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Original Article

In vivo effect of *Citrullus Lanatus* seeds extract on liver function on acute butylated hydroxytoluene induced oxidative stress in Albino rats

Edet Love Mendie[1], Etim Okon Effiom[1] and Modo Emmanuel Uchechukwu[2]

[1]Department of Biochemistry, Madonna University, Elele, Nigeria
[2]Department of Biochemistry, University of Calabar, Calabar , Nigeria

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ABSTRACT

Butylated Hydroxytoluene (BHT) is a synthetic antioxidant that exerts a pro-oxidant effect at high dose causing hepatotoxicity. In this study, the effect of the *Citrullus lanatus* seeds extract on acute BHT induced oxidative stress was investigated. The wistar rats were grouped into five. Group I was the control, group II was administered BHT only, group III, IV and V were administered BHT + 200, 400 and 600 mg/kg *Citrullus lanatus* seed extract respectively. Some biochemical parameters were measured in the serum using standard kits. The results showed that serum total bilirubin levels were higher in the BHT only group compared to the control and pre-treated rats. The BHT-only rats had lower total protein level than the control and pre-treated rats. The mean AST activity was higher in rats administered BHT only than those pre-treated. The ALT activity was significantly lower ($p<0.05$) in the BHT only rats compared to rats pre-treated with the extract. Furthermore, the BHT-only rats had significantly higher ($p<0.05$) mean ALP activity compared to those rats pre-treated These therefore suggest a possible liver injury via induced oxidative stress and *Citrullus lanatus* seeds extract exhibit a potential to ameliorate such damage though concentration dependent.

Keywords; *Citrullus lanatus*, Butylated Hydroxytoluene , oxidative stress, liver enzymes,total bilirubin and total protein.

INTRODUCTION

Citrullus lanatus (Watermelon) belongs to the family of *cucurbitaceae*. (Schipper, 2000). *C. lanatus* ancestor is the bitter fruit form of *C. vulgaris* Schrader (Mohr, 1986*)* and contains 6% sugar by weight, and rest being almost water; the seeds are excellent sources of protein (35%), oil (50%) and dietary fiber. Watermelon plays a very important role in Africa as it is used to quench thirst when there is shortage of water., It is also being used as worm expeller in recent years . Watermelon is an excellent source of vitamin A, B & C necessary for energy. Traditionally *Citrullus lanatus* is in use as energy source, cleanse and purify the kidney and bladder, lowers high blood pressure, prevent erectile dysfunction, act as antioxidant and used to treat enlarge liver &jaundice (Schpper.2000). The preliminary phytochemical analysis showed that the seeds contain bioactive constitute such as saponins, flavonoids, cyanogenic glycosides, alkaloids, tannins and oxalate (Braide *et al*, 2012). The seeds of watermelon can be bruised and rubbed up with water to form an emulsion, which can be used to cure catarrhal infections disorder of the bowels, urinary passage and fever. It is also being used as worm expeller, in recent years it has been used to expel tapeworms (Sodeke, 2005).

Butylated hydroxytoluene (BHT) is a synthetic phenolic antioxidant widely used as a food additive and known to increase the incidence of hepatocellular tumor in male mice or rats at high concentration. BHT is also documented as an antioxidant additive in such diverse products as cosmetics, pharmaceuticals, rubber, electrical transformer oil (at 0.35%) and embalming fluid. In the petroleum industry, BHT is known as the fuel additive. Also at high dosage, BHT is known to induce oxidative stress (Oikawa *et al*, 2003).

The liver is the largest gland in the body, and after the skin, the largest organ. It is involve in many metabolic activities among which are; detoxification of toxic substances, glycogen storage

***Corresponding Author Address:** Etim Okon Effiom, Department of Biochemistry, Madonna University, Elele, Nigeria; E-mail: okprince25@gmail.com*

and bile secretion (Keith *et al*, 2010). Also, most proteins are synthesized in the liver prior to entering the blood (Murray *et al*, 2009).

Since at high dose, BHTis known to cause hepato-toxicity by inducing oxidative stress in animals and *Citrullus lanatus* is reported to be a potent natural antioxidant, this work was therefore aimed at determining possible protective effect of *Citrullus lanatus* seed extract on the liver.

MATERIAL AND METHOD

Collection and preparation of extract: The *citrullus lanatus* seeds were air-dried at room temperature, pounded in a local mortar and finally reduced to fine powder using an electric blending machine. About 1110.36g of the powdered seeds was soaked in 3000ml of 80% methanol. The hydromethanolic extracts were concentrated using a rotary evaporator. Final weight was calculated as 86.292g with percentage yield of 7.77%. Extracts were dissolved in 25% dimethyl sulfoxide (DMSO) for assays.

Materials / Chemicals: Centrifuge, Spectrophotometer, Water bath, Methanol (BDH Laboratory Reagent), Butylated hydroxyl toluene (Qualikems Lab Reagent)**,** Chloroform (Sigma Aldrich,USA), Sodium chloride (Sigma), Randox Standard reagent kits and Dimethyl sufoxide (Qualikems Lab Reagent).

Experimental Design: Healthy male albino rats with average weight of 120 – 150g were used for this study. The animals were divided into group of five (5) based on weight differences.

- Group 1: Positive control (10ml/kg body weight of DMSO)
- Group 2: Negative control (1000mg/kg body weight of BHT only)
- Group 3: BHT + 200mg/kg body weight of *Citrullus lanatus* seeds extract
- Group 4: BHT + 400mg/kg body weight of *Citullus lanatus* seeds extract
- Group 5: BHT + 600mg/kg body weight of extract *Citrullus lanatus* seeds

The rats were kept at standard conditions with proper ventilation and acclimatized for four (4) weeks. Administration was carried out for 30 days. The animals were anaesthetized with chloroform and sacrificed 12 hours after the last dosage was given. Blood samples were collected in non-heparinised bottles and centrifuged at 3000rpm for 10minutes and serum collected for analysis.

Statistical Analysis: Data obtained from this study were analysed using the statistical package for social sciences (SPSS) version 18.0 for windows. Analysis of variance (ANOVA) were used to compare means, and values were considered significant at $p<0.05$.

EXPERIMENTAL ASSAYS:

Estimation of Serum AST Activity

This method is used for *in vitro* determination of aspartate amino transferase in serum.

Principle;

α-oxoglutarate+L-aspartate $\xrightarrow{\text{GOT}}$ L-glutamate+oxaloacetate

AST is measured by monitoring the concentration of oxaloacetate hydrazone formed with 2,4-dinitrophenylhydrazine.

Estimation of Serum ALT Activity

This method is used for in vitro determination of alanine aminotransferase (ALT) in serum.

Principle

α-oxoglutarate+L-alanine $\xrightarrow{\text{GPT}}$ L-glutamate+pyruvate.

Alanine aminotransferase is measured by monitoring the concentration of pyruvate hydrazone formed with 2,4-dinitrophenylhydrazine.

Estimation of Serum ALP Activity

This method is used for in vitro quantitative determination of alkaline phosphatase (ALP) in serum and plasma.

Principle

p-nitrophenylphosphate + H_2O $\xrightarrow{\text{ALP}}$ Phosphate + p-nitrophenol

ALP catalyzes the hydrolysis of p-Nitrophenyl phosphate (pNNP) to p-nitrophenol. pNPP is colorless but p-Nitrophenol has a strong absorbance at 405nm.

Bilirubin (BIL) Assay: This assay was intended to quantitatively determine *in vitro* the total and direct bilirubin in serum or plasma. Colorimetric method based on that described by Jendrassik and Grof (1938). Direct (conjugated) bilirubin reacts with diazotised sulphanilic acid in alkaline medium to form a blue coloured complex. Total bilirubin is determined in the presence of caffeine, which releases albumin bound bilirubin, by the reaction with diazotized sulphanilic acid.

Determination of Total Protein: Total protein content was determined using Tietz (1995) method.This was based on the principle that cupric ions in an alkaline medium, interact with protein peptide bonds resulting in the formation of a coloured complex. The intensity of this coloured complex is directly proportional to the protein complex in the sample; the characteristic colour

formed can be quantified by its absorbance at a wavelength of 540nm.

RESULTS

Effect of *Citrullus lanatus* Seeds Extract on Serum Total Bilirubin Levels in BHT-intoxicated Rats: As shown in figure I, the serum total bilirubin levels were higher in the BHT only rats (12.57 ± 0.19 mg/dl) compared to the normal control rats (11.21 ± 0.42 mg/dl), rats pre-treated with 200 mg/kg extract (11.46 ± 0.56 mg/dl) and 600 mg/kg extract (11.70 ± 0.83 mg/dl), however, the mean differences were not statistically significant (p>0.05).

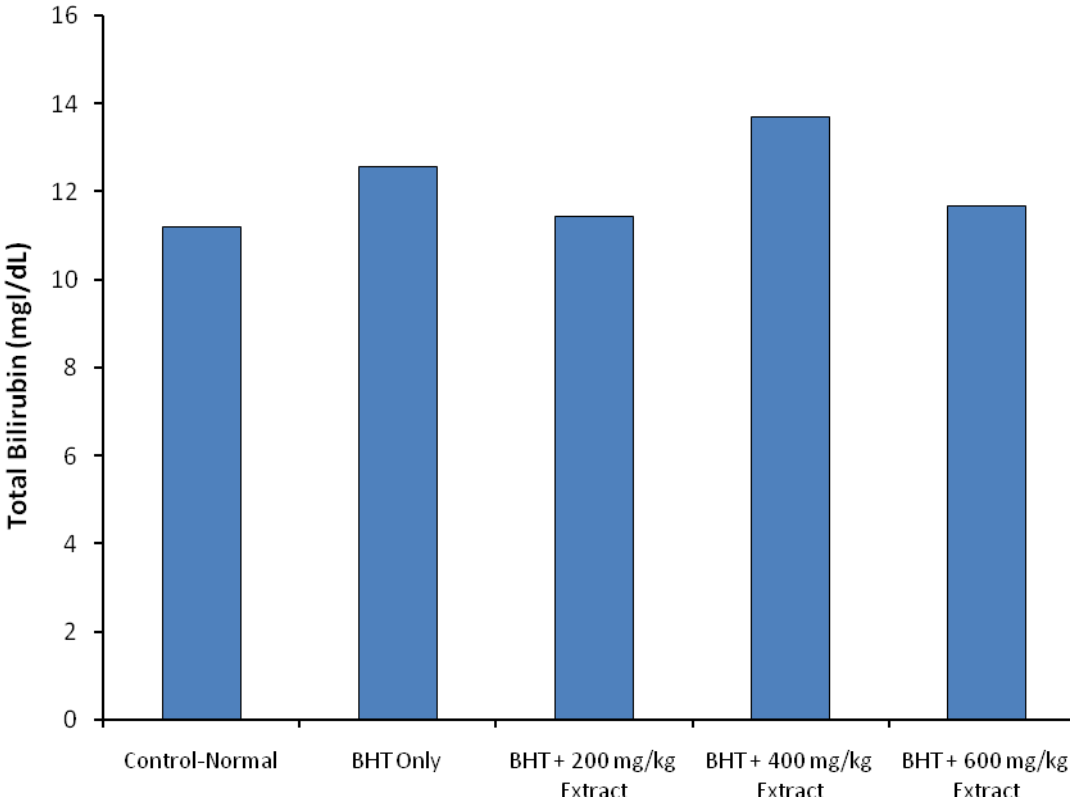

Figure I; Effect of *Citrullus lanatus* seeds extract on Serum Total Bilirubin Levels in BHT-induced Oxidative Stress. Data represented as Mean ± SEM

Effect of *Citrullus lanatus* Seeds extract on Serum Total Protein Levels in BHT- intoxicated Rats: As shown in the figure II below, the BHT-only rats had lower total protein level (3.81 ± 0.19 g/dL) than the normal control (4.93 ± 0.44 g/dL) and rats pre-treated with *Citrullus lanatus* extract (4.69 ± 0.80, 4.72 ± 1.35 and 5.00 ± 0.48 g/dL for those administered 200, 400 and 600 mg/kg respectively), although the mean differences were not statistically significant (p>0.05).

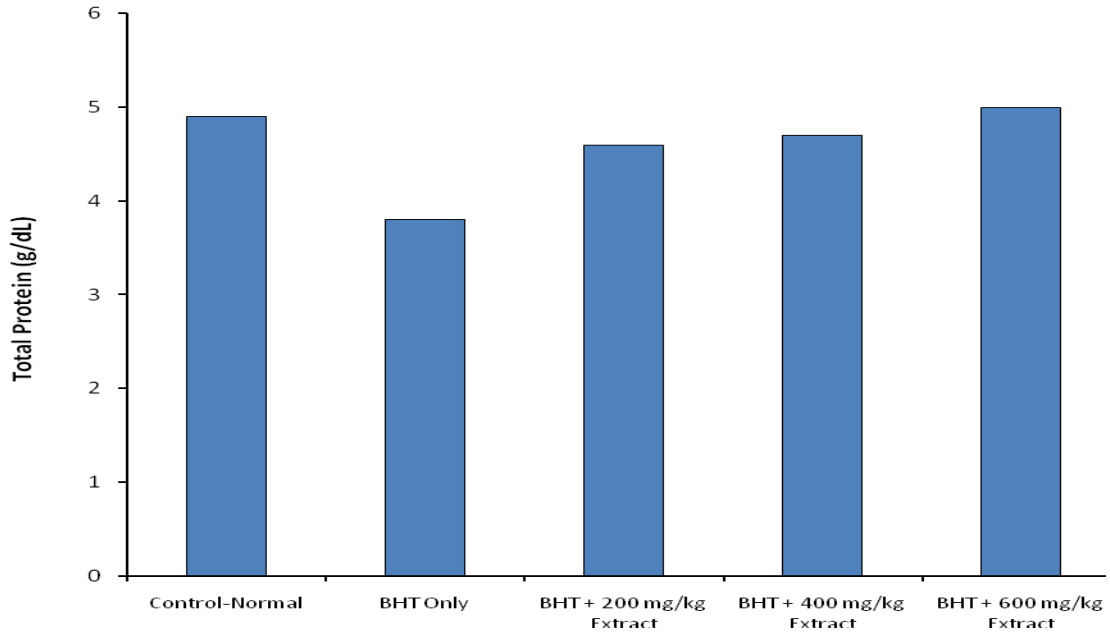

Figure II: Effect of *Citrullus lanatus* Seeds extract on Serum Total Protein Levels in BHT-induced Oxidative Stress**.** Data represented as Mean ± SEM\

Effect of *Citrullus lanatus* Extract on Alanine Aminotransferase (ALT) Activity in BHT-intoxicated Rats

As shown in figure III below, the ALT levels were significantly lower ($p<0.05$) in the BHT only rats (79.4 ± 2.5 U/L) compared to rats pre-treated with 200, 400 and 600 mg/kg extract (232.5 ± 1.3, 237 ± 6.1 and 237.2 ± 8.2 U/L).

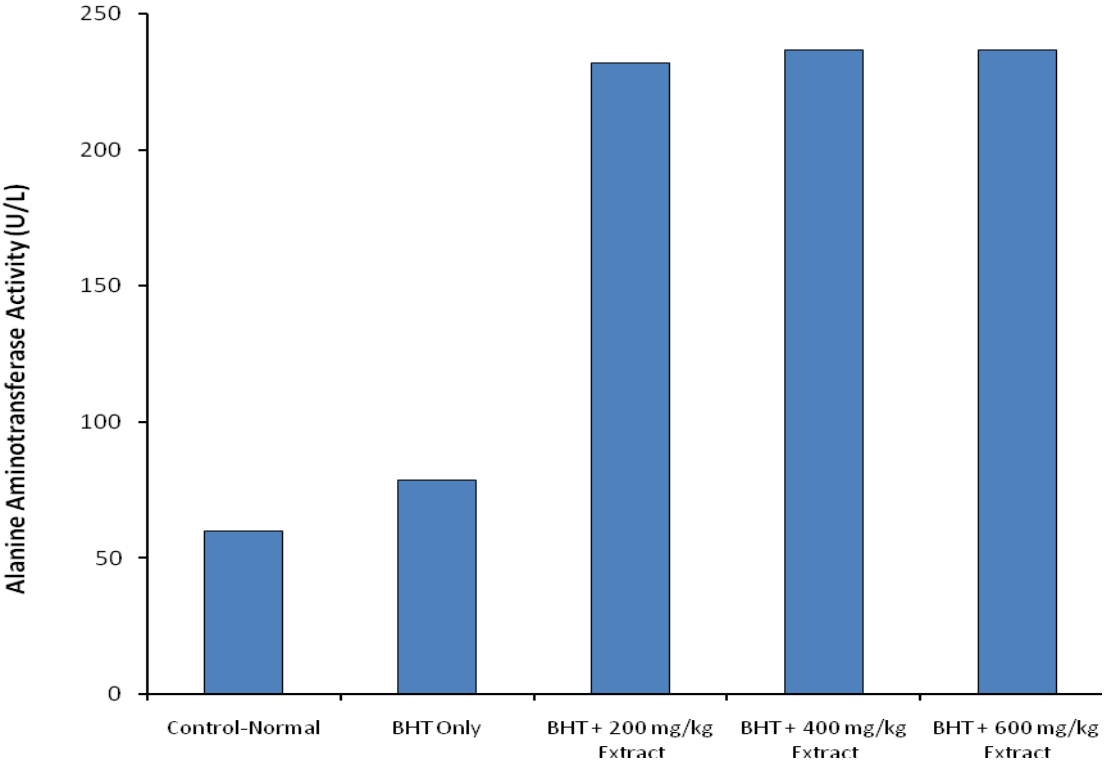

Figure III: Effect of *Citrullus lanatus* extract on ALT Levels in BHT-induced Oxidative Stress. Data represented as Mean ± SEM

Effect of *Citrullus lanatus* extract on Aspartate Aminotransferase (AST) Activity in BHT- intoxicated Rats: Figure IV below shows that the mean AST levels were higher in rats administered BHT only (373.6 ± 25.4 U/L) compared to the normal control (357.3 ± 17.5 U/L) and those pre-treated with 200, 400 and 600 mg/kg extract, although the mean differences were not statistically significant (p>0.05).

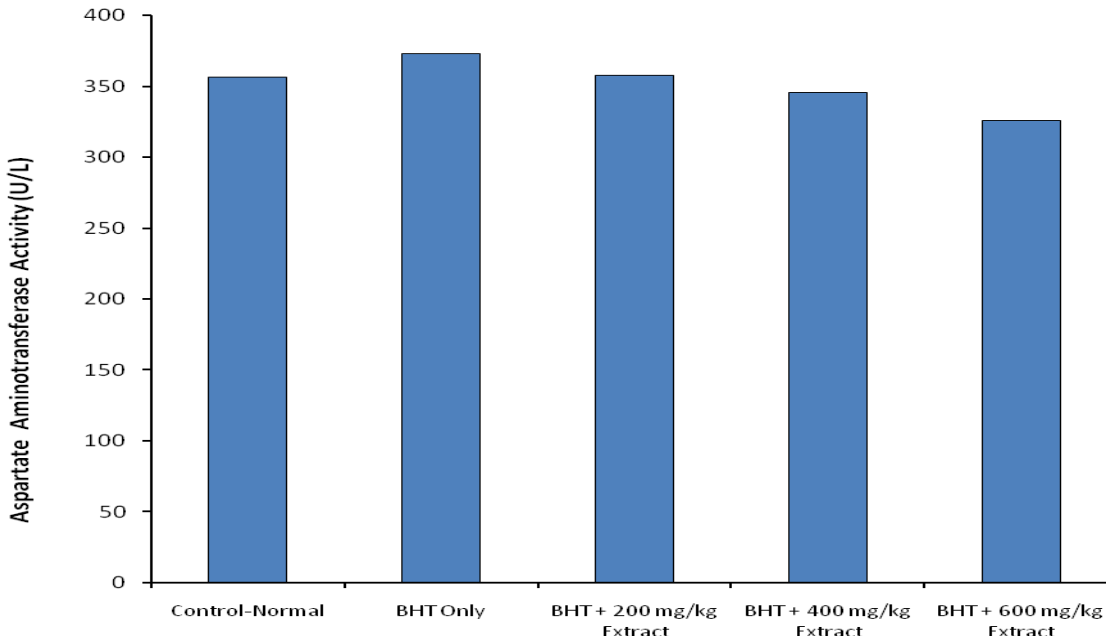

Figure IV; Effect of *Citrullus lanatus* **extract** on AST Levels in BHT-induced Oxidative Stress. Data represented as Mean ± SEM.

Effect of *Citrullus lanatus* extracts on alkaline phosphatase Levels in BHT- intoxicated Rats

As shown in figure V below, the BHT-only rats had significantly higher (p<0.05) mean ALP levels (1545.3 ± 44.9 U/L) compared to those rats pre-treated with 600 mg/kg *Citrullus lanatus* extract (1441.3 ± 9.3U/L).

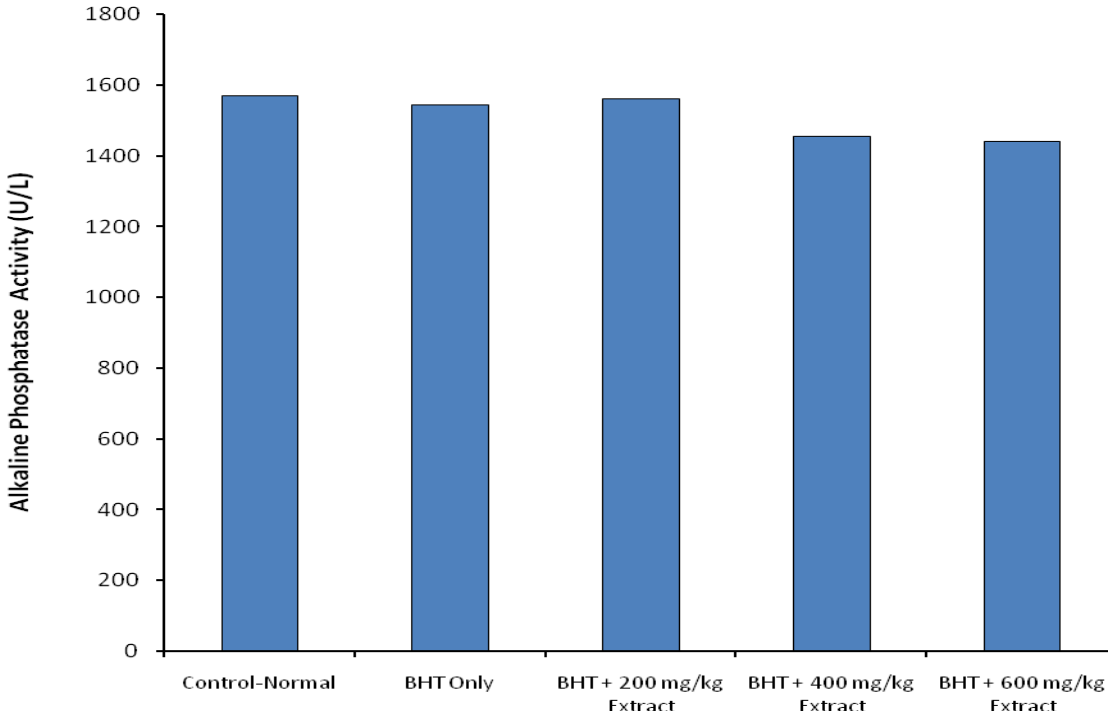

Figure V: Effect of *Citrullus lanatus* extract on ALP Levels in BHT-induced Oxidative Stress. Data represented as Mean ± SEM

DISCUSSION

The BHT- induced oxidative model has proven to be a valuable design for *in vivo* investigations on the potential use of several therapeutic agents for treatment of various diseases associated with oxidative stress. It has been demonstrated that several known anti-oxidant might have both antioxidant and oxidant action dependent on its concentration. (Cotelle, 2001)

Phytochemical screening of chemical constitutes of watermelon seeds indicates that secondary metabolites such as saponins, flavonoids, tannins, cyanogenic glycosides, alkaloids and oxalate are present in *Citrullus lanatus* seeds (Braide *et al*, 2012). These secondary metabolites are responsible for the pharmacological activities such as, analgesic and anti-inflammatory of seeds (Madhavi *et al*, 2012), anti-ulcerative activity (Alok, 2012), antioxidant activity (Naresh, 2011), laxative activity (Swapnil, 2011), hepatoprotective (Madhavi *et al*, 2012) and many other ethno-medicinal uses.

As shown in the figure above, the serum total bilirubin levels were higher in the BHT only rats (12.57 ± 0.19 mg/dl) compared to the normal control rats (11.21 ± 0.42 mg/dl), rats pre-treated with 200 mg/kg extract (11.46 ± 0.56 mg/dl) and 600 mg/kg extract (11.70 ± 0.83 mg/dl). This agrees with the fact that BHT is known to increase the incidence of hepato-toxicity in experimental animals (Faine *et al.*, 2006), which could be as a result of hepatic jaundice which led to increase in serum total bilirubin.

Also the BHT-only rats had lower total protein level (3.81 ± 0.19 g/dL) than the normal control (4.93 ± 0.44 g/dL) and rats pre-treated with *Citrullus lanatus* seeds extract (4.69 ± 0.80, 4.72 ± 1.35 and 5.00 ± 0.48 g/dL for those administered 200, 400 and 600 mg/kg respectively) though the differences were not significant. This is an indication that the normal control rats and pre-treated rats had a functional liver compared to the BHT-only rats because most proteins are synthesized in the liver prior to entering the blood (Murray *et al*, 2009).

The mean AST levels were higher in rats administered BHT only (373.6 ± 25.4 U/L) compared to the normal control (357.3 ± 17.5 U/L) and those pre-treated with 200, 400 and 600 mg/kg extract, although the mean differences were not statistically significant ($p>0.05$) as shown in figure IV and figure III shows that the ALT levels were significantly lower ($p<0.05$) in the BHT only rats (79.4 ± 2.5 U/L) compared to rats pre-treated with 200, 400 and 600 mg/kg extract (232.5 ± 1.3, 237 ± 6.1 and 237.2 ± 8.2 U/L). In figure V, the BHT-only rats had significantly higher ($p<0.05$) mean ALP levels (1545.3 ± 44.9 U/L) compared to those rats pre-treated with 600 mg/kg *Citrullus lanatus* extract (1441.3 ± 9.3). Results for AST and ALP suggest possible liver damage owing to oxidative stress with *Citrullus lanatus* seed extract showing a protective effect. *C. lanatus* seeds extract exhibited high antioxidant and free radical scavenging activities indicating that the plant is a significant source of natural antioxidant and its potential could, to a greater extent, be attributed to its phenolic contents(Etim *et al*.,2013).

CONCLUSION

Citrullus lanatus seeds extract from these results have shown to exhibit antioxidative and hepato – protective potentials toward liver damages caused by BHT-induced oxidative stress, hence recommended to be incorporated into our daily diets.

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