

Comparative Antioxidant Activities in CCL₄ induced Liver damage in Albino Rats Treated with Aqueous and Methanol Leaf Extracts of Vitex doniana and Bombax buonopense

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Abstract: Comparative antioxidant activities of aqueous and methanol leaf extracts of *Vitex doniana* and *Bombax buonopense* in carbon tetrachloride (CCl₄) liver damage in rats were carried out using 114 albino rats. Group A with 6 rats was used as the normal control and it received normal saline and rat feed. Group B to F were given a single dose of 2.5ml/kg of CCl₄ in olive oil (1:1 v/v) via intra-peritoneal cavity before treatment. Group B with 6 rats were given the CCl₄ without treatment and served as negative control. Group C received standard drug (Liv.52) daily for 7 days at the dose of 4ml/kg and served as positive control. Group D and E received aqueous and methanol extract of *Vitex doniana* while groups F and G received aqueous and methanol extract of *Bombax buonopense*. Group D, E, F and G were sub-divided into 4 different groups with 6 rats each by random design method and treated with the extract for 7 days at the doses of 100, 200, 400 and 800 mg/kg body weight. In acute toxicity study no death was recorded even at 1600mg/kg doses but LD₅₀ for the two leaf extracts were recorded at 2000 and 3000mg/kg body weight for *Vitex doniana* and *Bombax buonopense* respectively. The result showed significant ($p < 0.05$) increments in catalase (CAT), superoxide dismutase (SOD) and glutathione (GSH) levels in the rats treated with aqueous and methanol extracts of *Vitex doniana* and *Bombax buonopense* leaves. A significant ($p < 0.05$) decrease in malondialdehyde (MDA) level was recorded in the rats that were treated with extracts of aqueous and methanol extract of *Vitex doniana* and *Bombax buonopense*. The result also showed a significant ($p < 0.05$) increase in CAT, SOD and GSH levels in a dose dependent manner in rats treated with *Vitex doniana* leaf extracts when compared with those treated with *Bombax buonopense* leaf extract. This study has shown that *Vitex doniana* leaf extracts have more antioxidant potential effect than *Bombax buonopense* leaf extracts, though both have shown good antioxidant potential effect in rats.

Keywords: Antioxidant activities, *Vitex doniana*, *Bombax buonopense*, CCl₄, aqueous and methanol extract

I. Introduction:

Oxidative stress referred to an imbalance between the systemic manifestation of reactive oxygen species and ability of biological systems to detoxify the reactive intermediates or to repair the resulting damage (Rice-Evans and Gopintha, 1995). The major active oxygen group include: superoxide radical (O[•]), hydrogen peroxide (H₂O₂), hydroxyl radical (HO), singlet oxygen (O₂), hydroperoxyl radical (HOO[•]), nitric oxide (NO[•]), ferryl ion (Fe⁴⁺O) and others. A molecule that has one or more unpaired electrons in its outmost shells is called free radical (Rice-Evans and Gopintha, 1995). These radicals tend to be very reactive. They attack bio-molecules such as lipid, protein and DNA. So this oxidative stress indicates the effect of reactive oxygen species that tends to degrade bio-molecules (Rice-Evans and Gopintha, 1995). Lipid peroxidation is used as markers for oxidative stress which destruct cell membrane. Lipid peroxidation also involves the oxidation of unsaturated fatty acid in various pathological conditions to generate malondialdehyde (MDA) and 4-hydroxyalkanals upon decomposition (Esterbauer et al., 1991). Superoxide dismutase converts superoxide radicals into O₂ and H₂O₂. They can be found in extracellular fluid and also in almost all cells where they protect the cell exposed to oxygen. Catalase is also one of the antioxidant enzymes that are found in organism that are exposed to oxygen and it decomposes H₂O₂ to H₂O and O₂. High concentration of hydrogen peroxide is deleterious to cells and causes oxidation of cellular targets such as DNA, protein, and lipids, leading to mutagenesis and cell death (Chelikani et al., 2004).

When the body is exposed to chemical substances such as chlorobenzene, hexachlorobenzene, carbon tetrachloride (CCl₄) chloroform and heavy metals such as mercury, lead, arsenic and cadmium are toxic to the gastrointestinal tract, liver and gall bladder. Liver congested with this toxin will not allow flow of blood back to heart from any organ and these results to a stiffing situation leading to oxidative stress (Chelikani et al., 2004).

The rates at which people are exposed to these toxicants are in its increase. Leaf of *Vitex doniana* and *Bombax buonopense* are common medicinal plants used locally in the treatment of liver diseases. *Vitex doniana*

is commonly known as black plum. It is locally known as 'Uchakiri' in Igbo, 'Dinya' in Hausa, 'Oori-nla' in Yoruba, 'Tinya' in Fulani, 'Vabga' in Ghana, 'Mfuru' in Tanzanian and 'Muhonozi' in Uganda (Atawodi et al., 2003). *Bombax buonopense* is widely distributed in Africa. It belongs to the family of Malvaceae and its Common vernacular name include 'Vabga' in Ghana, 'Kurya' in Hausa, 'Akpu' in Igbo and 'Ogbolo' in Yoruba (Atawodi et al., 2003).

There is paucity of information on the protective effect of these local medicinal plants. Since there is high cost of available drugs for the treatment of liver related diseases, therefore result of this research work would contribute immensely in solving the health problem in both developed and developing countries of the world, hence the global research has turned toward the use of medicinal plants with little or no side effect in the prevention/treatment of diseases/ailments. This study therefore evaluated the comparative protective effect of *Vitex doniana* and *Bombax buonopense* leaves on toxic effect of CCl₄ in albino rats.

II. Materials and Methods

Materials

Collection of *Vitex doniana* and *Bombax buonopense* Leaves

This study was conducted in March, 2014 in Biochemistry Department, Ebonyi State University, Abakaliki. The leaf of *Vitex doniana* and *Bombax buonopense* were collected from Abakaliki in Abakaliki Local Government Area of Ebonyi State, Nigeria. The plants were identified and authenticated by a taxonomist in the Department of Applied Biology, Ebonyi State University, Abakaliki.

Preparation of *Vitex doniana* and *Bombax buonopense* leaves in powdered form: Fresh leaves of *Vitex doniana* and *Bombax buonopense* were collected, washed and dried at ambient temperature (25°C) and the leaves were turned severally to avoid fungi growth. After drying, the leaves were pulverized using electric blender. The leaf powders were stored in refrigerator in well labelled and air tight container prior for the extraction.

Preparation of *Vitex doniana* and *Bombax buonopense* leaves Extracts: Exactly 100g of powdered leaves of *Vitex doniana* and *Bombax buonopense* were weighed and soaked 1500 ml into conical flasks and allowed for 24 hours. After 24 hours of soaking the aqueous extracts were obtained by filtering the mixtures repeatedly with muslin cloth to obtain the filtrates and residues. The aqueous extracts were gotten by subjecting the filtrates into rotary evaporator. The same steps were taken to obtain the methanol extracts. The extracts were kept in sealed containers and stored in refrigerator at 2-4°C until required.

Experimental Animals: A total of 114 male albino rats weighing (200-300g) were used. They were acclimatized for two weeks and maintained at normal room temperature (25°C) in the animal house of Department of Biochemistry, Ebonyi state university, Abakaliki. Before starting the experiment all the animal were weighed weekly. They were kept in cages, fed on commercial rats feed and allowed free access to clean water.

III. Experimental Design:

Acute Toxicity Test:

Acute toxicity study of aqueous and methanol extracts of *Vitex doniana* and *Bombax buonopense* leaves were carried out according to the method of Sawadogo et al., (2006) and as was described by Adedapo et al., 2009.

Sub-Acute Toxicity Study:

In this study, 114 male rats were randomly assigned into 7 groups (i.e. Group A, B, C, D, E, F and G). Group A with 6 rats was used as the control and it received normal saline (vehicle) and normal rat feed. Group B to F were given a single dose of 2.5ml/kg of CCl₄ in olive oil (1:1 v/v) via intra-peritoneal cavity before treatment. Group B with 6 rats were given the CCl₄ without treatment and served as positive control. Group C received standard drug (Liv.52) daily for 7 days at the concentration of 4ml/kg. Group D, E, F and G received aqueous and methanol extract of *Vitex doniana* and *Bombax buonopense*. Group D, E, F and G were subdivided into 4 different groups with 6 rats each by random design method and treated with the extract for 7 days at the doses of 100, 200, 400 and 800 mg/kg body weight. The rats were starved for 24 hrs and then sacrificed for analysis. Also, the animals were weighed on the first day and on the last day of the study.

Collection of samples from animals: After the treatment period, the rats were anaesthetized using chloroform and dissected using dissecting tools. The bloods were drawn directly from the heart using syringe. Blood was stored in bottles without anticoagulant. The blood specimens were centrifuged at 3000rpm for 10 min to separate the serum from the red cells. After centrifugation, the serum were separated for biochemical assays and stored in specimen bottles ready for analysis.

Biochemical Analysis: MDA in serum was determined by the method described by Ohkawa et al., 1979. Reduced Glutathione (GSH), Catalase (CAT) and Superoxide Dismutase (SOD) were determined according to the method described by Oyedemi et al., 2010.

Statistical Analysis: Data obtained were subjected to one way analysis of variance (ANOVA). Means were compared for significance using Duncan's multiple range test ($p < 0.05$)

IV. Results and Discussion

The result showed significant ($p < 0.05$) increments in catalase (CAT), superoxide dismutase (SOD) and glutathione (GSH) levels in a dose dependent manner in rats treated with aqueous and methanol extracts of Vitex doniana and Bombax buonopense leaves as shown in Figure 1-4. A significant ($p < 0.05$) decrease in malondialdehyde (MDA) level was recorded in the rats that were treated with extracts of aqueous and methanol extract of Vitex doniana and Bombax buonopense as shown in Figure 1-4. The result also showed a significant ($p < 0.05$) increase in CAT, SOD and GSH levels in a dose dependent manner in rats treated with Vitex doniana leaf extracts when compared with those treated with Bombax buonopense leaf extract as shown in Figure 1-4.

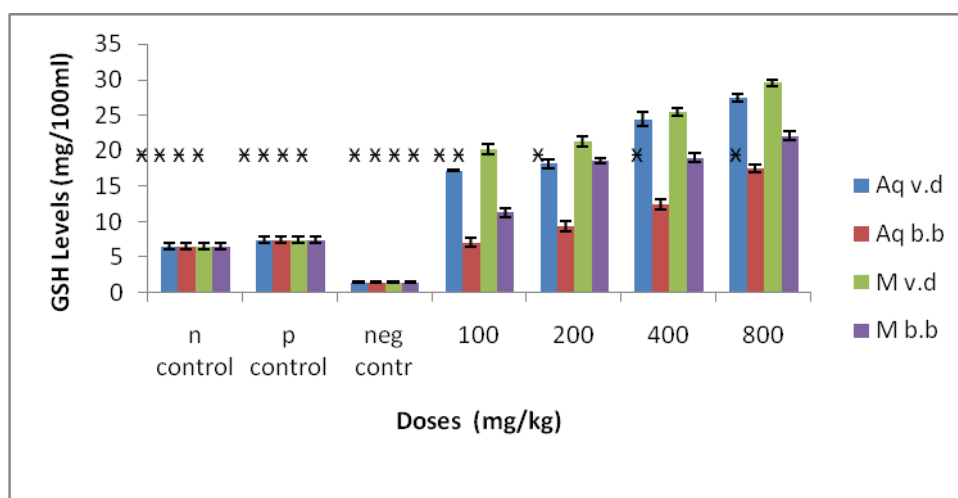


Figure 1: GSH Level in CCl₄ induced Liver damage in Albino Rats treated with aqueous and methanol extracts of Vitex doniana and Bombax buonopense leaf.

Data are shown as mean \pm S.D (n=6). Mean values in bars with (*) have significant differences ($p < 0.05$) when compared the normal controls.

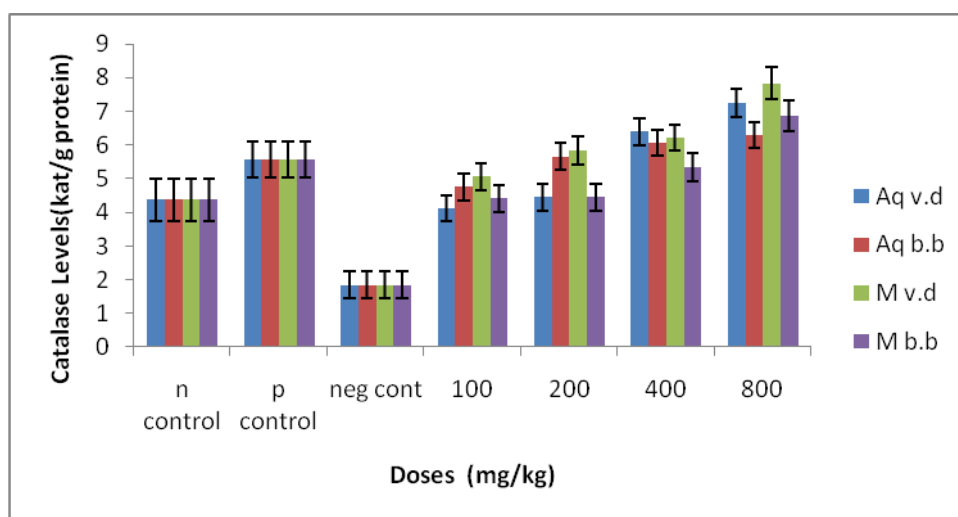


Figure 2: Catalase Level in CCl₄ induced Liver damage in Albino Rats treated with aqueous and methanol extracts of Vitex doniana and Bombax buonopense leaf.

Data are shown as mean ± S.D (n=6). Mean values in bars with (*) have significant differences (p<0.05) when compared with the controls.

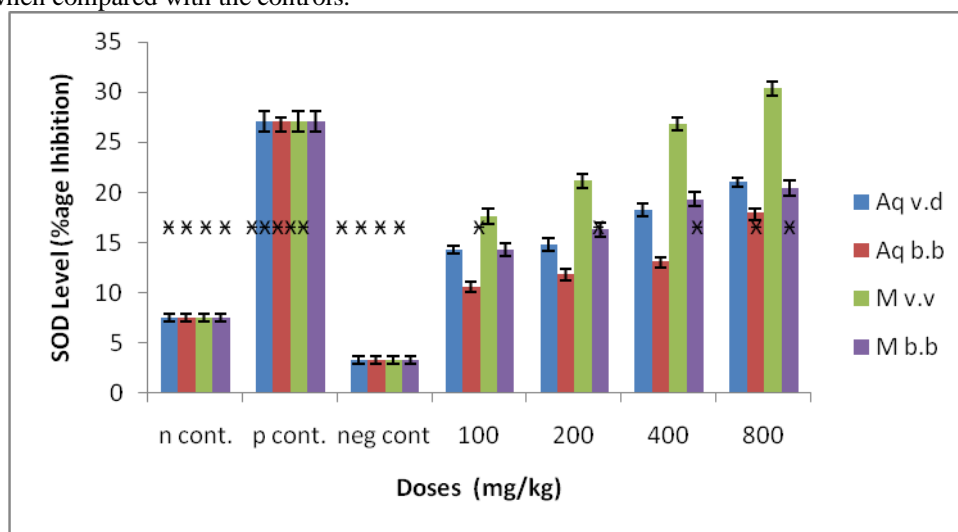


Figure 3: Level of SOD in CCl₄ induced Liver damage in Albino Rats treated with aqueous and methanol extracts of Vitex doniana and Bombax buonopense leaf

Data are shown as mean ± S.D (n=6). Mean values in bars with (*) have significant differences (p<0.05) when compared with the controls.

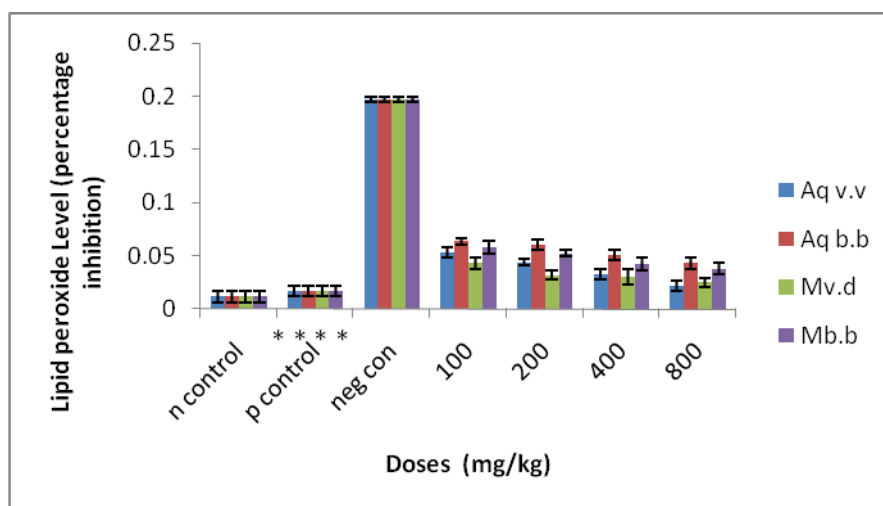


Figure 4: MDA Level in CCl₄ induced Liver damage in Albino Rats treated with aqueous and methanol extracts of Vitex doniana and Bombax buonopense leaf.

Data are shown as mean ± S.D (n=6). All show significant difference (p<0.05) when compared with the control.

Vitex doniana and Bombax buonopense leaf extracts significantly (P<0.05) increase the level of serum superoxide dismutase (SOD), glutathione (GSH) and catalase (CAT) in a dose dependent manner and significantly decreased the level of MDA in rats treated with the extracts. These findings are in correlation with the report of Aja et al. (2015) that mice treated with ethanol Leaf extracts of Cymbopogon citratus and Hyptis spicigera at 200,400, and 800 mg/kg body weight significantly (P<0.05) increased the level of reduced glutathione (GSH), peroxidase activity, activity of superoxide dismutase (SOD) and catalase (CAT) activity in doses dependent manner while the administration of the same extracts significantly (P<0.05) decreased the activity of lipid peroxidation marker(MDA) in a doses dependent manner. Aja, (2014) also reported that Moringa oleifera seed and leaf extracts significantly increased (P <0.05) serum superoxide dismutase (SOD), glutathione (GSH) and catalase (CAT) levels at all the doses. Lipid peroxidation is an established mechanism of cellular injury and is used as an indicator of oxidative stress. From the result obtained so far, lipid peroxidation decrease with increase in dose of the extract in the treated groups. In oxidative conditions, the level of the

glutathione can be considerably diminished through conjugation to xenobiotics, and be secreted as glutathione conjugates and glutathione disulfide from affected cells (Sies et al., 1994). These glutathione are antioxidant that provides excellent support to liver. Superoxide dismutase has been reported as one of the most important enzymes in the enzymatic antioxidant defence system (Oyedemi et al., 2010). Superoxide dismutase removes superoxide anion by converting it to hydrogen peroxide, and thus diminishing the effect caused by this radical.

These antioxidant enzymes depend on various essential trace elements and prosthetic groups for proper molecular organization and enzymatic action. Increase in SOD activity should accelerate the removal of the reactive oxygen species (El-Bahr, 2013). Catalases are enzymes involve in breaking the hydrogen peroxide produced by the action of SOD into water and oxygen. So action of SOD activity along with that of catalase explains the decrease in MDA level, which is an indicator of oxidative stress that persist in the cell, so the decrease in MDA level in the present study is as a result of increase in the activities of CAT and SOD as observed in the study. Any natural compound with anti-oxidant properties may help in maintaining healthy system when continuously taken as components of dietary foods and drugs.

V. Conclusion

This study has shown that *Vitex doniana* leaf extracts have more antioxidant potential effect than *Bombax buonopense* leaf extracts, though both have shown good antioxidant potential effect in rats. These effects may be responsible for the potential use of *Vitex doniana* and *Bombax buonopense* in the prevention and treatment of liver diseases.

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