Biochemical and Toxicological Studies of *Bridelia micrantha* [Berth] and *Mitracarpus villosus* [Swartz] DC Extracts used as Biofumigant Against Stored Produce Insect Pests on Albino Rats

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A B S T R A C T

Background:
In recent years, plant materials have been widely explored as sources of insect pest control agents with little or no study on their toxicity. The present study aimed to detect the biochemical alterations in liver and kidney associated with acute oral toxicity of the extracts of *B. micrantha* and *M. villosus* in albino rats.

Methods:
Twenty seven albino rats, weighing between 150-180g were used and divided into nine groups of three rats each, administered with different doses of each extracts (0, 500, 1000, 1500 and 2000mg/kg). The plasma and homogenates of liver and kidney of the rats were investigated for the activities of alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (ALT), total protein, urea and creatinine, using standard laboratory kits.

Results:
The administration of either extract did not cause death or any hazardous symptoms of acute toxicity, nor resulted in any evident changes in the body weight. However, the extracts caused significant decreases in the levels of ALT, AST, ALP and total protein, urea and creatinine in biochemical parameters. They also caused a significant decrease in the serum parameters of treated rats' liver and kidney at all doses.

Conclusions:
The results demonstrated that the oral administration of *B. micrantha* ethyl acetate extract and of *M. villosus* petroleum ether extract may be considered as moderately free of toxicity. This was based on our findings that two compounds were moderately safe with respects to their effects on the liver and kidney functions at concentrations of up to 2000 mg/kg body weight of the rats.

Keywords:
Biochemical Agent; Creatinine; Extracts; Homogenates; Plasma; Serum

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INTRODUCTION

Plant materials, as sources of bioactive compounds, play a leading role in controlling insect pests since old times in the Tropical and Subtropical continent of the world. Pesticide residues, environmental and health challenges associated with the adoption of synthetic insecticides have rekindled interest in plant derived natural insecticides as potential replacements for synthetic agents. Various plant products have offered numerous biological activities like antibacterial, antifungal and anti-insect activities.

B. micrantha (Family: Euphorbiaceae) and *Mitracarpus villosus* (Sw) DC (Family: Rubiaceae) are indigenous medicinal plant species in Nigeria and are used traditionally in the treatment of some aliments (1, 2). These plants also possess insecticidal activities against

Sitophilus zeamais, *Podagrica uniforma*, *Dysdercus superstitious,* and malaria vectors (3-7).

With the growing interests in the application of plant materials in the management of insect pests and coupled with the reported medicinal properties and insecticidal activity of certain plants, there is an urgent need for conducting scientific studies on their potential toxicities.

Liver is the principal site of the conversion and cleansing of xenobiotics, and; therefore, is expressly susceptible to these agents (8). Similarly, kidneys, as the major organs for the elimination of xenobiotics and the metabolites, are particularly predisposed to their harmful effects (9). Damage to these organs often results in rises in the clinical biochemical parameters, such as serum liver enzymes: AST, ALT and ALP, and total protein, and urea and creatinine, as markers of the impairment of renal functions (10). Adebayo *et al.* (11) and Anthonio *et al*. (12) have reported that AST, ALT and ALP levels in tissues and blood are important marker enzymes, used to assess the integrity of cell membrane, cytosolic activity and cell death in various organs.

This study was designed to investigate the biochemical alterations in rats' liver and kidneys secondary to the acute oral toxicity of *B. micrantha* ethyl acetate and *M. villosus* petroleum ether extracts, used as biofumigant against the effect of stored product insect pests on albino rats.

MATERIALS AND METHODS

Collection of Plant Materials and Preparation of Extracts: Leaves of *B. micrantha* and *M. villosus* plants were collected from Rufus Giwa Polytechnic, Owo Ondo State, Nigeria. The plant materials were identified at the Department of Forestry and Wood Technology, Rufus Giwa Polytechnic, Owo, Ondo State, Nigeria (Latitude 7° 11' N and Longitude 5° 35' E) were a specimen voucher was issued. The leaves were washed in clean water to remove dirt and dried in shade at room temperature for two weeks. To avoid volatilization, the materials were pulverized using electric blender (Binatone model; Japan) and packaged in a polythene bag until used.

Two hundred grams (200g) of the plant powder was sequentially extracted with a series of solvents in order of increasing polarity viz., petroleum ether, hexane, ethyl acetate, acetone, chloroform and methanol, using Soxhlet apparatus (India). The solvents were removed using rotary vacuum evaporator under reduced pressure at 60°C.The residue was dissolved in a known volume of methanol and assessed for insecticidal potential by fumigant toxicity. The active extract which showed maximum effect, was evaluated for the biochemical and toxicological activity in albino rats.

Experimental Animals: The 27 healthy, adult albino rats weighing between 150-180g were housed in cages under ambient environmental conditions of alternating 12h light/dark cycles with free access to rat food and clean water *ad libitum*. The animals were acclimatized for a period of two weeks before the starting the study. All animals were handled according to the standard ethical provisions for using experimental animals (13).

Experimental Design and Acute Toxicological Tests: After acclimatization, animals were arranged in a completely randomized order, comprising three rats per experimental and control groups with free access to food and water *ad libitum* as follows:

Group I: Control rats.

Group II: Rats treated with 500 mg/kg *B. micrantha* ethyl acetate extract

Group III: Rats treated with 1000 mg/kg *B. micrantha* ethyl acetate extract

Group IV: Rats treated with 1500 mg/kg *B. micrantha* ethyl acetate extract

Group V: Rats treated with 2000 mg/kg *B. micrantha* ethyl acetate extract

Group VI: Rats treated with 500 mg/kg *M. villosus* petroleum ether extract

Group VII: Rats treated with 1000 mg/kg *M. villosus* petroleum ether extract

Group VIII: Rats treated with 1500 mg/kg *M. villosus* petroleum ether extract, and

Group IX: Rats treated with 2000 mg/kg *M. villosus* petroleum ether extract.

Doses of each extract were administered through oral cannula to protect the oral cavity from being injured. All of the experimental animals were separately observed for mortality or signs of toxicity throughout the experiments.

Biochemical Analysis: At the end of the experimental period, the final body weights of the animals were measured and were then sacrificed by cervical decapitation after an overnight fast. The blood samples were centrifuged at 3000rpm for 5min. and the supernatants (plasma) were separated. The liver and kidneys homogenates were centrifuged at 2000rpm for 15 minutes and the supernatants (serum) were stored at -20°C until they were used for the biochemical analyses (14). The plasma and sera from the homogenates of liver and kidneys were tested for the levels of alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (ALT), total protein, urea, and creatinine, using standard laboratory kits (Randox, UK). The biochemical activities were measured following standard laboratory assays and procedures (15-16).

Statistical Analyses: The data thus obtained were statistically analysed by one-way analysis of variance (ANOVA) and significant treatment means were separated at ($p<0.05$), using the Tukey's multiple regression tests. Treatment data were presented as the means ± standard error of the mean (SEM).

RESULTS

Effect of B. Micrantha and M. Villosus Extracts on Body Weight: There was no observed significant differences ($p>0.05$) among the experimental groups

versus the control with respect to the body weight gain in rats administered with various dosages of *B. micrantha* ethyl acetate and *M. villosus* petroleum ether extracts (Table 1). Although, rats administered with 1000 mg/kg *M. villosus* extract had the least body weight gains (0.85%). Also, irrespective of the extract dosages, no signs of behavioral changes, mortality or morbidity were observed throughout the study groups. Based on the data, it was evident that high concentrations of either extracts (2000mg/kg) were not lethal to the rats.

Table 1. Effect of *B. micrantha* and *M. villosus* extracts on body weight of Albino rats

Treatment Group	Initial weight (g)	Final weight (g)	% Weight gain
I	178.34	181.48	1.76
II	169.51	173.03	2.07
III	172.07	175.67	2.09
IV	167.92	170.04	1.26
V	170.40	172.68	1.34
VI	171.41	173.37	1.14
VII	169.77	171.22	0.85
VIII	173.55	175.86	1.33
IX	171.09	174.82	2.18

Each value is the mean for 3 rats per group.

Liver Biochemical Indices: The result of the biochemical effects of *B. micrantha* and *M. villosus* extracts on albino rats indicated that the responses were does-dependent. The levels of AST, ALT, ALP and total protein decreased with increasing the extracts doses (Table 2). Rats administered with 500mg/kg of the extracts showed the highest biochemical activities, while those treated with 2000mg/kg had the lowest values. However, AST, ALT, and ALP at dose of 2000mg/kg showed significantly different activity compared with those noted in the control group ($p<0.05$). No significant differences were detected between doses at 500mg/kg and 1000mg/kg. The total protein concentration had a significant reduction compared to those for the control group at all doses ($p<0.05$).

Toxicity of B. Micrantha and M. Villosus Extracts on Serum Biochemical Parameters: Serum levels of AST, ALT and ALP in rats administered with varying doses of *B. micrantha* and *M. villosus* extracts are presented in Table 3. The result showed that the serum levels progressively and significantly ($p<0.05$) increased with the increasing dosages of the extracts. Rats treated with 2000mg/kg showed the maximum levels of the liver enzymes. However, in spite of the increases in the serum levels of all treated rats, insignificant increases were noted in the groups treated with 500 and 1000mg/kg compared to those in the control group.

Table 2. Toxicity of *B. micrantha* and *M. villosus* extracts on some Liver Biochemical Indices of Albino Rats.

Plant Extracts/ Conc. (ml)	Aspartate aminotransferase (AST) IU/g	Alanine aminotransferase (ALT) IU/g	Alkaline phosphatase (ALP) IU/g	Total protein min (x 10^{-1})
500mg/kg				
B. micrantha	170.25±4.20^{b}	122.50±1.11^{b}	28.50±1.11^{b}	1.62±0.01^{b}
M. villosus	165.25±4.20^{b}	117.50±2.11^{b}	22.00±1.11^{b}	1.53±0.02^{b}
1000mg/kg				
B. micrantha	163.25±4.20^{b}	116.50±2.10^{b}	28.50±1.10^{b}	1.50±0.02^{b}
M. villosus	162.00±4.21^{b}	115.00±2.21^{b}	22.00±1.11^{b}	1.50±0.04^{b}
1500mg/kg				
B. micrantha	148.50±4.10^{a}	102.75±2.28^{a}	10.75±1.08^{a}	0.80±0.03^{a}
M. villosus	143.75±4.28^{a}	107.25±2.20^{a}	9.00±1.10^{a}	0.80±0.03^{a}
2000mg/kg				
B. micrantha	146.25 ±4.20^{a}	100.50±2.10^{a}	9.25±1.10^{a}	0.75±0.02^{a}
M. villosus	137.25 ±4.20^{a}	100.50±2.10^{a}	8.75±1.14^{a}	0.70±0.02^{a}
Untreated	172.75 ±2.17^{b}	125.25±2.20^{ab}	28.50±1.11^{b}	1.64±0.01^{b}

Each value is the mean ± standard error for three replicates. Mean in the same column with the similar superscripts are not significantly different at ($p<0.05$) using the New Duncan's Multiple Range Test.

Table 3. Toxicity of *B. micrantha* and *M. villosus* extracts on some serum biochemical parameters of Albino rats

Plant Extracts/ Conc. (mg/kg)	Aspartate aminotransferase (AST) IU/g	Alanine aminotransferase (ALT) IU/g	Alkaline phosphatase (ALP) IU/g
		500mg/kg	
B. micrantha	48.50±2.10^{a}	40.25±2.20^{a}	24.00±1.10^{a}
M. villosus	49.50±2.10^{a}	45.50±2.10^{a}	27.25±1.20^{a}
		1000mg/kg	
B. micrantha	52.00±2.10^{a}	47.00±2.10^{a}	25.25±1.20^{a}
M. villosus	55.25±2.20^{a}	48.25±2.20^{a}	29.25±1.20^{a}
		1500mg/kg	
B. micrantha	68.00±2.10^{b}	60.25±2.20^{b}	40.00±2.10^{b}
M. villosus	76.00±2.10^{b}	71.50±2.10^{b}	40.50±1.15^{b}
		2000mg/kg	
B. micrantha	88.50 ±2.15^{c}	83.00±2.10^{c}	56.50±2.15^{c}
M. villosus	89.25 ±2.20^{c}	88.00±2.10^{c}	60.00±2.10^{c}
Untreated	45.00 ±2.21^{a}	38.50±2.10^{ab}	19.00±1.11^{b}

Each value is the mean ± standard error for three replicates. Means in the same columns with similar superscripts are not significantly different at ($p<0.05$) using the New Duncan's Multiple Range Test.

Toxicity of B. Micrantha and M. Villosus Extracts on the Kidney Biochemical Parameters: The administration of *B. micrantha* and *M. villosus* extracts was associated with changes in kidney biochemical serum parameters (Tables 4 and 5). The data showed a significant dose-dependent response in treated rats compared to the control group ($p<0.05$).

Table 4. Toxicity of *B. micrantha* and *M. villosus* Extracts on some Kidney Biochemical Parameters of Albino Rats.

Plant Extracts/ Conc. (mg/kg)	Urea (mmol/l)	Creatinine (mmol/l)
	500mg/kg	
B. micrantha	31.00±2.10^{b}	164.25±3.20^{b}
M. villosus	29.00±2.10^{b}	157.50±3.15^{b}
	1000mg/kg	
B. micrantha	25.25±2.20^{b}	159.00±3.10^{b}
M. villosus	24.25±2.20^{b}	153.00±3.10^{b}
	1500mg/kg	
B. micrantha	20.00±2.10^{a}	132.25±3.20^{a}
M. villosus	19.50±2.15^{a}	126.50±3.15^{a}
	2000mg/kg	
B. micrantha	18.50±2.15^{a}	127.00±3.10^{a}
M. villosus	17.25±2.20^{a}	123.25±3.20^{a}
Untreated	33.50±2.15^{a}	166.25±3.20

Each value is the mean ± standard error for three replicates. Means followed by the same letter along the column are not significantly different (P>0.05), using New Duncan Multiple Range Test.

Table 5. Toxicity of *B. macrantha* and *M. villoscus* Extracts on some Serum Biochemical Parameters of Albino Rats.

Plant Extracts/ Conc. (mg/kg)	Urea (mmol/l)	Creatinine (mmol/l)
500mg/kg		
B. macrantha	16.00±1.10^{a}	51.50±2.15^{a}
M. villoscus	19.25±1.20^{a}	53.25±3.20^{a}
1000mg/kg		
B. macrantha	19.50±2.15^{a}	54.00±2.10^{a}
M. villoscus	24.00±1.10^{a}	55.50±2.15^{a}
1500mg/kg		
B. macrantha	44.00±2.10^{b}	89.50±2.15^{b}
M. villoscus	45.25±2.20^{b}	91.00±2.10^{b}
2000mg/kg		
B. macrantha	59.00±2.10^{c}	114.50±3.15^{a}
M. villoscus	62.00±2.10^{a}	115.50±3.15^{a}
Untreated	13.25±1.20^{a}	45.00±2.10

Each value is the mean ± standard error of three replicates. Means followed by the same letter.

DISCUSSION

B. micrantha and *M. villosus* have been shown to have insecticidal properties (5, 7) suggesting that they could be used for the development of new chemical compounds for the management of insect pests. In spite of its efficacy, there are growing concerns about the toxicity of plant products in last decade due to their bioactive contents. However, few toxicological studies have been conducted to determine if the plants' bioactive molecules are safe, particularly if the primary intention is to protect food grains for human consumption.

The insignificant differences ($p>0.05$) noted in the body weight gain at the end of the experiments in all of the doses of extracts administered to the rats compared to those in the controls, suggest that the extracts were not lethal to the rats. This observation also suggests that the extract did not alter the metabolic processes of the treated rats, which might have subsequently affected the hormones and body weight (17). The observed increases in body weight could be attributed to the extracts' nutritional properties (18) without significant alterations in the energy metabolism of the rats (19). This is consistent with the findings reported by Oshomoh *et al.* (20) who showed that weight changes in rats orally administered with aqueous extract of *B. micrantha* were not significantly different between the control and the treated groups. Konate (21) also observed insignificant differences in weights and no other detectable clinical signs in rats treated with the aqueous and acetone extracts *of Cienfuegosia digitate.*

Liver is a vital organ actively involved in many metabolic and biochemical processes, and is the frequent target for many toxins (22). Hepatic damages are linked to alterations in the metabolic functions of this organ (23). Renal and hepatic function analyses are very useful for the screening of the toxicity of drugs and plant extracts, as both are important for the survival of an organism (24). Our data revealed that the extracts mildly impaired the kidney functions in the treated rats since the given doses slightly increased the levels of urea and creatinine.

The increase in ALP level suggests the likelihood of the extracts at high concentrations caused cell membrane damage in the liver. However, increased ALP activity is needed during stress to produce adequate amount of phosphate groups for oxidative phosphorylation leading to ATP generation. This in turn, is required for the phosphorylation of some biomolecules, such as ethanolamine and choline to form phosphatidyl ethanolamine and choline, which are vital to the stability of cellular plasma membrane (11). Apart from the significant decreases and increases in AST, ALT and ALP levels in the liver and serum biochemical indices, no deviation of these parameters was detected in rats treated with various doses of the extracts. Consequently, liver impairment did not occur in rats, which further suggests that both extracts were most likely safe. The lack of adverse effect of *B. micrantha* and *M. villosus* extracts on renal function indices in rats may suggest that the typical functioning of the nephrons at the glomeruli level was not affected.

When administered at a dose of 2000mg/kg, the extracts caused either significant increases or decreases in the levels of AST, ALT ALP and total protein in serum and liver biochemical parameters, respectively. This is consistent with the findings of Oshomoh (20); Mohammed et al. (25) and Manal et al (26) who reported a similar trend in rats administered with the aqueous extract of *B. micrantha*, butanol fraction of *Buchholzia coricea* and ethyl acetate extracts of *Maerua psuedopetalosa*.

Protein is crucial for growth and replacement of worn-out tissues. The rate of cell divisions is determined chiefly by the rate of protein synthesis (27). Serum or plasma is a very complex mixture of proteins and their determination is clinically valuable, which reveals the systemic and biochemical status of the individual. The decline in total proteins ($p<0.05$) in this study suggests that the extracts reduced the liver's synthetic capacity for proteins in rats treated with *B. micrantha* ethyl acetate and *M. villosus* petroleum ether extracts compared to those in the control rats. This corroborate the findings of Atere and Ajoa (28) who reported a decreasing trend in serum total proteins in rats administered with the crude alkaloidal fraction of *Gnestis ferruginea* roots. These results also suggest liver impairment secondary to the plant extract treatments (29). Dhanotiya (30) believes that liver damage decreases plasma protein, which might be the result of a decline in the protein absorption from the small intestine (31), which is also considered as a sign of impaired synthetic function of the liver. The lower the doses of extracts, the higher were the urea and creatinine activities of the kidney biochemical parameters. In the serum, the higher the extract doses, the higher were the urea and creatinine levels. In this study, increased serum urea was documented in treated animals. This may indicate impaired renal function due to the adverse effects of the extracts. This is consistent with the findings of Tanuja *et al*. (32). Serum urea level differs directly with protein intake and inversely with the rate of excretion. Creatinine is the waste product formed in the muscles by its metabolism and is synthesized in the liver, passes into the circulation and is taken up almost entirely by the skeletal muscles (33). An increase in the creatinine level may indicate the renal damage, and increase in serum creatinine reflects the extent of tubular necrosis (34). Generally, our results suggest that the toxicity of both extracts against serum and biochemical parameters is dose dependent. This is consistent with the findings of Poonkodi, *et al.* (35) who reported that the anti-inflammatory effect of *M. villosus* extract was dose dependent.

CONCLUSIONS

The findings of this study revealed that ethyl acetate and petroleum ether extracts of *B. micrantha* and *M. villosus* had mild to moderate effects on the treated rats. The extracts slightly increased and reduced the levels of biochemical and serum parameters linked to the liver and kidney functions, respectively. Therefore; our findings may suggest that both extracts exerted mild toxicity effects. However, for the ultimate safety, future investigations on the genotoxicity, mutagenesis and reproductive toxicity are recommended to elucidate the adverse effects of the extracts evaluated in this study.

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CONFLICT OF INTERESTS

The authors declare no conflict of interests in the course of conducting this study.

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