Aqueous Extract of *Hibiscus sabdariffa* calyx Showed Antioxidative Effects And Ameliorate Acetaminophen (Paracetamol)- Induced Hepatotoxicity And Nephrotoxicity in Rats.

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Abstract: Drug-induced hepatotoxicity and nephrotoxicity accounts for frequent hospital acquired acute liver and kidney injuries. This study investigated the possible anti oxidative and ameliorative potentials of aqueous extract of *Hibiscus sabdariffa calyx in* Acetaminophen (Paracetamol)-induced toxicity in rats. Forty (40) Wistar rats weighing between 150-200g were randomly selected into 8 groups, first group served as negative control, second, third and fourth groups received 200mg/kg, 400mg/kg and 600mg/kg body weight extract respectively, fifth group received 2000mg/body weight of Acetaminophen only while the sixth, seventh and eighth groups received 200mg/kg, 400mg/kg, 600mg/kg body weight of extract respectively following induction of toxicity with 2000mg/kg body weight of Acetaminophen. Malondialdehyde (MDA), reduced glutathione (GSH) concentrations as well as superoxide dismutase (SOD) activities were measured in liver and kidney homogenates while total protein (TP), albumin (ALB), total bilirubin (TB), conjugated bilirubin (C.BIL),urea and creatinine concentrations as well as Alanine aminotransferase (ALT), Aspartate aminotransferase (AST) and Alkaline phosphatase (ALP) activities were measured in plasma spectrophotometrically using standardized methods after administration of extract for 12 days. Results showed, that rats orally pretreated with extracts at 200,400 and 600mg/kg body weight elicits significant ($p<0.05$) decreases in TB, C.BIL, urea, creatinine, MDA concentrations, AST,ALT and ALP activities as well as significant increases in SOD activity, GSH, albumin and total protein concentrations in a concentration-dependent manner. Intoxicated rats however showed significant decreases ($p<0.05$) in GSH, TP, albumin concentrations and SOD activities with elevated levels of MDA, TB, C.BIL, urea, creatinine and AST, ALT and ALP activities. Interestingly, animals treated with the extract after intoxication with Acetaminophen showed significant increases ($p<0.05$) in concentrations of TP, ALB, GSH and SOD activities with corresponding decrease in TB, C.BIL, urea, creatinine and MDA concentrations as well as AST, ALT and ALP activities compared with Acetaminophen treated controls in a dose dependent manner. Conclusively, properties exhibited by extract are suggestive of its antioxidative potentials which accounts for its ameliorative effects on acetaminophen induced hepatotoxicity and nephrotoxicity.
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1. Introduction

About 60 percent of hospital –acquired acute liver and kidney injury can be accounted for by drug-induced toxicities, which is a main cause of mortality and morbidity. Several options such as dose adjustment based on tissue functions, hydration and avoidance of these agents, have been proposed to prevent or ameliorate drug –induced toxicity. Nevertheless, drug –induced toxicity remains a major problem for health care professionals as several studies have shown that analgesics toxicities is caused by increased Reactive Oxygen Species (ROS) and other oxidants in tissues and several cellular components.

Liver is one of the largest organs in human body. It is a vital organ that has a wide range of functions in the body, including biotransformation and detoxification of endogenous and exogenous harmful substances, plasma protein synthesis, and glycogen storage (Cemek *et al*., 2010). Liver injury is a common clinical problem. Some cases of liver injuries and failures result from adverse effect of high doses of

drugs, excessive consumption of alcohol, infection and autoimmune disorders (Racknagel, 2003). Metabolism of chemicals takes place largely in the liver and this accounts for the organ's susceptibility to metabolism –dependent, drug –induced injury (Kaplowitz *et al*., 1999). Liver may be damaged by free radicals through mechanisms of covalent binding and lipid peroxidation with subsequent tissue injury and elevated levels of its marker enzymes (Nagalekshmi et al., 2011).

The use of nephrotoxic drugs has been implicated as a causative factor in up to 25 percent of all cases of acute renal failure in critically ill patients (Pannu and Nadim, 2008), this is probably because the kidney is supplied with a large volume of blood accounting for 20 percent of total cardiac output, hence the kidney is likely to be affected by secondary effects of drugs and their metabolites that are accumulated through the urine concentration mechanism (Marieb, 2006). Also the kidney performs several functions which includes excretion of waste products from blood, reabsorption of vital nutrients, acid-base homeostasis, osmolality regulation, blood pressure regulation and secretion of hormones (Sembulingam, 2003). Hence, with the impairment of the nephron, filtration and reabsorption no longer take place in the kidney, which gives rise to accumulation of metabolic waste in the plasma which is observed by high level of urea and creatinine in plasma (Guo et al., 2002). Irrespective of their clinical use nephrotoxicity is significantly induced by various drugs and are important cause of renal failure (Agarwal, 2004; Poormoosavi et al, 2010).

Some studies have shown that tissue toxicity is mediated by oxidative stress (Chitturi *et al*., 2001; Mehta *et al*., 2002). Oxidative stress can be described as excess formation and/or insufficient removal of highly reactive molecules such as reactive oxygen species and reactive nitrogen species (Maritim *et al*., 2003).

In the past two decades, it has become increasingly clear that oxidative stress plays a major role in the pathogenesis of a number of human diseases such as renal failure, liver failures, ischemia, neurodegenerative diseases, hypertension, cancer and diabetes mellitus (Halliwell et al., 1992; Mates *et al*., 1999). Also, oxidative stress have been linked with the activities of many drugs as part of the mechanism associated with their pharmacological actions and this have been shown to constitute a major mechanism in the pathogenesis of Acetaminophen - induced liver and renal damage in experimental animals as toxic overdose of Acetaminophen were reported to have life –threatening impact on the kidney and the liver of both human and experimental animals, hence early protection from Acetaminophen induced toxicity has life saving importance (Ghosh et al., 2010; Demirbag et al., 2010).

Acetaminophen also called Paracetamol (PCM) is an effective, cheap, safe and widely available analgesic and anti-pyretic drug when used at its recommended dose (up to 4 g/day) in adults (Rumack, 2004). However, an acute or cumulative overdose can cause severe liver injury with the potential to progress to liver failure (Lee, 2004). Damage to the liver does not result from Acetaminophen itself, but one of the metabolites *N*-acetyl-*p*-benzo guinoneimine (NAPQI). NAPQI depletes the natural antioxidant and directly damage cells of the liver, leading to liver failure (Mitchell, 1973). NAPQI is generally stabilized through conjugation with glutathione (GSH) and eliminated via the kidney, however with over dose of Acetaminophen, the production of NAPQI exceed the capacity of GSH to detoxify it with excess NAPQI leading to renal damage associated with oxidative stress (Ozkaya et al., 2010).

Therefore, supplementation with anti oxidants is very crucial to delay, prevent or remove oxidative damage associated with drug metabolism as there are numerous reports indicating that Acetaminophen-mediated oxidative stress or tissue toxicity is attenuated by the use of naturally occurring antioxidants and or free radical scavengers such as vitamins, medicinal plants with rich bioactive contents that have potential beneficial effects on human health (Janbaz *et al*., 2004; El-Ridi and Rahmy, 2005; Ajith *et al*., 2007). However, almost all organisms possess antioxidant defense repair systems, which quench or minimizes the production of oxygen-derived species, these protective systems are insufficient to entirely prevent the damages (Simic, 1998) caused by endogenous or exogenous oxidants (Sun, 1990). The use of medicinal plants in the treatment of diseases has therefore generated renewed interest in recent times, as herbal preparations are increasingly being used in both human and animal health care system (Eisenberg *et al*., 1998).

World Health Organization (WHO) reported that 80 percent of the emerging world's population depend on medicinal plants as their main source of therapeutics (WHO, 2008).

Hibiscus sabdariffa is a species of hibiscus native to West Africa (Chau *et al*., 2000). It is an annual or perennial herb, growing to 2-2.5m (7-8ft) tall. It belongs to the family Malveveae (Morton, 1987; Hirunpanich *et al*., 2006; Alarcon-Aguilar *et al*., 2007).

The beverages made from the calyx are known as Zobo in Yoruba, Zoborodo in Hausa, and Karkade in Egypt with several medicinal uses such as antihypertensive, anti-cancer, anti-stress, hypo

lipidemic, anti plasmodic, diuretic and anti diarrheal activities (Joshi *et al*., 2006).

This study therefore investigated the anti oxidative and possible ameliorative potentials of aqueous extract of *Hibiscus sabdariffa* calyx on acetaminophen- induced hepatotoxicity and nephrotoxicity in rats.

2. Material and Methods

2.1 Materials

Some of the materials used in this study include: Wistar rats, Acetaminophen injection, Whatmann filter paper, cages and rat feeds, cotton wool, Hand gloves and Universal bottles, Needles and syringes, Electric weighing balance and scissors, Lithium heparin bottles, Oral cannula, blender, measuring cylinders, beakers, test tubes, automatic micropipettes, pipettes, spatula, incubator, centrifuge, funnel, petri dishes, refrigerator, water bath, spectrophotometer, refrigerator, washing brushes, detergent, permanent marker, feeding trough, aqueous extract of *Hibiscus sabdariffa*, surgical blades, mortar and pestle, cotton wool and commercial rat feeds.

2.2 Reagents

Acetaminophen injection with NAFDAC registration number: 04-041, Laboratory assay kits for total protein, aspartate aminotransferase (AST), alanine aminotransferase (ALT), alkaline phosphate (ALP), Washing buffer, Homogenising buffer, Ellman's reagent, bromocresol green, biuret reagent, normal saline, thiobarbituric acid, Sulphanilic acid, Sodium nitrite, Caffeine benzoate reagent, Alkaline tartrate reagent, Trichloroacetic Acid (TCA), Tris Kcl Buffer (P^H 7.4), carbonate buffer (pH 10.2), Adrenalin, GSH working standard, Phosphate buffer (P^H 7.4),all were of quality analytical grade obtained from Sigma.USA.

2.3 Plant Materials and its aqueous extract preparation.

The fresh flowers of *Hibiscus sabdariffa* were purchased at Igbona Market, in Oshogbo and authenticated at the Botany Department of the Obafemi Awolowo University Ile-ife with herbarium number 16750 deposited. The sample was dried at room temperature while the extraction of the red petals of *H. sabdariffa* was performed by the method described by (Obi *et al.*, 2005). Hundred grams (100g) of dried calyx was added to 500 ml of distilled water and boiled for 15 minutes. The boiled materials were then filtered after cooling. The aqueous filtrate was then evaporated to dryness and stored in a sealed bottle at 4^0 C until required for preparations of various concentrations of aqueous extract used for this study.

2.4 Experimental Animals and Experimental Design

Forty male Wistar rats (weighing 150-200g) obtained from the Department of Biomedical Sciences, Faculty of Basic Medical Sciences, Ladoke Akintola University of Technology (LAUTECH), Ogbomoso were used for the experiment. They were maintained and housed in cages in the Departmental animal house, fed on standard pellet and provided with water ad-libitum. The handling of the animals were based on the international rules and ethics based on handling of experimental animals as obtained in my institution. The animals were maintained under standard laboratory conditions (temperature 24-28^o C, relative humidity 60-70% and 1:1 dark and light cycle). They were randomly selected into eight groups with five animals in each group. The dried *Hibiscus sabdariffa* extract (HSE) was dissolved in distilled water and administered orally at different concentrations as shown in various groups while Acetaminophen (2000mg/kg) was administered intra peritoneally.

Group A: (Negative control): normal diet only

Group B: Animals were treated with only 200mg/kg body weight of HSE for 12 days

Group C: Animals were treated with 400mg/kg body weight of HSE for 12 days

Group D: Animals were treated with 600mg/kg body weight of HSE for 12 days

Group E (Positive control): Animals were intoxicated with 2000mg/kg body weight of Acetaminophen

Group F: Animals were treated for 12 days with 200mg/kg body weight of HSE after intoxication with 2000mg/kg body weight of Acetaminophen.

Group G: Animals were treated for 12 days with 400mg/kg body weight of HSE after intoxication with 2000mg/kg body weight of Acetaminophen.

Group H: Animals were treated for 12 days with 600mg/kg body weight of HSE after intoxication with 2000mg/kg body weight of Acetaminophen.

2.5 Animal sacrifice, sample collection and preparation of tissue homogenates

The experimental animals were sacrificed 12 hours after the last treatment by cervical dislocation. The animals were carefully cut open and blood drained from the heart using a syringe and needle (heart puncturing), collected into heparin bottle. The liver and kidney were then excised, weighed and washed in a beaker containing 5mls of washing buffer. The washed tissues were then homogenized in 4ml homogenizing buffers separately. The homogenates were centrifuged at 10,000g for 20 minutes to collect the supernatants used for various biochemical assays.

2.6 Biochemical analysis

The activities of plasma ALT and AST were estimated using the method described by Bergmeyer *et al.*, (1986) while determination of plasma activity of

Alkaline phosphatase (ALP) was estimated as described by Rec, (1972). Estimation of plasma bilirubin was done using Jendrassik and Grof method as described by Garber, (1981). Biuret method was used for the estimation of plasma total protein as described by Kingsley and Frankel, (1939) while plasma albumin estimation was done using bromocresol green binding method as described by Doumas *et al*., (1971). Plasma Urea concentrations were determined according to the method of Berthelot-Searcy as described by Henry, (1991) while creatinine was determined by the method described by Bartels and Bohmer, (1972).

2.7 Antioxidant parameters and marker of lipid peroxidation

Malondialdehyde (MDA) was estimated spectrophotometrically by thiobabitturic acid-reacting substances (TBARS) as described by the procedure of Varshney and Kale, (1990). Determination of GSH concentration was done using the method described by Beutler *et al.,* (1963) while SOD activity was determined by the method of Misra and Fridovich, (1972).

2.8 Statistical Analysis

The results were reported as means ± SD from five repeated determinations and evaluated with the analysis of variance followed by turkey post Hoc test to compare test groups with control groups. Differences were considered to be statistically significant at $P<0.05$.

3. Results

Table 1: Plasma total protein (TP) and albumin(ALB) concentrations in various treatment groups.

Treatment Groups	Total Protein ± SD (g/l)	Albumin ±SD (g/l)
Normal feed only	81.97±3.10	36.55±3.54
200mg/kg of the extract only	82.50±4.13	38.55±3.53
400mg/kg of the extract only	84.63±3.79	41.27±1.80
600mg/kg of the extract only	92.17±2.07	47.87±2.01
Acetaminophen only	40.33±7.11	14.17±1.32
Acetaminophen +200mg/kg of the extract	43.75±3.68	17.75±2.03
Acetaminophen +400mg/kg of the extract	46.10±3.89	*24.98±1.93
Acetaminophen +600mg/kg of the extract	*52.61±5.28	*32.49±2.93

Values were given as mean ± Standard deviation of five determinations with p value <0.05 considered statistically significant.
*P-value <0.05 Considered significant when compared with Acetaminophen control group.

Table 2: Plasma total bilirubin (T.BIL) and conjugated bilirubin (C.BIL) concentrations in various treatment group.

Treatment Groups	T.BIL(µmol/L)	C.BIL(µmol/L) ±SD
Normal feed only	10.69±2.76	3.55±1.06
200mg/kg of the extract only	11.09±3.74	3.29±1.55
400mg/kg of the extract only	11.99±2.98	4.63±1.63
600mg/kg of the extract only	11.74±1.89	5.85±0.85
Acetaminophen only	30.13±216	11.95±2.86
Acetaminophen +200mg/kg of the extract	24.54±2.81	10.33±2.69
Acetaminophen +400mg/kg of the extract	*23.09±3.09	*8.07±1.72
Acetaminophen +600mg/kg of the extract	*13.82±2.85	*4.53±1.70

Values were given as mean ± Standard deviation of five determinations with p value <0.05 considered statistically significant.
*P-value <0.05 Considered significant when compared with Acetaminophen control group.

Table 3: Plasma Aspartate aminotransferase, Alanine aminotransferase and Alkaline phosphatase activities in various treatment groups.

Treatment Groups	AST±SD (U/L)	ALT±SD (U/L)	ALP±SD (U/L)
Normal feed only	22.57±3.10	21.15±2.24	53.91±4.01
200mg/kg of the extract only	23.35±2.67	21.52±2.74	53.21±3.48
400mg/kg of the extract only	25.39±2.30	21.27±1.98	53.02±2.78
600mg/kg of the extract only	28.40±5.55	21.37±1.16	52.15±2.02
Acetaminophen only	95.86±8.40	67.99±2.11	86.38±3.87
Acetaminophen +200mg/kg of the extract	95.17±2.76	66.89±10.58	85.14±3.24
Acetaminophen +400mg/kg of the extract	91.46±3.76	59.01±13.64	82.71±4.45
Acetaminophen +600mg/kg of the extract	*82.62±4.87	*51.44±6.33	*74.33±211

Values were given as mean ± Standard deviation of five determinations with p value <0.05 considered statistically significant.
*P-value <0.05 Considered significant when compared with Acetaminophen control group.

Table 4: Malondialdehyde (MDA), Reduced glutathione (GSH) concentrations and Superoxide dismutase activities (SOD) in the liver homogenates of different treatment group.

Treatment Groups	MDA± SD(µg/mg)	SOD±SD (U/mg)	GSH ±SD(µmol/mg)
Normal feed only	33.12±2.09	25.81±2.11	6.86±1.11
200mg/kg of the extract only	31.54±1.54	27.23±1.33	7.17±1.32
400mg/kg of the extract only	30.29±2.46	30.31±2.53	8.08±3.37
600mg/kg of the extract only	21.90±0.79	34.24±5.22	10.97±1.82
Acetaminophen only	57.99±4.77	7.56±1.74	1.10±0.53
Acetaminophen +200mg/kg of the extract	57.50±1.24	10.55±1.80	1.88±0.71
Acetaminophen +400mg/kg of the extract	*44.41±4.36	*15.23±1.93	2.51±0.53
Acetaminophen +600mg/kg of the extract	*40.14±2.26	*15.15±3.67	*6.61±0.56

Values were given as mean ± Standard deviation of five determinations with p value <0.05 considered statistically significant.
*P-value <0.05 Considered significant when compared with Acetaminophen control group.

Table 5: Plasma total protein,Urea and Creatinine concentrations in various treatment groups

Treatment Groups	Plasma Protein concentration (g/l)	Plasma Urea Concentration (mmol/l)	Plasma Creatinine Concentration (µmol/L)
Normal feed only	81.97±3.10	6.63±0.13	130.57±2.12
200mg/kg of the extract only	82.50±4.12	6.37±0.16	127.06±2.19
400mg/kg of the extract only	84.38±2.41	6.11±0.74	124.18±2.17
600mg/kg of the extract only	92.17±2.07	5.88±0.36	122.48±2.17
Acetaminophen only	40.33±7.11	16.29±1.42	233.37±31.31
Acetaminophen +200mg/kg of the extract	43.75±3.68	9.53±0.71	180.15±4.21
Acetaminophen +400mg/kg of the extract	*46.10±3.89	*8.75±0.21	170.70±1.75
Acetaminophen +600mg/kg of the extract	*52.61±5.28	*7.62±0.47	*150.85±1.72

Values were given as mean ± Standard deviation of five determinations with p value <0.05 considered statistically significant.
*P-value <0.05 Considered significant when compared with Acetaminophen control group.

Table 6: Superoxide dismutase activity (SOD), Reduced glutathione (GSH) and Malondialdehyde (MDA) Concentrations in the kidney homogenate of various treatment groups.

Treatment Groups	SOD Activity (U/mgProtein)±SD	GSH Concentration (µmol/mg protein)±SD	MDA Concentration (µg/mg protein)±SD
Normal feed only	5.39±0.040	5.78±0.44	8.88±1.28
200mg/kg of the extract only	5.84±0.28	6.78±0.76	8.39±0.89
400mg/kg of the extract only	6.15±0.43	7.50±0.53	8.57±0.92
600mg/kg of the extract only	6.94±1.00	8.23±0.53	8.06±0.53
Acetaminophen only	3.06±0.41	3.19±0.16	20.14±1.96
Acetaminophen +200mg/kg of the extract	4.84±0.59	4.26±0.42	16.30±0.90
Acetaminophen +400mg/kg of the extract	4.98±0.49	*5.03±0.50	14.06±1.14
Acetaminophen +600mg/kg of the extract	*5.29±0.42	*5.33±0.67	*11.02±1.14

Values were given as mean ± Standard deviation of five determinations with p value <0.05 considered statistically significant.
*P-value <0.05 Considered significant when compared with Acetaminophen control group.

4. Discussions

Health is the subject of priority as far as life is concerned, but despite effort to maintain good health, humans are continuously exposed to different kinds of chemicals such as food additives, industrial chemicals, pesticides and other undesirable contaminants in the air, food and soil (Stavric, 1994). Most of these chemicals induce a free radical-mediated lipid peroxidation leading to disruption of biomembranes and dysfunction of cells and tissues (Cho *et al*., 2003). Acetaminophen (PCM) is a well-known model compound producing tissue injury (Daly *et al*., 2008). It gets converted into reactive toxic electrophilic metabolite known as *N*-acetyl-*p*-benzo quinoneimine (NAPQI) by hepatic microsomal cytochrome P_{450} enzyme system. NAPQI causes peroxidative degradation in the adipose tissue resulting in fatty infiltration of hepatocytes (Mayuren *et al*., 2010).

In an attempt to protect the cell membrane, living organisms have different mechanisms that speed up termination of lipid peroxidation by scavenging free radicals. One of such mechanisms is antioxidant defense system (Parma *et al.*, 2010).

In this study the possible antioxidant and ameliorative potentials of aqueous extract of *Hibiscus sabdariffa* calyx against Acetaminophen- induced tissue toxicity or oxidative damage in rats were investigated. The results of the study showed that the liver function indices such as the plasma levels of AST, ALT, ALP, T.BIL and C.BIL in groups B, C & D (groups administered with 200mg/kg, 400mg/kg and 600mg/kg of the extract only respectively) were similar to the negative control (group with feed only) while plasma levels of total protein, albumin as well as the tissue GSH concentrations and SOD activities were significantly ($p<0.05$) increased in a dose dependent manner (Table 1,2,3 and 4). The significant increase ($p< 0.05$) in the values of total protein and albumin is likely due to the availability of diverse number of amino acids reported in the extract (Morton, 1987) which is used in the synthesis of albumin with subsequent increase in total protein.

Conversely, the administration of the extract decreased the tissue MDA levels suggesting that *Hibiscus sabdariffa* calyx is a safe medicinal plant with a significant antioxidant effects. These results were in agreement with the results obtained by Abbas *et al.,* (2011) who reported that *Hibiscus sabdariffa* is probably a safe medicinal plant as they cause no significant change in cholesterol, triglyceride, BUN, serum creatinine, sodium and potassium levels.

However, the intoxication of the animals positively indicated the possibility of acetaminophen mediated oxidative stress in the rats' hepatocytes. This is sequel to the sharp increase in plasma Alanine aminotransferase (ALT) activity (table 3) which is considered to be a significant indicator of acetaminophen-induced acute liver damage (Thapa and Walia, 2007).

Furthermore, significant increases ($p<0.05$) in the plasma Alanine amino transferase activity (67.99 ± 2.11 U/L) of Acetaminophen-intoxicated rats compared with the negative control (21.15 ± 2.22 U/L) (table 3) signifies Acetaminophen-induced acute liver damage to have developed. In these rats, Aspartate aminotransferase (AST) and Alkaline phosphatase (ALP) activities were also found to be increased significantly ($p< 0.05$) when compared with the negative control group. Plasma AST and ALP activities increase not only in liver damage but also in case of various other tissues and organs damage such as the kidney and the placenta (Thapa and Walia, 2007).

Acetaminophen intoxication also significantly decreased ($p< 0.05$) plasma total protein and albumin (table 1), with increased plasma bilirubin (table 2) compared with the negative control group. The liver is the major source of most of the plasma proteins, in which the parenchymal cells are responsible for synthesis of albumin, fibrinogen and other coagulation factors and most of the α- and β-globulins (Pawlikowska-Pawlega *et al*., 2007). The observed decrease in albumin by acetaminophen could be a result of a decline in the number of cells responsible for albumin synthesis in the liver through necrosis. The direct interference with the albumin-synthesizing mechanism in the liver as a result inflammation may also be implicated for decrease in albumin. Acetaminophen overdose is known to be associated with inflammation, marked by an increase in the inflammatory cytokines; tumor necrosis factor -α (TNF-α), interleukin-1α and interleukin-1β, as well as the up regulation of nitrogen oxide (NO) from macrophages and hepatocytes (Jaeschke *et al*., 2003 and Ghosh *et al*., 2010). Such cytokines produced during inflammation shunt amino acids to increase the synthesis of proteins important to the inflammatory process, thus decreasing albumin synthesis as it is not essential to inflammation.

Accumulation of bilirubin is a measure of alterations in binding, conjugation and excretory capacity of hepatocytes. The elevated level of bilirubin is usually an indication of biliary obstruction, hemolysis, and in some cases renal failure (Pawlikowska-Pawlega *et al*., 2007). The observation of increased level of plasma bilirubin in Acetaminophen - intoxicated rats (table 2) are suggestive of Acetaminophen -induced hepatic damage.

Also in the present study, Acetaminophen intoxication caused a significant elevations ($p<0.05$) in MDA levels and reduction in GSH levels with

simultaneous inhibition in the activities of antioxidant enzyme; SOD in the rats' hepatocytes (table 4). Lipid peroxidation is a well-established mechanism of cellular injury and is used as an indicator of oxidative stress in cells and tissues. Lipid hydro peroxides (LOOH) are byproducts of lipid peroxidation and increased levels of lipid peroxidation products are associated with a variety of chemical-induced toxicities including Acetaminophen (Choi *et al*., 2010 and Yousef *et al*., 2010). Lipid peroxidation (LPO) is known to cause cellular injury by inactivation of membrane enzymes and receptors, de polymerizaton of polysaccharide, as well as protein cross linking and fragmentation (Luqman and Rizvi, 2006). Kanbur and coworkers have reported LPO contents to increase in the liver tissues of Acetamonophen -induced liver damage in rats (Kanbur *et al*., 2009). Overproduction of free radicals in Acetaminophen -intoxicated rats might have upshot the hepatic lipid peroxidation, and consequently increased malondialdehyde concentrations. This may also be implicated with diminished hepatic glutathione (GSH) concentrations, as to combat the over production of free radicals, hepatic GSH stores might have been exhausted. This findings correlate with the report of Knight *et al*., (1987).

Reduced glutathione is a substrate for glutathione related enzymes, and a regenerator for alpha tocopherol; therefore, it plays an important role in the antioxidant defense system (Meister, 1991). It is well known that a large dose of acetaminophen causes hepatic GSH depletion because NAPQI reacts rapidly with glutathione (Masubuchi *et al*., 2005), which consequently exacerbates oxidative stress in conjunction with mitochondrial dysfunction.

Oxidative stress plays a critical role in the cellular toxicity and is implicated as a major factor in the pathogenesis of several diseases (Parodi, 2007 and Sener *et al*., 2005). It is known that when ROS generation over loads the antioxidant defense, the free radicals can then interact with endogenous macromolecules and alter the cellular functions. Administration of Acetaminophen alone had also resulted in diminished antioxidant enzymes activities in the rats' hepatocytes as decrease in SOD activity in the intoxicated rats may be due to the overproduction of superoxide radical anions.

Furthermore, rats treated with the extract (200mg/kg, 400mg/kg and 600mg/kg body weight) following Acetaminophen -intoxication restored the liver damage as demonstrated by the significant decrease ($p<0.05$) in plasma AST, ALT and ALP activities, with consequent increases in the plasma levels of total protein and albumin, when compared with the intoxicated rats. The improvement against Acetaminophen induced liver damage seemed to be dose dependent; with 600 mg/kg doses being more effective. This recovery towards normalization of the enzymes suggests the capability of the extract to accelerate parenchymal regeneration, thus protecting against membrane fragility and subsequently decreasing leakage of marker enzymes into the circulation. Takeda and Yasui, (1985) earlier reported the presence of quercetin in *H. sabdariffa* calyx which has been known for their protective effect against different toxicants (Janbaz *et al*., 2004; Choi *et al*., 2010; Yousef *et al*., 2010).

Concerning the hepatic antioxidant markers, there was significant and dose dependent increase ($p<0.05$) in hepatic GSH and SOD activities, which consequently reduced the MDA levels compared with the Acetaminophen intoxicated control (table 4). This result is in concordance with the report of El-Beshbishy *et al*., 2010.

Interestingly, similar biochemical indices investigated to ascertain the state of the kidney due to this intoxication showed similar trends with that of the results obtained in the liver as significant $p<0.05$ increases in plasma levels of urea and creatinine were obtained in the intoxicated group (Table 5) compared with other treatment groups which are suggestive of strong correlation between nephrotoxicity and oxidative stress and are in agreement with reports of Isik et al., (2006) who noticed an elevation in these parameters with the administration of acetaminophen. The elevated H_2 O_2 and O_2^- production alters the filtration surface area and modifies the filtration coefficient; while both factors could decrease the glomerular filtration leading to accumulation of urea and creatinine in the blood. The elevated activities of ALP (Table 3) as related to the kidney may also be due to increased in lipid peroxidation of biomembranes which causes leakage of cellular components into the blood (Matsuo et al., 1989). Also, all the antioxidant parameters examined such as MDA, GSH concentrations and SOD activities in the kidney homogenates elicit similar effects as obtained in the liver (Table 6), all pointing to the toxic effects of acetaminophen at the treated dosage. However in all the treated group with different concentrations of the extract, their ameliorative effects were observed in a concentration –dependent manner.

In accordance with these findings, aqueous extract of *Hibiscus sabdariffa* calyx in this study demonstrated a strong ameliorative potential in Acetaminophen induced tissue injuries especially to the liver and the kidney. The ameliorative effect of *Hibiscus sabdariffa* could be correlated directly with its ability to reduce activity of plasma enzymes and enhance antioxidant defense status, which may be as a result of the high presence of phenolic compounds in the plant as earlier reported by Subramanian and Nair,

(1972). The results of this study are indicative of the anti oxidant property of *Hibiscus sabdariffa* calyx, an indication of its bioactive constituents suggesting that it may be used as a safe, cheap, and effective alternative chemopreventive and protective agent in the prevention, treatment and management of diseases associated with oxidative tissue damage or injuries as a result of prolonged chemotherapy.

Conclusion

In conclusion, additional investigation is required to elucidate the molecular mechanism(s) of the properties shown by the plant even as common drinks, beverages and food supplements in order to support the clinical use of the plant in the design of pharmaceutical agent and consequent use as a potential therapeutic agent against the ailments where tissue damage is manifested while there is also need to increase the awareness of the potential toxic effects of Acetaminophen overdose in hospitals and other organs of health care.

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