



Cholinergic and Antioxidant Properties of *Blighia sapida*: Potential as a Biological Pest Controlling Agent



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Abstract

Natural products which are capable of reducing the activities of cholinergic and metabolic enzymes are safer pest-controlling alternatives to synthetic ones. This study compared the ability of *Blighia sapida* stem-bark extract and Rambo, (a synthetic pesticide), to interfere with acetylcholinesterase (AChE), glutathione S-transferase (GST), and biomarkers in the brain, liver, and blood of Wistar rat. The brain and liver of rats were excised and blood was collected into heparinized tubes at the end of a 28-day experiment for biochemical investigations. The activities of AChE and GST decreased at a dose-dependent rate ($P < 0.05$). There was no significant difference in the alkaline phosphatase (ALP) of the treated groups and a dose-dependent increase in total protein concentration was detected. The stem-bark extract did not significantly alter liver biomarkers like ALT, AST, and ALP, particularly at repeated low doses of 50 and 100 mg/kg. The extract of *Blighia sapida* reduced AChE and GST activity; a property that could be exploited in the formulation of pest control agents.

Keywords: Hydro-alcohol extract, Acetylcholinesterase, Glutathione S-transferase, Biomarkers

INTRODUCTION

Since ancient times, man has engaged in agriculture to meet his food needs and used pesticides to prevent pre-and post-harvest losses of crops due to pests (Saroj *et al.*, 2020). Crop infestation by pests is a threat to global food security and its control with synthetic pesticides is associated with health and environmental problems (Wilson and Huber, 2021). Pesticides are chemicals formulated to destroy, control, or prevent pests but because their mode of action is not specific, they also affect non-target organisms including humans (Kaur *et al.*, 2019; Nartop *et al.*, 2020). Synthetic pesticides exhibit harmful effects such as neurological, behavioral dysfunctions, hormonal imbalances, kidney and liver disorders, genotoxicity, and others, due to their persistence in the environment and absorption by humans/animals (Ravindran *et al.*, 2016; Malhat *et al.*, 2018). The environmental and health effects of synthetic pesticides have

led to the search for safer alternatives from natural sources (Chowdhary *et al.*, 2018).

Plants exhibit pesticide activity by interfering with cholinergic and antioxidant enzymes such as acetylcholinesterase and glutathione S-transferase in target pests. Acetylcholinesterase (AChE) terminates nerve impulses by catalyzing the hydrolysis of a neurotransmitter, acetylcholine into acetic acid and choline in the nervous system of various organisms, while GST play an important role in pesticide resistance and detoxification (Yang *et al.* 2008; Gullner *et al.*, 2018). The primary cytosolic enzymes known as glutathione S-transferases catalyze the conjugation of electrophile molecules with reduced glutathione (GSH), resulting in the transformation of potentially hazardous compounds into more water-soluble and less toxic ones (Dobritzsch *et al.*, 2020).

Culturally, *Blighia sapida* stem-bark powder is usually mixed with seeds of African finger (*Abelmoschus esculentus*) and pearl millet

(*Pennisetum glaucum*) before sowing to prevent insect attacks. To the best of our knowledge, there is no scientific support for this practice, hence this study.

MATERIALS AND METHODS

Preparation of Hydro-alcohol extract

Blighia sapida stem bark was obtained, identified, authenticated, and milled to powder form using an electrical Grinding Machine (SR-14733, Marlex, Daman). The powder material (1066.71 g) was macerated in 70 % (v/v) ethanol/water for 72 hours at room temperature using the method of Handa *et al.* (2008). The resulting suspension was filtered and concentrated with a rotary evaporator at 40°C to yield a residue termed ethanol extract (EE).

Experimental Animals

Thirty Wistar rats, weighing between 150 and 220 g were obtained from the Department of Anatomy, University of Ibadan, Ibadan, Nigeria. The rats were acclimatized to the laboratory conditions for two weeks in the Animal House. Rats were fed with standard pellets obtained from Ladokun Feeds, Ibadan, Oyo State, Nigeria, and allowed free access to clean water. The principle of laboratory animal care (NIH publication No. 85–23) guidelines and procedures were followed for the study (NIH Publication Revised, 1996).

Experimental Design

Thirty (30) rats were randomly divided, following the method of Adekola *et al.* (2020), into five groups, and each group contained six rats.

Group 1–received 1.0 ml of distilled water; Group 2 –received Rambo pesticide 10% (w/v); Group 3 –received 50 mg/kg EE; Group 4 –received 100 mg/kg EE; Group 5 –received 150 mg/kg EE

Based on the result of the acute test (LD₅₀) conducted, three doses (50, 100, and 150 mg/kg) of *B. sapida* ethanol extract were chosen for the study. The rats were treated by gavage administration of the extract and insecticide ‘Rambo’, which was obtained from market, containing 0.6% permethrin (synthetic pesticide) every 2 days for 28 days. On the 28th day, the animals were made to fast overnight before

being sacrificed under light chloroform anesthesia. The blood was obtained through the cardiac puncture, and organs of interest were excised. Collected blood samples were kept in separate heparinized tubes, while the organs were stored in plain sample bottles. The samples were labeled and used for the estimation of biochemical parameters.

Determination of biochemical parameters

In the liver and brain homogenates, the biochemical parameters were measured using the techniques outlined by Klein and Kaufman (1967) for ALP, Lowry *et al.* (1951) for total protein, Habig *et al.* (1974) for GST, and Voss and Sachsse (1970) for AChE.

Histopathological examination

Liver samples were cleared from adhering tissues, fixed in 10% formal saline, dehydrated through ascending grades of alcohol (50%, 70%, 80%, 90%, and 100%) - and cleared in xylene. The wax-infiltrated tissues were embedded in paraffin wax and 5µm thick sections were prepared from the tissues using a LEICA rotary microtome. These sections were floated in a water bath (45 °C) to allow spreading of the folded sections and mounted on glass slides. Dewaxed slide sections were then rehydrated in descending grades of alcohol (100%, 90%, 80%, 70%, and 50%) and stained with hematoxylin and eosin. Sections were differentiated in a mixture (1% hydrochloric acid in 70% alcohol) to remove excess dye from the tissues. The stained tissues were observed under the microscope (LEICA DM750) interfaced with a LEICA (ICC50) camera.

Statistical analysis

The data obtained were analyzed using one-way analysis of variance (ANOVA) followed by Tukey–Kramer multiple comparisons test using the software Graph pad Prism 5. The statistical significance was set at $p < 0.05$ level of significance. Values were expressed as mean $\pm$ standard error of the mean (SEM).

RESULTS

The acute toxicity test revealed that the extract was non-toxic at the highest dose (5000 mg/kg) tested. No toxic symptoms or mortalities were observed in the two phases of the acute toxicity

test ($LD_{50} > 5000$ mg/kg). There was a significant decrease in AChE activities of the experimental animals that received a high dose (150 mg/kg). There was also a significant decrease in the plasma GST activities at 100 and 150 mg/kg extract administration (Table 1). The decrease in these enzymes was similar to that observed for the Rambo pesticide treatment, which was significant when compared to the control group. However, there was no significant difference in the activity of alkaline phosphatase in the test groups as compared to the control group. A dose-dependent increase in protein concentration was observed (Table 1). Histopathological investigation of liver sections in control rats showed normal cellular integrity and normal lobular architecture with central veins and radiating cords of hepatocytes, separated by blood sinusoids of the liver. However, no marked lesion or congestion was observed in the groups treated with 50, 100, and 150 mg/kg of *B. sapida* extract. Mild lesions and portal congestion were observed in the group that was administered with 10% (w/v) Rambo pesticide (Figure 1).

DISCUSSION

In this study, no death was recorded during the acute and sub-acute toxicity tests with graded doses of ethanol extract of *B. sapida*. No symptom of toxicity was noticed and no significant changes in appearances were observed when the treated groups were compared with the control group. The extract of *B. sapida* decreased the activity of cholinergic and antioxidant enzymes in Wistar rats administered with high doses in a similar pattern as a synthetic pesticide (Rambo). This effect is typical of botanicals that interrupt the proper functioning of insect nervous system, causing uncontrollable excitation, tremor, and, death as a result of an accumulation of acetylcholine (Malhat *et al.*, 2018).

Accumulation of acetylcholine at neuron synapses and neuromuscular junctions has been reported to cause effects such as paralysis and muscle weakness (Yilmaz *et al.*, 2016).

Glutathione S-transferases (GST) is the main enzyme that catalyzes the conversion of foreign chemicals to hydrophilic, less toxic, and easily excreted materials from the system (Lee *et al.*, 2015; Dobritsch *et al.*, 2020). A low dose (50 mg/kg) of the extract exhibited no significant reduction in GST activities, however, higher doses of 100 mg/kg and 150 mg/kg caused a significant reduction in the activity of GST. The findings of this study are similar to that of Oni *et al.* (2019) in which administration of *Acalypha wilkesiana* extract reduced GST and caused mortality of *Callosobruchus maculatus*. Similarly, Ebadollahi *et al.* (2013) and Tarigan *et al.* (2016) have reported pesticidal activity of different botanical extracts that reduced GST activity. The observed effects of the *B. sapida* extract on the activities of AChE and GST indicate that the metabolites in the extract may serve as an effective botanical pesticide.

Alkaline phosphatase is a membrane-bound enzyme and it plays a critical role in the transportation of metabolites across the cell membrane, conditions such as liver damage and bone disease lead to its alteration which affects the transport of metabolites and membrane permeability (Simon *et al.*, 2019). Alkaline phosphatase has been referred to as a family of zinc metalloenzymes, and an increase in its activity is an indication of biliary process obstruction (Shirode *et al.*, 2019). The ALP activity of the rats treated with different doses of ethanol extract of *B. sapida* showed no significant difference when compared to the control rats. This indicates that the extract may not cause cellular leakage of the enzyme and obstruction of normal biliary process. The results of ALP in this study implied that phytoconstituents in the plant extract were neither toxic nor caused damage to the membrane at the doses considered, hence, the transportation of metabolite remains intact. This result was also confirmed by the histopathological examination of the liver (Figure 1), in which the micrograph of the liver of rats treated with different doses of the plant extract did not have lesions.

Table 1: Effect of hydro-alcohol extract of *Blighia sapida* on biochemical parameters of Wistar rats

Groups	Control	Synthetic	Extract	Extract	Extract
Parameters	0 mg/kg	10 % (w/v)	50 mg/kg	100 mg/kg	150 mg/kg
AChE (U/L)	240.14 ± 6.18*	175.67 ± 30.42***	226.85 ± 2.46*	224.43 ± 4.47*	210.16 ± 2.03**
GST (µmol/mg)	256.48 ± 12.73*	134.37 ± 7.42***	211.57 ± 7.06*	209.61 ± 20.09**	196.64 ± 8.44**
ALP (U/L)	0.022 ± 0.003	0.024 ± 0.008	0.017 ± 0.001	0.019 ± 0.001	0.022 ± 0.003
AST (U/L)	290.87 ± 5.83	302.94 ± 10.49	300.02 ± 11.48	302.37 ± 5.87*	308.19 ± 13.54*
ALT (U/L)	8.79 ± 1.09	19.07 ± 0.97**	9.46 ± 1.50	17.47 ± 1.30*	19.13 ± 0.84**
TP Liver (g/L)	126.26 ± 0.75	135.15 ± 6.58	103.79 ± 0.62**	117.42 ± 7.17*	125.03 ± 6.97

Values are presented as Mean ± SEM of seven (7) replicates.

Values with () are significantly different at $p < 0.05$ when compared to the control group (group 1).*

AChE: Acetylcholinesterase, ALP: Alkaline phosphatase,

GST: Glutathione S-transferases, TP: Total protein,

AST: Aspartate aminotransferase, ALT: Alanine aminotransferase.

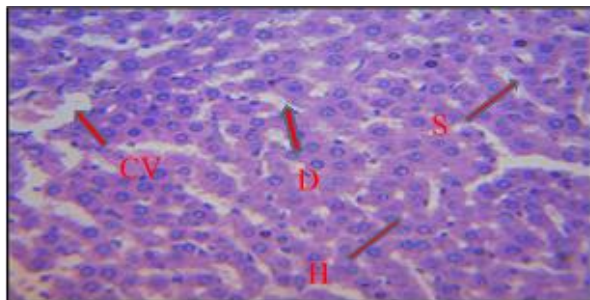


Plate 1A: Micrograph of the liver of rats in control group showing no lesion (x 300)

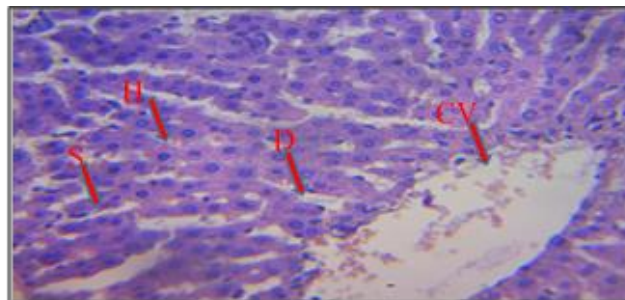


Plate 1B: Micrograph of the liver of rats treated with 10% synthetic Rambo showing mild portal congestion (x300)

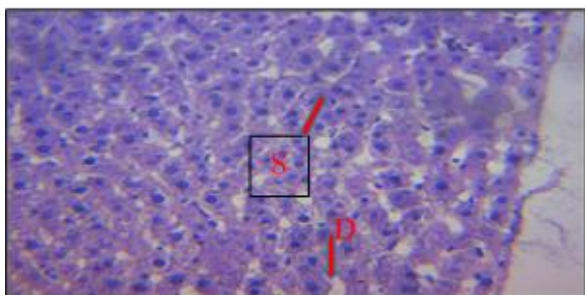


Plate 1C: Micrograph of the liver of rats treated with 50mg/kg of BSE showing no lesion (x300)

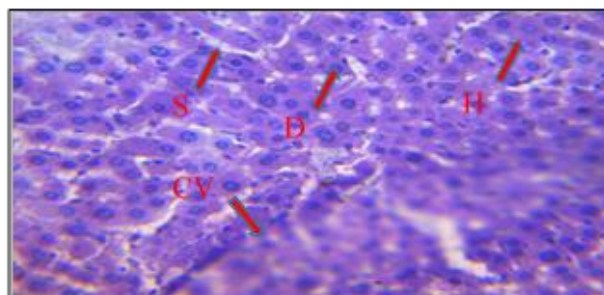


Plate 1D: Micrograph of the liver of rats treated with 100mg/kg of BSE showing no lesion (x 300)

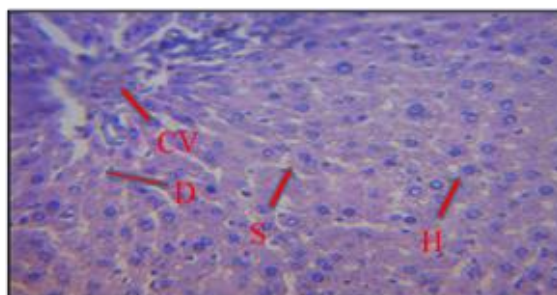


Plate 1E: Micrograph of the liver of rats treated with 150mg/kg of BSE showing no lesion (x300)

Figure 1: Photomicrographs of liver sections from control, 10% Rambo and *B. sapida* treated rats

NOTE: Central veins (CV), Hepatocytes (H), Sinusoids (S), Bile duct (D) and Magnification (x300)

Plasma alkaline phosphatase is one of the liver biomarker enzymes, whose evaluation provides details of pathological damage to the tissue (Mu'azu *et al.*, 2020). A high level of plasma ALP activity has been linked with hepatobiliary dysfunction, which is likely a result of hepatobiliary injury and cholestasis (Ore and Olayinka, 2015). The non-significant effects on ALP activities observed suggests that the doses of the extract considered may not affect the hepatobiliary function of the liver.

The liver is mainly involved in the synthesis of plasma protein, damage to which causes a reduction in the protein concentration (Adeoti *et al.*, 2017). A dose-dependent increase in protein concentration was observed in the groups that were administered with different doses of ethanol extract of *B. sapida* which implies that the extract is not toxic at the doses considered because a decrease in the level of total protein has been associated with damage to the liver function caused by toxicity (Simon *et al.*, 2019). The histopathological examination of the liver in control rats showed normal cellular integrity and normal lobular architecture with central veins (CV) and radiating cords of hepatocytes (H), separated by blood sinusoids (S) of the liver. However, no marked lesion or congestion was observed in the groups treated with 50, 100, and 150 mg/kg of *B. sapida* extract. A mild lesion and portal congestion was observed in the group that was administered with 10% (w/v) Rambo pesticide. However, Adekola *et al.* (2020) reported distinct hepatocytes, clear central vein, and mildly congested sinusoids in rats administered with 250 mg/kg ethanol extract of *B. sapida*.

CONCLUSION

The hydro-alcohol extract of *B. sapida* lowered the level of acetylcholinesterase and glutathione-S-transferases activities. This suggest that *B. sapida* has a neurodegenerative ability peculiar to pesticide agents. Hence, the use of the plant extract as an agent for the mitigation of pests in agriculture could be employed.

ABBREVIATIONS

ACH_E: Acetylcholine esterase; GST: Glutathione S-transferases; ALP: Alkaline phosphatase; DEET: Diethyltoluamide; GSH: Glutathione; mg/kg: Milligram/kilogram; EE: Ethanol extract; w/v: Weight/volume; SEM: Standard error of the mean.

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