasm (Nyblom *et al.,* 2006). Liver disease is the most important cause of increased ALT activity and a common cause of increased AST activity. In hepatocellular injury or necrosis they leak into the circulation and raise the serum level of the enzymes. The major diagnostic use of AST is in myocardial infarction, while ALT is used in viral hepatitis and acute pancreatitis Reichling and Kaplan (1988).

The groups treated with *H. sabdariffa* ethanolic extract showed no significant difference in serum and liver levels of AST and ALT compared to the water control. This is an indication of reduced toxicity of the *H. sabdariffa* to the liver. When DNPH treated group was compared with the water control, there was a significant increase in the serum activities of the enzyme with decrease in activities in liver. The increase activity of these enzymes in the serum is a clear indication of the toxicity of DNPH on the liver. The toxicity is sequel to the free radicals generations and repression of protein synthesis in this organ, Ologundudu, *et al.,* (2009). Prior administration of *H. sabdariffa* ethanolic extract followed by treatment with DNPH resulted in significant decrease in serum activities and an increase in the liver activities of the enzymes compared with those treated with DNPH only. Since the toxicity of DNPH is related to its ability to generate free radicals, it follows therefore that the prophylactic administration of these ethanolic extracts built up defense and thus reducing significantly the oxidative damage provoked by DNPH administration. Tissues necrosis that usually characterizes oxidative damage is therefore minimized and the tissue activities of ALT and AST are preserved and therefore, their serum activities normalized.

Lactate Dehydrogenase exists in body tissues as tetramer. Two monomers, H and M, can combine in various proportion with results that five isoenzymes of LDH are known. The determination of lactate dehydrogenase activity has a wide variety of clinical uses. As an intracellular enzyme, its increase indicates tissue damage with its consequent release to the blood stream. The damage can range from simple anoxia with small cell damage and cytoplasm loss to severe cellular necrosis causing various degrees of enzyme activity increase.

In Acute Myocardial Infarction, the total LDH activity (along with that of CK and AST) constitutes an important diagnostic element. The activity starts increasing 12-24 hours after the infarction and reaches a peak between 48-72 hours, remaining high up to the seventh or tenth day. On the other hand, an LDH activity increase is observed in patients with hepatic necrosis (produced by toxic agents or acute infections such as viral hepatitis) even accompanying renal tubular necrosis, pyelonephritis, etc. In blood tumors like leukemia and lymphoma increased levels of LDH are also observed. In the cerebrospinal fluid (CSF) normal value is approximately 10% of its value in serum, markedly increasing its value in bacterial meningitis. In viral meningitis, LDH increases its value only in 10% of cases. Increase in LDH activities in serum indicated that DNPH is agent which renders the membrane of LDH proliferated, as results increase the enzyme activities in the serum. *H. Sabdariffa* helps in minimising the hypertoxicity caused by DNPH to the liver.

The serum level of bilirubin could also give an indication of liver damage. Hyperbilirubinemia is indicative of impairment of liver function.

Neither the low nor high dose of the ethanolic extract showed a clear indication of toxicity to the liver. *H. sabdariffa* show high protective valued against the effect of DNPH. Bilirubin is formed by the breakdown of haemoglobin in the spleen, liver and bone marrow. In the liver, bilirubin is conjugated with glucuronic acid to form a soluble compound. The conjugated bilirubin passes down the bile duct and is excreted into the gastrointestinal tract. An unconjugated, albumin bound form is present in the circulation. It is insoluble and does not normally pass through the kidneys into the urine.

An increase in bilirubin concentration in the serum or tissues is called jaundice. Jaundice occurs in toxic or infectious diseases of the liver e.g. hepatitis B or obstruction of the bile duct and in rhesus incompatible babies.

Useful information may be obtained by determining which form of bilirubin is elevated.

Human alkaline phosphatase (AlkP, EC.3.1.3.1) consists of a group of at least five tissue-specific isoenzymes which catalyzes the hydrolysis of phosphate mono-esters at alkaline pH. A variety of disease processes can result in the release of increased quantities of alkaline phosphatase into the blood. Increased level of ALP is associated with a variety of bone and liver disorders (Dufour *et al.*, (2000). Increased level in serum indicates that DNPH causes obstruction or damage to the liver, thereby causing decrease enzyme activities in the liver.

Gamma-glutamyltransferase in serum originates primarily from the hepatobiliary system. Therefore, it is elevated in all forms of liver diseases and has been shown to be more sensitive than alkaline phosphatase in detecting obstructive jaundice, cholangitis and cholecystitis. High levels of GGT are also seen in patient with primary and secondary liver cancer. Increased levels are also observed in cases of alcohol abuse and in alcoholic liver cirrhosis. In patients receiving anticonvulsant drugs such as phenytoin and phenobarbital, increased level of the enzymes in serum may reflect induction of the new enzymes activity and the toxic effects of alcohol and other drugs on the microsomal structures in liver cells. Although GGT is the most sensitive enzymatic indicator of hepatobiliary diseases it cannot be used to differentiate between different types of hepatobiliary diseases. However, GGT can be used in combination with other biochemical markers to discriminate between different types of hepatobiliary diseases. Phenylhydrazine has been studied to undergo both phase I (where it is hydroxylated at the Para position) and phase II (where it could be conjugated with glucuronic/ mercapturic acid) (Mc Isaac *et al.*,), one proposed mechanism (Maduka *et al.*, 2003) explains that liver damage by DNPH stimulates high release of gamma glutamyl transferase in the serum. The ethanolic extract of H. Sabdariffa administered with DNPH, the ethanolic extract show suppressive action against the toxicant.

CONCLUSION

In conclusion, the various results obtained in this study substantiated the claim that ethanolic extract of *Hibiscus sabdariffa* have hepatoprotective agents. DNPH is known to induce in a variety of interrelated mechanism and yields different unfavourable consequence. The ability of ethanolic extract suppresses the toxic effect of this toxicant reinforce their stands in the class of hepatoprotective agents, and this would suggest that it could be employed for therapeutic purposes. It is therefore recommended that more encouragement should be given to the consumption of this ethanolic extract for its anti-hepatotoxicity properties.

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