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Toxicity, anti-lipid peroxidation, invitro and invivo evaluation of antioxidant activity of Annona muricata ethanol stem bark extract

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Abstract: The presence of natural antioxidant capacity in plants has been well documented world over. There is an increasing demand for natural antioxidant to replace synthetic additives in the food and pharmacologicals. The objective of this study is to evaluate the *invivo* antioxidant potential of ethanol extract of *Annona muricata* against CCl_4. induced toxicity in rats as well as its *invitro* antioxidant effect and lipid peroxidation. The extract was prepared by cold maceration using absolute ethanol. The *invitro* antioxidant properties of the extract was determined using DPPH (2,2-diphenyl-1-picrylhdrazyl) radical and *invivo* antioxidant enzymes were assayed to evaluate the biological activities of the extract. The polyphenol content of the extract was determined and it contained alkaloids, tannin, flavonoids, phenol in appreciable amount. In the *invivo* studies, the animals were grouped into three (3) groups of 15 rats each. Group 1 served as control and received 1ml/kg b.w of olive oil orally for 28 days. Group 2 rats were orally administered 1ml/kg CCl_4 mixed with olive oil (1:10) daily for 28 days while group 3 rats were administered 1ml/kg CCl_4 and 200 mg/kg b.w of *Annona muricata* stem extract. Three of the rats from each group were sacrificed on days 1, 8, 15, 22 and 28. The plant extract showed remarkable hepatoprotective and antioxidant activity against carbon tetrachloride (CCl_4) induced oxidative stress as revealed from serum enzyme markers. CCl_4 induced a significant rise ($p<0.001$) in aspartate amino transferase (AST), alanine amino transferase (ALT), alkaline phosphatase (ALP) and MDA (malondialdehyde) level in the serum with a reduction in catalase activity. Treatment of rats with the plant extract (200mg/kg b.w) significantly altered both serum enzymes activities and oxidant levels to near normal against CCl_4 – treated rats. The *invivo* and *invitro* rapid radical scavenging studies were positive for the stem bark extract. This study suggests that the possible mechanism of the exhibited biological activities of the extract may be due to free radical scavenging owing to the presence of polyphenols in the extract. The plant extract possesses, antioxidant, anti-lipid peroxidation effect and is hepatoprotective. These may be the rationale for its folkloric uses and pharmacological effects.

Keywords: *Annona muricata*, Antioxidant, DPPH (2,2-Diphenyl-1-Picrylhydrazyl), Wistar Rats, *Invitro* And *Invivo*

1. Introduction

The human body has a complex system of natural enzymatic and non-enzymatic antioxidant defenses which counteract the harmful effects of free radicals and other oxidants. Free radicals are responsible for causing a large number of diseases including cancer (Kinnula and Crapo, 2004), cardiovascular disease (Singh and Jialal, 2006), neural disorders (Sas *et al.,* 2007), Alzheimer's disease (Smith *et al.,* 2000), mild cognitive impairment (Guidi *et al.,* 2006), Parkinson's disease (Bolton *et al.,* 2000), alcohol induced liver disease (Arteel, 2003), ulcerative colitis (Ramakrishma *et al.,* 1997), aging (Hyun *et al.,* 2006) and atheroscterosis (Upston *et al.,* 2003).

Over 50% of all modern clinical drugs are of natural product origin (Stuffness and Douros, 1982) and natural products play an important role in drug development programmes in the pharmaceutical industry (Baker *et al.,* 1995). Herbal drugs have gained importance in recent years because of their efficacy and cost effectiveness. Thus, there is an increasing interest in the measurement and use of plant antioxidant for scientific research as well as industrial purposes. The antioxidant reactions involve multiple steps

including the initiation, propagation, branching and termination of free radicals. Free radicals are created when cells use oxygen to generate energy. These by-products are generally reactive oxygen species (ROS) such as superoxide anion, hydroxyl radical and hydrogen peroxide that result from the cellular redox process. At low or moderate concentrations, ROS exert beneficial effects on cellular responses and immune function but at high levels, free radicals and oxidants generate oxidative stress, a deleterious process that can damage cell structures, including lipids, proteins, and DNA (Pham-Huy *et al.,* 2008).

Protection against free radicals can be enhanced by ample intake of dietary antioxidants. Substantial evidence indicates that foods containing antioxidants and possibly in particular the antioxidant nutrients may be of major importance in disease prevention. There is, however, a growing consensus among scientists that a combination of antioxidants, rather than single entities, may be more effective over the long term. Antioxidants may be of great benefit in improving the quality of life by preventing or postponing the onset of degenerative diseases. In addition, they have a potential for substantial savings in the cost of health care delivery.

These antioxidants acts as free radical scavengers by preventing and repairing damages caused by ROS, and therefore can enhance the immune defense and lower the risk of cancer and other degenerative diseases (Pham-Huy *et al.,* 2008).

Antioxidants have been reported to prevent oxidative damage caused by ROS by reacting with free radicals, chelating, and catalytic metals and also by acting as oxygen radical scavengers (Shahidi and Wanasundra, 1992; Buyukokuroglu *et al.,* 2001). The antioxidant in biological system can be either enzymatic or non-enzymatic. The enzymatic antioxidants include catalase, superoxide dismutase, glutathione which catalyse neutralization of many types of free radicals (Jacob, 1995), while the non-enzymatic antioxidants include Vitamin C, selenium, Vitamin E, carotenoids, and polyphenols.

There is growing evidence that antioxidants play a pivotal role in the prevention of heart disease, cancer, DNA degeneration, pulmonary disease, and neurological disorder (Percival, 1998).

Recently, there has been an upsurge of interest in the therapeutic potential of plants as antioxidants in reducing oxidative tissue injuries (Patel *et al.,* 2010). Plants, herbs, and spice, rich in phenolic compounds like flavonoids, have been demonstrated to have anti-inflammatory, anti-allergic, anti-viral, anti-aging, and anti carcinogenic activities which can be attributed to their antioxidant properties (Agil *et al.,* 2006).

In the continuation of the strategy of new drug discovery for the management of oxidative stress related diseases, the plant *Annona muricata* have been studied for its antioxidant activity and phytoconstituents.

Annona muricata belongs to the family *Annonaceae*. It is commonly called "Graviola or Soursop" in English and other various local names. *A. muricata L* is indigenous to most of the world's tropical rainforest areas of South and North America as well as West African Countries like Nigeria, Ghana, Ivory Coast and Gambia (Harrison and Rhett, 2005). All parts of *Annona muricata* tree are used in natural medicine in the tropics including the stem bark, leaves, and root, fruit and fruit seeds. *A. muricata* L has been used as phyto-therapy for various ailments such as cancer, as broad spectrum internal and external anti microbial to treat bacterial and fungal infections, anti- helmintic etc. Other uses of the plant documented by traditional medicine practitioners are: antiviral, digestive stimulant, anti-malarial, vermifuge (Das *et al.,* 1999).

Despite the wide use of this plant as medicinal plant all over the globe, the literature contains few reports of antioxidant activity. The present study is aimed at establishing the *invitro* and *Invivo* antioxidant potentials of the ethanol stem bark extract of *Annona muricata* , anti-lipid peroxidation effect as well as the relationship of the phyto-chemical content with antioxidant activity.

2. Materials and Methods

2.1. Chemicals Used

All chemicals and reagents used were of analytical grade procured from reputable companies. 2, 2 – Diphenyl-1-Picrylhydrazyl (DPPH) was obtained from Sigma Aldrich Co., St. Louis, USA. Enzymes assay kits were obtained from Randox Laboratories Ltd, Crumlin, Co., Antrim, UK. Malondialdehyde (MDA) assay kit was obtained from Northwest Life Science Specialist, LLC Vancouver, USA. Ethanol (BDH).

2.2. Plant Material

The plant sample i.e. *Annona muricata* was collected from Anyigba town, Kogi State, Nigeria and was authenticated. The stem bark was removed and cleaned from dirt. It was dried under shade at room temperature for 3 weeks.

2.3. Preparation of Extract

The dried stem bark of *A. muricata* was powdered using mechanical blender and 100g of the powdered sample was extracted using cold maceration with 1L of absolute ethanol for 7 days. The extract solution was filtered through whatman filter paper No. 1. The extract was concentrated under reduced pressure (bath temperature 50^{o}C). The dried extract was stored in airtight container until used.

2.4. Experimental Animals

Albino rats of Wister strain, weighing between 200 – 230g maintained on normal rat pellet and water *ad libitum*, were divided into 3 groups of 15 rats each. They were obtained from the Department of Zoology, University of Nigeria, Nsukka, and kept in the Animal House of the Biochemistry Department, Kogi State University, Anyigba. The animals were allowed to acclimatize for two weeks before the

commencement of the experiment. The animals were kept in well-ventilated cages at room temperature and under natural light/darkness cycles. They were maintained in accordance with the recommendation of the Guide for the care and use of laboratory animals (DHHS, 1985).

The relevant authorities in the Department of Biochemistry, Kogi State University has approved the use of the animals for the purpose of the experiment.

2.5. Phytochemical Screening

Phytochemical screening was carried out with ethanol extract of the stem bark of *A. muricata* for the detection of various phytochemicals. The extract was tested for the presence of Tannins, Saponins, Glycosides, Alkaloids, Flavonoids, and Phenolic compounds using the standard procedures described by Trease and Evans, 2002.

2.6. Determination of Physico-Chemical Properties

The physico-chemical properties of *A. muricata* were determined following the method described by A.O.A.C (1999).

2.7. Invitro Antioxidant Activity Studies

Reducing Power

The reducing power was evaluated by method as described by Nabsree and Bratati (2007). Different concentrations of the extract (25, 50, 75 and 100 mg/ml) were prepared in methanol. Sample solution (03ml: 100/gml^{-1}) was mixed with reagent solution (3ml; 0.6m sulphoric acid, 2.8mM sodium phosphate and 4mM Ammonium molybdate). A blank composed of 3ml of reagent solution and methanol was also prepared. All test tubes were capped and incubated in boiling water bath at 95^{o}C for 90 minutes. Absorbances of samples were read against blank at 695nm. The antioxidant activity of sample was expressed as mg ascorbic acid equivalent.

DPPH Free Radical Scavenging Activity

The method described by Adesegun *et al.,* 2008 was followed in the determination of DPPH radical scavenging activity of *Annona muricata* ethanol stem bark extract. Different concentrations of the extract (25, 50, 75, and 100 mg/ml) were prepared using methanol. 1ml of 0.002% DPPH solution was mixed with 3ml of all the concentrations of the extract separately. The mixture was shaken vigorously and allowed to stand at room temperature in the dark for 20 minutes. The absorbance was read against blank at 577nm. Percentage scavenging activity was calculated using the expression.

$$\%\ \text{Scavenging Activity} = \frac{\text{Absorbance control} - \text{Absorbance of sample}}{\text{Absorbance of control}} \times 100$$

2.8. Experimental Design/Animal Treatment

Forty-five (45) rats weighing between 200-230g were sorted randomly into 3 groups of 15 rats each. Group 2 (control) received 1ml/kg of olive oil orally throughout duration of the experiment, group 2 were administered orally 1 ml/kg CCl_4 mixed with olive oil in ratio 1:10 daily while group 3 rats were given 1ml/kg CCl_4 in same vehicle and then treated orally with 1ml (200 mg/kg) of ethanol stem bark extract of *A. muricata*. Three (3) of the rats from each group were sacrificed on days 1, 8, 15, 22 and 28.

2.9. Preparation of Serum

The animals were sacrificed by severing the jugular vein with a surgical blade. Blood was allowed to flow freely into plain bottles. The blood was allowed to clot and then centrifuged at 1500 x g for five (5) minutes after which the serum was separated and used for enzyme assays.

2.10. Biochemical Estimations

Estimation of Catalase Activity

Catalase activity was estimated by determining the decomposition of H_2O_2 at 240nm in an assay mixture containing phosphate buffer (Dhalwal *et al.,* 2008). The change in absorbance/minute for each assay was calculated and the result expressed as CAT/Unit mg.

$$\text{CAT (U)}/100\text{mg of sample} = \frac{\text{Absorbance/min x } 0.003}{38.3956\ \text{x}10^{-6}}$$

Estimation of Serum Aspartate Amino Trasferase (AST)

AST in serum was determined by the method of Tietz (1994).

Estimation of Alkaline Phosphatase (ALP)

ALP activity was estimated using the method described by Rosalki *et al*., 1993.

Estimation of Alanine amino trasferase (ALT). The determination of alanine amino transferase activity was carried out following the method of Friedman and Young, (1997).

Estimation of Malondialdehyde (MDA).

Measurement of the lipid peroxidation using MDA as marker was adopted and assayed following the procedure described by Botsoglou *et al.,* 1994.

2.11. Statistical Analysis

Data analyses were run in triplicates. Data were transformed by analysis of variance (ANOVA). Statistical analysis was performed by the student's t-test and by ANOVA.

3. Results

3.1. Phytochemical Investigation

It was found that ethanol extract of the stem bark of *A. muricata* contained alkaloid, saponins, tannins, flavonoids, phenolic compounds and glucosides (Table 1).

Table 1. *Phytochemical Composition of A. muricata Stem Bark Extract*

Phytochemical	Indication in Plant
Alkaloid	++
Saponins	+
Tannins	++
Flavonoids	++
Phenolic compounds	+++
Glycoside	+

Key: +++ = Presence of bioactive compound in very high concentration
++ = High concentration
+ = Presence of bioactive compound
– = Absence of bioactive compound.

3.2. Proximate Composition

The proximate analysis revealed that the plant sample is low in mineral element (ASH, 8.5%) and richer in carbohydrate (50.79%) as presented in Table 2.

Table 2. *Proximate Composition of A. muricata Ethanol Stem Bark Extract*

Moisture %	Ash %	Crude Fibre %	Fat %	Protein %	Carbohydrate %
13.26	8.5	13.20	6.0	8.31	50.79

3.3. Invitro Antioxidant Activity

Table 3. *DPPH Free Radical Scavenging Activity of A. muricata Ethanol Stem Bark Extract*

Concentration of Extract	25 µg/ml	50 µg/ml	75 µg/ml	100 µg/ml
Absorbance	1.565±0.003	1.318±0.004	1.107±0.003	1.015±0.003
% Inhibition	21.3%	33.7%	44.3%	49.0%

Results are expressed as mean ± S.D., n = 3.

In table 3, the ethanol stem bark extract of *A. muricata* exhibited free radical scavenging activity in a concentration dependent fashion.

Table 4 shows the reducing power of *A. muricata* ethanol stem bark extract on molybdenum (vi). The absorbance increased with increase in concentration of the extract.

Table 4. *Reducing Power of Annona muricata Ethanol Stem bark Extract on Molybdenum*

Concentration of Extract	25 µg /ml	50 µg /ml	75 µg /ml	100 µg/ml
Absorbance	0.044±0.004	0.061±0.002	0.074±0.003	0.096±0.002
Mg ($AAEg^{-1}$)	0.48	0.67	0.81	1.05

Results are expressed as mean ± S.D., n = 3.

Table 5. *Effect of Annona muricata Ethanol Stem Bark Extract on Liver ALT Activity in CCl_4 – Induced Oxidative Stress in Rats (U/L)*

Days	Control	CCl_4 Treated Rats	Treated with AMESBE
Day 0	64.35±0.78	66.87±1.13	66.03±0.68
Day 7	66.13±0.88	70.35±0.66^{a,b}	66.48±0.77^{c}
Day 14	66.99±0.61	98.42±1.28^{a,b}	72.66±2.01^{c}
Day 21	69.97±0.33	126.95±2.4^{a,b}	77.92±1.76^{d}
Day 28	74.43±1.20	159.99±1.64^{a,b}	79.69±0.89

Results are expressed as mean ± S.D., n = 3.
^{a} p<0.001 Vs control, ^{b} p<0.001 Vs AMESBE Treated, ^{C} p<0.01; ^{d} p<0.05 Vs control (AMESBE – *Annona muricata* Ethanol Stem Bark Extract).

There is significant different (p<0.001) between the values of ALT in the CCl_4 -induced oxidative stress group and that of the control and *Annona muricata* extract treated group (Table 5).

Table 6. *Effect of A. muricata Ethanol Stem Bark Extract on Liver AST Activity in CCl_4 – Induced Oxidative Stress in Rats (U/L)*

Days	Control	CCl_4 Treated Rats	Rat Treated with AMESBE
Day 0	98.23±0.001	100.34±0.001	99.07±0.002
Day 7	97.20±1.88	112.90±2.42^{a,b}	102.66±2.65^{c}
Day 14	100.75±0.50	140.15±0.36^{a,b}	111.25±0.50^{c}
Day 21	103.42±0.33	171.83±1.86^{a,b}	116.83±0.76^{c}
Day 28	108.71±0.38	195.50±1.34^{a,b}	123.50±1.89

Results are expressed as mean ± S.D., n = 3.
^{a} p<0.001 Vs control, ^{b} p<0.001 Vs AMESBE Treated, ^{C} p<0.01 Vs control.

As presented in Table 6, serum activity of AST was higher and significantly different in the *Annona muricata* treated group than in control animals in day 7 to 21 of the experiment, although there was no significant increase in day 28 compared with control value.

Table 7. *Effect of Annona muricata Ethanol Stem Bark Extract on Liver ALP Activity in CCl_4 – Induced Oxidative Stress in Rats (U/L)*

Days	Control	CCl_4 Treated Rats	Rat Treated with AMESBE
Day 0	121.05±0.82	138.73±0.62	134.05±1.70
Day 7	122.72±2.50	159.72±0.31^{a,b}	136.43±3.51^{c}
Day 14	123.21±2.60	183.26±2.08^{a,b}	136.38±0.93^{c}
Day 21	125.38±0.70	198.46±0.55^{a,b}	139.38±1.66^{d}
Day 28	126.29±0.89	222.72±2.67^{a,b}	138.39±1.03

Results are expressed as mean ± S.D., n = 3.
^{a} p<0.001 Vs control, ^{b} p<0.001 Vs AMESBE Treated, ^{C} p<0.01; ^{d} p<0.05 Vs control.

As presented in Table 7, ALP activity is markedly elevated in CCl_4 treated animals, indicating liver damage. Serum activity of ALP was higher and significantly different in *Annona muricata* treated group than in control animals in day 7 to 21, although there was no significant increase in day 28 compared to the control value.

Table 8. *Effect of Annona muricata Ethanol Stem Bark Extract on the Activity of Catalase in CCl_4 – Intoxicated Rats (U/100 mg)*

Days	Control	CCl_4 Treated Rats	Rat Treated with AMESBE
Day 0	23.69±0.13	22.85±0.91	23.50±0.25
Day 7	23.14±0.45	21.72±1.06	22.57±0.63
Day 14	23.78±0.73	19.26±0.87^{a,b}	21.64±0.74^{c}
Day 21	24.05±2.31	17.57±0.56^{a,b}	20.53±1.31^{c}
Day 28	23.83±1.01	15.72±0.84^{a,b}	20.47±1.21^{c}

Results are expressed as mean ± S.D., n = 3.
^{a} p<0.001 Vs control, ^{b} p<0.001 Vs AMESBE Treated, ^{C} p<0.01 Vs control.

As presented in Table 8, CCl_4 treatment caused a significant (p<0.001) decrease in the level of catalase. Treatment with *A. muricata* resulted in a significant increase in catalase activity when compared with CCl_4 treated rats.

Table 9. *Effect of Annona muricata Ethanol Stem Bark Extract on Lipid Peroxidation (MDA) in CCl_4 – Intoxicated Rats (µmol of MDA/L)*

Days	Control	CCl_4 Treated Rats	Rat Administered AMESBE
Day 0	45.23±0.32	46.21±0.52	45.13±0.71
Day 7	45.35±0.17	60.57±0.49[a,b]	51.68±0.40[c]
Day 14	46.07±0.05	67.61±0.56[a,b]	53.89±0.71[c]
Day 21	46.52±0.25	71.94±0.46[a,b]	54.58±0.45[c]
Day 28	46.76±0.05	79.97±0.08[a,b]	56.08±0.87[c]

Results are expressed as mean ± S.D., n = 3.
[a] $p<0.001$ Vs control, [b] $p<0.001$ Vs AMESBE Treated, [C] $p<0.01$ Vs control.

Estimation of lipid peroxidation levels by thiobarbituric acid reaction showed a significant ($p<0.001$) increase in the serum level of MDA (Table 9) of rats intoxicated with CCl_4. *Annona muricata* significantly ($p<0.001$) prevented the increase in MDA and was brought to near normal.

4. Discussion

Virtually all plants have one or more phytochemical resident in their leaf, stem, root, fruit and flowers. Ethanol stem bark extract of *A. muricata* contains phytochemicals including tannins, flavonoids, saponins and alkaloids which are known to exhibit medicinal as well as physiological activities. Flavonoid are hydroxylated phenolic substances known to be synthesized by plants in response to microbial infection and they have been found to be anti-microbial substances against wide array of microorganisms *invitro* (Marjorie, 1996). They are also effective antioxidants and show strong anti-cancer activities (Okwu, 2004). The presence of these phytochemicals in *A. muricata* could be contributory to its antioxidant activity observed in this investigation. In the present experiment the order of increasing relative abundance of these phytochemical in the ethanol stem bark extract of *A. muricata* is Glycoside Saponin Alkaloid Flavonoid Total phenolic compounds (Table 1).

Traditional medicine all over the world is nowadays revalued by an extensive activity of research on different plant species and their therapeutic principles. Experimental evidence suggests that free radical and reactive oxygen species can be involved in a high number of diseases (Sajeesh *et al.,* 2011). As plants produce a lot of antioxidants to control the oxidative stress caused by sunbeams and oxygen, they can represent a source of new compounds with antioxidant activity.

Antioxidants (free radical scavengers) are chemicals that interact with and neutralize free radicals, thus preventing them from causing cellular damage in the biological system (Diplock *et al.,* 1998). The body makes some of the antioxidants it uses to neutralize free radicals. These antioxidants are called endogenous antioxidants. However, the body also relies on external sources, primarily the diet,to obtain the rest of the antioxidants it needs (Valko *et al.,* 2007). These exogenous antioxidants are commonly called dietary antioxidants. Fruits, vegetables, and grains are rich sources of dietary antioxidants (Bouayed and Bohn, 2010).

The *invitro* antioxidant potential of AMESBE was assayed using DPPH photometric assay and reducing power assay, while the *invivo* antioxidant potential was evaluated using serum catalase activity, and malondialdehyde level assay.

DPPH is a stable free radical at room temperature and accepts an electron or hydrogen radical. The reduction capability of DPPH radicals was determined by decrease in its absorbance at 517nm, which is induced by antioxidant.

The significant decrease in DPPH radical was due to the scavenging activity of *Annona muricata* ethanol stem bark extract (AMESBE). The reducing power of AMESBE was based on the ability of the extract to reduce molybdenum (VI) to molybdenum (V). The *invitro* antioxidant assay of AMESBE revealed that it has a potent antioxidant activity. DPPH is generally used to monitor chemical reactions involving radicals, most notably antioxidant assay (Sharma and Bhat, 2009).

The antioxidant compounds neutralize the free radical character of DPPH by transferring either electrons or hydrogen atoms to DPPH (Naik *et al.,* 2003), thereby changing the colour from purple to the yellow coloured stable diamagnetic molecule – diphenylpicrylhydrazine. The degree of discoloration indicates the scavenging potential of the extract or antioxidant in terms of hydrogen donating ability (Mosquera *et al.,* 2007). The antioxidant activity of AMESBE observed in this study could be due to the presence of phenolic compounds detected in it.

Other antioxidants work against the molecules that form from radicals, destroying them before they can begin the domino effect that leads to oxidative damage. For example, certain enzymes in the body such as superoxide dismutase, work with other chemical to transform free radical into harmless molecules.

The *invivo* antioxidant assay showed that AMESBE increased the activity of serum catalase. Catalase is an ubiquitous enzyme that catalyzes the decomposition of hydrogen peroxide, a reactive oxygen species (ROS) which is a toxic product of both normal aerobic metabolism and pathogenic ROS production (Kohen and Nyska, 2002). The increased serum activities of catalase as observed in this investigation suggest that AMESBE has an *invivo* antioxidant activity and is capable of ameliorating the effect of ROS in biologic system (Bakirel *et al.,* 2008).

ROS react with all biological substance; however, the most susceptible ones are polyunsaturated fatty acids. Reactions with these cell membrane constituents lead to lipid peroxidation (Bakirel *et al.,* 2008). In this investigation, the level of MDA is used as a marker of lipid peroxidation (LPO). The increase in MDA level in serum of intoxicated rats by CCl_4 suggests enhanced lipid peroxidation leading to tissue damage and failure of antioxidant defense mechanism to prevent formation of excessive free radicals. Treatment with AMESBE significantly reversed these changes (Table 9). Malondialdehyde is an endogenous genotoxic product of enzymatic and ROS – induced LPO whose adducts are known to exist in DNA isolated from healthy human being (Niedernhofer *et al.,* 2003).

In our study, the increase in the activity of catalase could lead to the inactivation of LPO reactions, hence decrease in MDA (Table 9).

Enzymes are used also as markers for toxicity. Tables 5, 6 and 7 showed the activities of such enzymes following administration of a toxicant (CCl_4) as against the herbal drug (AMESBE).

Administration of CCl_4 caused a significant ($p<0.001$) elevation of enzyme level such as AST, ALP and ALT when compared with control. There was a significant ($p<0.001$) restoration of these enzymes levels on administration of AMESBE. The reversal of the increased serum enzymes in CCl_4 – induced liver damage by AMESBE may be due to the prevention of the leakage of intracellular enzymes by its membrane stabilizing activity. This is in agreement with the commonly accepted view that serum levels of transaminases return to normal with the healing of hepatic parenchyma and the regeneration of hepatocytes (Thabrew and Joice, 1987).

5. Conclusion

Based on this study, we safely conclude that AMESBE possesses *invitro* and *invivo* antioxidant activity, and anti lipid peroxidation effect and may be employed in protecting tissue from oxidative stress. These may be the rationale behind some of its folkloric uses and also may be responsible for some of its pharmacologic effects.

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