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Research Article
Biochemical Effects of the Aqueous Extract of *Hibiscus sabdariffa* on Liver Marker Enzymes and Lipid Profiles in Acetaminophen-challenged Rats

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Abstract

Background and Objective: *Hibiscus sabdariffa* is one of the medicinal plants that have been shown to possess antihypertensive and cardioprotective effects in rats. This study was conducted to determine the hepato-regenerative properties of aqueous extract of *Hibiscus sabdariffa* leaves in acetaminophen induction of the liver by using rat model. **Materials and Methods:** Twenty Wistar Albino rats were used for this study and were divided into four groups. Group 1 rats were normal control group 2 (positive control) rats were administered acetaminophen only at a dose of 750 mg kg^{-1} b.wt., i.p. Both rats of group 3 and 4 were administered low and high doses respectively at 600 mg kg^{-1} b.wt., of the extract after induction of acetaminophen. The results were analyzed using one way analysis of variance using SPSS version 20. **Results:** Group 2 rats treated with acetaminophen only showed significant increase ($p<0.05$) in ALT, AST and ALP activities compared to group 1 rats. The administration of *Hibiscus sabdariffa* extract after the induction of acetaminophen significantly ($p<0.05$) decreased the ALT and AST activity when compared with group 2 (acetaminophen-induced alone). There was a significant decrease ($p<0.05$) in the total protein and albumin concentration of the group 2 rats when compared to the treatment groups. Also treatment with extract of *Hibiscus sabdariffa* showed significant reduction ($p<0.05$) in the LDL concentration of group 3 and 4 when compared with group 2 rats. Conversely, significant increase ($p<0.05$) was observed in total cholesterol, HDL and triacylglycerol concentration of the group 2 rats when compared with group 1 rats. However, treatment with *Hibiscus sabdariffa* caused a significant ($p<0.05$) reduction in the total cholesterol, HDL and triacylglycerol concentrations of the rats in group 3 and 4 when compared to the group 2 rats. **Conclusion:** The present study revealed that the extract of *Hibiscus sabdariffa* could have the ability to protect the liver against paracetamol induced hepatocellular injury, reduce blood cholesterol levels and this could be due to its free radical scavenging property and the presence of natural antioxidants.

Key words: *Hibiscus sabdariffa*, acetaminophen, hepato-regenerative, cholesterol

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Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Liver diseases have become one of the major causes of morbidity and mortality all over the world. However, drug induced liver injury is one of the most common causative factors of liver disorders that posses a major clinical and regulatory challenge. The manifestations of drug-induced hepatotoxicity are highly variable, ranging from asymptomatic elevation of liver enzymes to fulminant hepatic failure[1]. Acetaminophen (paracetamol) is the most commonly used analgesic and antipyretic drug that is available over the counter in many countries. At the same time, acetaminophen overdose is the most common cause of acute liver failure and the leading cause of liver failure requiring liver transplantation in developed countries[2]. Acetaminophen overdose causes a multitude of interrelated biochemical reactions in liver cells producing multitude of outcomes. Among those are covalent modification and inhibition of enzyme activity, protein oxidation, lipid peroxidation, DNA fragmentation and deregulation of Ca^{2+} homeostasis, each contributing to acetaminophen-induced liver damage[3]. However these metabolic disorders caused by acetaminophen at an overdose makes it a suitable agent in researching about liver related disorders using animal models.

Inspite of tremendous advances in modern medicine, there are hardly any reliable drugs that protect the liver from damage and/or help in regeneration of hepatic cell[4]. However, many active plants extracts are frequently utilized to treat a wide variety of clinical diseases including liver disease[5]. In traditional medicine, *Hibiscus sabdariffa* has good features useful in several applications, such as antidotes to poisonous chemicals (acids, alkali and pesticides) and venomous mushrooms. Previous phytochemical investigations of this plant show the presence of phenolic compounds, anthocyanins, flavonols, protocatechuic acid (PCA), etc[6,7]. Some recent studies[8] have demonstrated the positive effect of *Hibiscus sabdariffa* in reducing blood pressure in experimental animals and humans. El-Saadany *et al.*[9] confirmed the hypo-cholesterolemic activity of the plant when they observed lowering effect in the different lipid fraction levels of hypercholesterolemic rat. In various studies, pre-treatment of *H. sabdariffa* extract to animals suffering from liver injury induced by tert-butyl hydroperoxide (t-BHP), lipopolysaccharide and azathioprine[10-12] was found to have blocked the elevated levels of liver marker enzymes (alanine aminotransferase, ALT and aspartate aminotranferase, AST) and improved the abnormality of liver histology. In addition, the extract also has the capability to protect liver from radiation[13] and counteract the over-dosage effect of acetaminophen (paracetamol, PCM)[14].

The continuous interests in this plant up to now have led to many scientific discoveries that exceed the traditional medicinal belief. However, the present study examined the hepato-regenerative potential of *H. sabdariffa* on paracetamol induced liver damage in Wistar Albino rats using the liver enzymes and lipid profile levels as biomarkers.

MATERIALS AND METHODS

Materials

Plant materials: Fresh leaves of *Hibiscus sabdariffa* were purchased from Ogige market, Nsukka, Enugu State of Nigeria and were identified by Mr. Alfred Ozioko of the herbarium Botany Department, University of Nigeria, Nsukka.

Animals: Adult male Wistar albino rats of 10-16 weeks and average weight of 160±15 g were obtained from the Animal House of the Faculty of Biological Sciences, University of Nigeria, Nsukka. The animals were acclimatized for a duration of 7 days under standard environmental conditions with a 12 h light/dark cycle maintained on a regular feed (vital feed) and water *ad libitum*.

Chemicals/reagents/samples: All chemicals used in this study were of the analytical grade and products of May and Baker, England; BDH, England and Merck, Darmstadt, Germany. Reagents used for all the assays were commercial kits and products of Randox, USA, QCA, Spain, Teco (TC), USA, Biosystem Reagents and Instruments, Spain.

Methods

Preparation of acetaminophen (paracetamol) sample: The stock concentration of acetaminophen was prepared by dissolving 600 mg of the standard drug in 2 mL of distilled water bringing the stock concentration to 60 mg mL^{-1}. The dose used was 750 mg kg^{-1} b.wt.[15].

Extraction of the active agents of *Hibiscus sabdariffa*: The leaves of *Hibiscus sabdariffa* were air-dried separately at room temperature, then into powdery form using electrical grinding machine. The ground samples extracted with aqueous solvent, using cold maceration techniques. The samples were filtered using Whatman filter paper. The filtrates (that is the active agents of the extract) concentrated to solid matter using rotary evaporators, which then become the stock sample of the aqueous leaf extract which were used for the analysis. These extracts were stored in the refrigerator compartment to prevent microbial growth.

Experimental design: Twenty male Albino Wistar rats were acclimatized at the same conditions of temperature and pressure and the same animal feeds were used for all the rats. The rats were divided into four groups of five rats each as shown below:

Group 1 = Normal/negative rats (control)
Group 2 = Positive control (acetaminophen treated rats)
Group 3 = Acetaminophen treated+400 mg kg^{-1} b.wt., of aqueous leaf extract *Hibiscus sabdariffa*
Group 4 = Acetaminophen treated+600 mg kg^{-1} b.wt., of aqueous leaf extract *Hibiscus sabdariffa*

After the experiment the animals where sacrificed and blood was collected and used for biochemical analysis.

Assay of aspartate aminotransferase activity (AST): A Randox Commercial Enzyme kit according to the method of Reitman and Frankel[16] was used in the study.

Assay of alanine aminotransferase activity (ALT): A Randox Commercial Enzyme Kit based on the methods of Reitman and Frankel[16] was used for the study.

Assay of alkaline phosphatase activity (ALP): This was done using the QCA commercial enzyme kit which is based on the phenolphthalein monophosphate method of Klein *et al.*[17].

Total cholesterol concentration (TCL): The total cholesterol concentration was determined according to the method of Allain *et al.*[18].

Low density lipoprotein-cholesterol concentration (LDL): The low density lipoprotein-cholesterol concentration was determined according to the method of Assmann *et al.*[19].

High density lipoproteins (HDL): The low density lipoprotein-cholesterol concentration was determined according to the method of Albers *et al.*[20].

Estimation of triacylglycerol concentration (TGL): The triacylglycerol concentration was determined according to the method of Trinder[21].

Determination of total protein concentration (TP): The determination of protein concentration was carried out according to the method of Slater[22].

Determination of serum albumin concentration (SA): The concentration of serum albumin was determined using the method of Doumas *et al.*[23].

Statistical analysis: The results were expressed as Mean±SD and test of statistical significance was carried out using one-way analysis of variance (ANOVA) where $p<0.05$ was considered statistically significant[24]. The statistical packaged used was the statistical package for social sciences (SPSS), version 20.

RESULTS AND DISCUSSION

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, rats and mice with toxic doses[25,26]. This study examined the role of *Hibiscus sabdariffa* aqueous extract on liver marker enzymes and lipid profile in acetaminophen-induced rats. Acetaminophen is metabolized in the liver by hepatic microsomal cytochrome P450 mixed function oxidase system to metabolites that are excreted by kidney[2]. According to Jaeschke *et al.*[27], at overdose acetaminophen induces a substantial mitochondrial oxidant stress and this oxidant stress which precedes cell injury after some hours of intake and free radical scavengers attenuate acetaminophen-induced liver injury. The modified mitochondrial proteins and high levels of cytosolic calcium can depress mitochondrial respiration and adenosine triphosphate (ATP) synthesis and induce mitochondrial oxidant stress[28]. Acetaminophen stimulates the division of liver cells and causes increases in serum ALP, AST and ALT enzymes which are accepted as indicators of hepatotoxicity[29].

In the present study, Table 1 and 2 shows that administration of acetaminophen at the dose of 750 mg kg^{-1} b.wt., intraperitoneal caused a significant ($p<0.05$) increase in the activity of AST, ALT and ALP when compared to the normal control. This may be due to the actions of the toxic metabolite N-acetyl-p-bezoquinone imine (NAPQI) which binds covalently to the liver cells leading to increase in lipid peroxidation and production of free radicals in turn increases the liver marker enzymes indicating hepatocyte damage and loss of pharmacological activity. More so the elevated levels of ALP can be as a result of intrahepatic and extra hepatic obstruction to bile flow. In the liver, alkaline phosphatase is found histo-chemically in the microvilli of bile canaliculi and in the sinusoidal surface[30].

Table 1: Effects of aqueous leaf extract of *Hibiscus sabdariffa* on aspartate transaminase (AST) and alanine transaminase (ALT) activity against acetaminophen-induced liver damage in Wistar Albino rats

Groups	AST (IU L^{-1})	ALT (IU L^{-1})
Negative control	46.0±4.45*	30.33±3.50*
Positive control	76.0±3.76	55.00±2.67
Paracetamol+400 mg kg^{-1} b.wt., of extract	47.3±4.74*	41.33±3.43*
Paracetamol+600 mg kg^{-1} b.wt., of extract	41.0±2.76*	30.00±4.60*

Values are expressed as Mean±SEM, n = 3 animals in each group, where p<0.05 is considered, *Significant when compared with positive control group using one-way analysis of variance

Table 2: Effects of aqueous leaf extract of *Hibiscus sabdariffa* on alkaline phosphatase (ALP) against acetaminophen-induced liver damage in Wistar Albino rats

Groups	ALP (IU L^{-1})
Negative control	72.66±2.72*
Positive control	96.66±4.65
Paracetamol+400 mg kg^{-1} b.wt., of extract	86.66±3.65*
Paracetamol+600 mg kg^{-1} b.wt., of extract	72.80±3.89*

Values are expressed as Mean±SEM, n = 3 animals in each group, where p<0.05 is considered, *Significant when compared with positive control group using one-way analysis of variance

Table 3: Effects of aqueous leaf extract of *Hibiscus sabdariffa* on HDL and LDL concentrations against acetaminophen-induced liver damage in Wistar Albino rats

Groups	HDL (mg dL^{-1})	LDL (mg dL^{-1})
Negative control	0.967±0.05*	2.00±0.15*
Positive control	0.567±0.06	2.30±0.13
Paracetamol+400 mg kg^{-1} b.wt., of extract	1.127±0.09*	1.63±0.12*
Paracetamol+600 mg kg^{-1} b.wt., of extract	1.460±0.07*	1.30±0.14*

Values are expressed as Mean±SEM, n = 3 animals in each group, where p<0.05 is considered, *Significant when compared with positive control group using one-way analysis of variance, HDL: High density lipoprotein, LDL: Low density lipoprotein

The administration of mid and high doses (400 and 600 mg kg^{-1} b.wt.) of aqueous extract of *Hibiscus sabdariffa* after intoxication with acetaminophen at an overdose as can be seen in Table 1 and 2 caused a significant (p<0.05) reduction in the AST ALT and ALP activities which indicates that *Hibiscus sabdariffa* leave extract at the dose of 400 mg kg^{-1} showed efficacy in ameliorating the liver cells. *Hibiscus sabdariffa* is known to contain a number of bioflavonoids and some bioactive phytochemicals such as arabinogalactans, rhamnogalacturans, riboflavin, β-carotene, phytosterols which are well known as potent free radical scavengers[31]. The results of this study is in line with the study of Adeyemi *et al.*[32] and Odigie *et al.*[33] where the anti-hepatotoxic activities of *Hibiscus sabdariffa* L. in animal model of streptozotocin diabetes-induced liver damage were determined and the calyxes extract of *Hibiscus sabdariffa* tend to reverse the change in lipid peroxidation activity, indicating decreased lipid peroxidation and damage to cells and tissues respectively.

Table 3 shows that administration of acetaminophen caused a significant increase (p<0.05) in the low density lipoprotein concentration and also caused a significant (p<0.05) reduction in the high density lipoprotein concentration of the group 2 rats when compared to the negative control (group 1). However, treatment with the extract caused a significant (p<0.05) reduction in the low density lipoprotein and a significant (p<0.05) increase in the high density lipoprotein concentration of the group 3 and 4 animals when compared to the positive control group 2. Inferences from the results of this study revealed that, to a large extent that the aqueous extract of *Hibiscus sabdariffa* possess the ability to stimulate the immune system *in vivo*. If more LDL is present in the blood than can be quickly taken up by cells, the LDL and its cholesteryl esters accumulate in the walls of large blood vessels and produce chemical signals that attract white blood cells. The white blood cells produce inflammation, leading to formation of a plaque. This plaque can become coated in calcium, resulting in the hardening of the arteries associated with atherosclerosis. Eventually, the blood vessel can become constricted and can trigger formation of a blood clot. If the blood vessels supplies the heart with blood, the result is a heart attack. If the blood vessel supplies the brain with blood, the result is a stroke[34]. Since HDL helps clear excess cholesterol from the body in a process of inverse cholesterol transport, results of the study which shows increase in HDL concentration helps the body in its function of excretion. Also, the extract, as it is taken in humans as a beverage may be of benefit in enhancing immunity. Further studies need to be done to evaluate the exact mechanism of action. This result is consistent with the findings of Sundari *et al.*[35] who observed that there is a significant decrease (p<0.05) in LDL concentration and a significant

Table 4: Effects of aqueous leaf extract of *Hibiscus sabdariffa* on total cholesterol and triacylglycerol concentrations against acetaminophen-induced liver damage in Wistar Albino rats

Groups	Total cholesterol (mg dL^{-1})	Triacylglycerol (mg dL^{-1})
Negative control	3.2±0.15*	1.23±0.20*
Positive control	6.5±0.35	3.50±0.16
Paracetamol+400 mg kg^{-1} b.wt., of extract	3.6±0.14*	1.72±0.14*
Paracetamol+600 mg kg^{-1} b.wt., of extract	3.7±0.25*	1.70±0.35*

Values are expressed as Mean±SEM, n = 3 animals in each group, where $p<0.05$ is considered *Significant when compared with paracetamol control group using one-way analysis of variance

Table 5: Effects of aqueous leaf extract of *Hibiscus sabdariffa* on total protein and albumin concentrations against acetaminophen-induced liver damage in Wistar Albino rats

Groups	Total protein (gm dL^{-1})	Albumin concenttration (gm dL^{-1})
Negative control	6.70±0.32*	4.80±0.26*
Positive control	4.26±0.58	2.56±0.20
Paracetamol+400 mg kg^{-1} b.wt., of extract	8.16±0.33*	4.60±0.26*
Paracetamol+600 mg kg^{-1} b.wt., of extract	7.96±0.58*	4.90±0.15*

Values are expressed as Mean±SEM, n = 3 animals in each group, where $p<0.05$ is considered *Significant when compared with paracetamol control grp using one-way analysis of variance

increase ($p<0.05$) in HDL concentrations of rats treated with aqueous extract of *Hibiscus sabdariffa* after exposure to acetaminophen.

Table 4 shows a significant ($p<0.05$) increase in the levels of total cholesterol and triacylglycerol concentration of the positive control group when compared to the negative control group. The administration of different doses (400 and 600 mg kg^{-1} b.wt.) of aqueous extract of *Hibiscus sabdariffa* leaves after exposure to acetaminophen significantly reduced ($p<0.05$) the concentrations of total cholesterol and triacylglycerol when compared to the untreated group (group 2). This shows that the extract has the capacity to reduce cholesterol levels by improving the synthesis of HDL from the liver. Improving the synthesis of HDL from the liver could be as a result of regeneration of liver cells or mopping up of free radicals from the system. The results of this study is in line with the study of Usoh *et al.*[36] where the antioxidant and hepatoprotective effects of dried flower extracts of *Hibiscus sabdariffa* L., on rats treated with carbon tetrachloride.

Table 5 shows that administration of high dose of acetaminophen caused a significant ($p<0.05$) decrease in the total protein and albumin concentrations of the group 2 (untreated) animals when compared to the normal control. However treatment with the aqueous extract of *Hibiscus sabdariffa* at the dose of 400 and 600 mg kg^{-1} b.wt., caused a significant ($p<0.05$) increase in the total protein and albumin concentrations of the group 3 and 4 animals when compared to the group 2 rats. These results suggest that the extract through the activity of its antioxidants has the ability to inhibit the breakdown of membrane proteins. This breakdown is caused by the free radicals generated by the toxic NAPQI as a result of acetaminophen overdose. The results of this study is in correlation with the study of Rajkapoor *et al.*[37] where the protective effect of *Phyllanthus polyphyllus* on acetaminophen induced Hepatotoxicity in rats were determined.

CONCLUSION

From the results of this study, it is deduced that the aqueous extract of *Hibiscus sabdariffa* possesses hepato-regenerative properties which is shown in the ability of the extract to improve the functionality of the liver cells, inhibit the actions of free radicals and reduce cholesterol levels significantly in the treatment groups. These results have revealed the ethno-medicinal claim of the use of the plant in the management of cellular damage and liver toxicity.

SIGNIFICANCE STATEMENTS

This study proved the local use of *Hibiscus sabdariffa* as a medicinal plant. However the present research could be a pointer to the discovery of new hepato-regenerative drugs and anti-cholesterol drugs.

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REFERENCES

1. Reid, A.B., R.C. Kurten, S.S. McCullough, R.W. Brock and J.A. Hinson, 2005. Mechanisms of acetaminophen-induced hepatotoxicity: Role of oxidative stress and mitochondrial permeability transition in freshly isolated mouse hepatocytes. J. Pharmacol. Exp. Therapeut., 312: 509-516.

2. Chun, L.J., M.J. Tong, R.W. Busuttil and J.R. Hiatt, 2009. Acetaminophen hepatotoxicity and acute liver failure. J. Clin. Gastroenterol., 43: 342-349.
3. Jaeschke, H. and M.L. Bajt, 2006. Intracellular signaling mechanisms of acetaminophen-induced liver cell death. Toxicol. Sci., 89: 31-41.
4. Parmar, S.R., H.V. Patel and K. Kiran, 2010. Hepatoprotective activity of some plants extract against paracetamol induced hepatotoxicity in rats. J. Herb. Med. Toxicol., 4: 101-106.
5. Ukegbu, C.Y., O. Arome, E. Affiong, A. Ogechukwu and C. Ike, 2016. Anti-diabetic effect of the methanolic leaf extract of *Axonopus compressus (P. Beauv)* in alloxan induced diabetic rats. Int. J. Biochem. Res. Rev., 12: 1-5.
6. Seca, A.M.L., A.M.S. Silva, A.J.D. Silvestre, J.A.S. Cavaleiro and F.M.J. Domingues *et al*., 2001. Phenolic constituents from the core of kenaf (*Hibiscus cannabinus*). Phytochemistry, 56: 759-767.
7. Fakeye, T.O., A. Pal, D.U. Bawankule and S.P.S. Khanuja, 2008. Immunomodulatory effect of extracts of *Hibiscus sabdariffa* L. (Family Malvaceae) in a mouse model. Phytother. Res., 22: 664-668.
8. Herrera-Arellano, A., S. Flores-Romero, M.A. Chavez-Soto and J. Tortorie-Ilo, 2004. Effectiveness and tolerability of a standardized extract from *Hibiscus sabdariffa* in patients with mild to moderate hypertension: A controlled and randomized clinical trial. Phytomedicine, 5: 375-382.
9. El-Saadany, S.S., M.Z. Sitohy, S.M. Labib and R.A. El-Massry, 1991. Biochemical dynamics and hypocholesterolemic action of *Hibiscus sabdariffa* (Karkade). Mol. Nutr. Food Res., 35: 567-576.
10. Liu, C.L., J.M. Wang, C.Y. Chu, M.T. Cheng and T.H. Tseng, 2002. *In vivo* protective effect of protocatechuic acid on *tert*-butyl hydroperoxide-induced rat hepatotoxicity. Food Chem. Toxicol., 40: 635-641.
11. Lin, W.L., Y.J. Hsieh, F.P. Chou, C.J. Wang, M.T. Cheng and T.H. Tseng, 2003. Hibiscus protocatechuic acid inhibits lipopolysaccharide-induced rat hepatic damage. Arch. Toxicol., 77: 42-47.
12. Amin, A. and A.A. Hamza, 2005. Hepatoprotective effects of *Hibiscus*, *Rosmarinus and salvia* on azathioprine-induced toxicity in rats Life Sci., 77: 266-278.
13. Adaramoye, O., B. Ogungbenro, O. Anyaegbu and M. Fafunso, 2008. Protective effects of extracts of *Vernonia amygdalina*, Hibiscus sabdariffa and vitamin C against radiation-induced liver damage in rats. J. Radiat. Res., 49: 123-131.
14. Olaleye, M.T. and B.T.J. Rocha, 2008. Acetaminophen-induced liver damage in mice: Effects of some medicinal plants on the oxidative defense system. Exp. Toxicol. Pathol., 59: 319-327.
15. Araya, H., T. Horie, M. Hayashi and S. Awazu, 1987. An alteration in the liver microsomal membrane of the rat following paracetamol overdose. J. Pharm. Pharmacol., 39: 1047-1049.
16. Reitman, S. and S. Frankel, 1957. A colorimetric method for the determination of serum glutamic oxalacetic and glutamic pyruvic transaminases. Am. J. Clin. Pathol., 28: 56-63.
17. Klein, B., P.A. Read and L.A. Babson, 1960. Rapid method for the quantitative determination of serum alkaline phosphatase. Clin. Chem., 6: 269-275.
18. Allain, C.C., L.S. Poon, C.S.G. Chan, W. Richmond and P.C. Fu, 1974. Enzymatic determination of total serum cholesterol. Clin. Chem., 20: 470-475.
19. Assmann, G., H.U. Jabs, U. Kohnert, W. Nolte and H. Schriewer, 1984. LDL-cholesterol determination in blood serum following precipitation of LDL with polyvinylsulfate. Clin. Chim. Acta, 140: 77-83.
20. Albers, J.J., G.R. Warmick and M.C. Cheng, 1978. Quantitation of high density lipoproteins. Lipids, 13: 926-932.
21. Trinder, P., 1969. Determination of blood glucose using an oxidase-peroxidase system with a non-carcinogenic chromogen. J. Clin. Pathol., 22: 158-161.
22. Slater, R.J., 1986. Experiments in Molecular Biology. Humana Press, Clifton, NJ., USA., ISBN: 978-1-60327-405-0, pp: 269.
23. Doumas, B.T., W.A. Watson and H.G. Biggs, 1971. Albumin standards and the measurement of serum albumin with bromcresol green. Clin. Chim. Acta, 31: 87-96.
24. Sokal, R.R. and F.J. Rohlf, 1995. Biometry: The Principles and Practice of Statistics in Biological Research. 3rd Edn., W.H. Freeman and Co., New York, USA., ISBN: 0-7167-2411-1, Pages: 887.
25. Roberts, J.L. and J.D. Morrow, 2001. Analgesic-Antipyretic and Anti-Inflammatory Agents and Drugs Employed in the Treatment of Gout. In: Goodman and Gilman's, the Pharmacological Basis of Therapeutics, , Hardman, J.G., L.E. Limbird, A.G. Gilman, (Eds.). McGraw Hill Co., New York, pp: 687-731.
26. Abraham, P., 2005. Vitamin C may be beneficial in the prevention of paracetamol-induced renal damage. Clin. Exp. Nephrol., 9: 24-30.
27. Jaeschke, H., T.R. Knight and M.L. Bajt, 2003. The role of oxidant stress and reactive nitrogen species in acetaminophen hepatotoxicity. Toxical. Lett., 144: 279-288.
28. Chun, L.J., M.J. Tong, R.W. Busuttil and J.R. Hiatt, 2009. Acetaminophen hepatotoxicity and acute liver failure. J. Clin. Gastroenterol., 43: 342-349.
29. Ali, B.H., H.M. Mousa and S. El-Mougy, 2003. The effect of a water extract and anthocyanins of *Hibiscus sabdariffa* L. on paracetamol-induced hepatoxicity in rats. Phytother. Res., 17: 56-59.
30. Rosalki, S.B. and N. McIntyre, 1999. Biochemical Investigations in the Management of Liver Disease. In: Oxford Textbook of Clinical Hepatology, Bircher, J., J.P. Benhamou, N. McIntyre, M. Rizzetto and J. Rodes (Eds.). 2nd Edn., Oxford University Press, New York, USA., ISBN-13: 978-0192625151, pp: 503-521.

31. Mahadevan, N., Shivali and P. Kamboj, 2009. *Hibiscus sabdariffa* Linn.-An overview. Nat. Prod. Radiance, 8: 77-83.
32. Adeyemi, D.O., V.O. Ukwenya, E.M. Obuotor and S.O. Adewole, 2014. Anti-hepatotoxic activities of *Hibiscus sabdariffa* L. in animal model of streptozotocin diabetes-induced liver damage. BMC Complement. Altern. Med., Vol. 14. 10.1186/1472-6882-14-277.
33. Odigie, I.P., R.R. Ettarh and S.A. Adigun, 2003. Chronic administration of aqueous extract of *Hibiscus sabdariffa* attenuates hypertension and reverses cardiac hypertrophy in 2K-1C hypertensive rats. J. Ethnopharmacol., 86: 181-185.
34. Christie, W., 2013. Plasma lipoproteins: Composition, structure and biochemistry. AOCs Lipid Library, 6: 55-80.
35. Sundari, K., G. Govindaraju and B. Bharathi, 2011. Hepatoprotective effect of ethanolic extracts of *Sphaeranthus indicus* (Linn) on paracetamol-induced liver toxicity in rats. Int. J. Applied Biol. Pharm. Technol., 2: 315-321.
36. Usoh, I.F., I.S. Ekaidem, O.E. Etim, H.D. Akpan, E.J. Akpan and A. Fakoya, 2012. Antioxidant and hepatoprotective effects of dried flower extracts of *Hibiscus sabdariffa* L. on rats treated with carbon tetrachloride. J. Applied Pharm. Sci., 2: 186-189.
37. Rajkapoor, B., Y. Venugopal, J. Anbu, N. Harikrishnan, M. Gobinath and V. Ravichandran, 2008. Protective effect of *Phyllanthus polyphyllus* on acetaminophen induced hepatotoxicity in rats. Pak. J. Pharm. Sci., 21: 57-62.