EUROPEAN JOURNAL OF BIOMEDICAL AND PHARMACEUTICAL SCIENCES
http://www.ejbps.com

ISSN 2349-8870
Volume: 9
Issue: 4
09-18
Year: 2022

ASSESSMENT OF THE AMELIORATIVE POTENTIALS OF DICHLOROMETHANE EXTRACT OF *MUSA ACUMINATA* CAVENDISH BRACT IN INDOMETHACIN ADMINISTERED WISTAR RATS

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Article Received on 26/01/2022 **Article Revised on 15/02/2022** **Article Accepted on 07/03/2022**

ABSTRACT
This study investigated the ameliorative potentials of dichloromethane extract of *Musa acuminata* Cavendish bract in indomethacin administered Wistar rats. Forty five adult male Wistar rats (120-180g) were divided into five groups of nine rats each and a single dose of Indomethacin (40mg/kg bodyweight) was orally administered to study groups and control. Concentrations of 100mg, 150mg and 200mg/kg bodyweights dichloromethane extract of *M. acuminata* Cavendish bract were orally administered to the animals for three weeks. Dichloromethane extract of sample bract was analysed by Gas chromatography-mass spectrometry (GC-MS) screening while faecal occult blood (FOB) of rats was carried out using immunochromatographic method. The effect on some biochemical indices, hematological indices and histopathology of kidney, stomach and liver of rats were analysed. GC-MS analyses showed the presence of fatty acids; n-Hexadecanoic acid, 9,12-Octadecadienoic acid and 9-Octadecanoic acid as the predominant compounds in the sample analysed. FOB tests were positive (+) at week 0 and week one, then negative (-) at weeks two and three in all treated groups. Compared to the control (21.70 ± 0.12%, 7.24 ± 0.04g/dl, and 3.75 ± 0.05x10^9/L), a significant dose-dependent increase ($p \leq 0.05$) in packed cell volume, hemoglobin and red blood count was observed in treated groups (36.80 ± 0.17%, 12.27 ± 0.06g/dl and 5.70 ± 0.11 x10^9/L) respectively. Serum levels of Aspartate-aminotransferase, Alanine-aminotransferase, Alkaline-phosphatase, creatinine and urea showed a dose-dependent decrease significantly at ($p \leq 0.05$) in treated groups compared to the untreated control. Histology examination of tissues showed no degenerative changes in study groups. The study confirmed that *M acuminata* Cavendish at 100mg, 150mg and 200mg/kg improved hematological indices of rats and had no deleterious effect on the liver and kidney tissues of Wistar rats administered indomethacin.

KEYWORDS: Gas chromatography-mass spectrometry (GC-MS), *Musa acuminata* cavendish bract, indomethacin, gastric injury, faecal occult blood, liver and kidney function.

1. INTRODUCTION
Musa acuminata Cavendish is a perennial herb belonging to the family *Musaceae.* Cavendish cultivars belong to the AAA genome group, which includes the cultivars that have three sets of chromosome inherited from the wild species *Musa acuminata* as ancestor (Schiota, 1993). It is a widely consumed fruit in the tropical and subtropical regions mostly for its great taste and medicinal value. The flowers are used in ethnomedicine to treat bronchitis and diabetes, the young leaves are applied to the body to relieve pain from burns, the roots are used to treat digestive problems, and the sap has been used in treating epilepsy, fevers, diarrhoea, and can also relieve haemorrhoids, insect bites and stings (Lia *et al.*, 2017). The potassium in *M. acuminata* Cavendish fruit have been reported to help prevent muscle cramp (Fingolo *et al.*, 2012). The fruit peel and pulp also have antifungal and antibiotic properties and sap from the leaf is used medicinally as a remedy for red eyes, and young leaves for skin swelling (Lia *et al.*, 2017). According to Pushpangadan *et al.* (1999), *M. acuminata* Cavendish leaves are used by the tribes of India for bandaging cuts, blisters, ulcers and burns, and leaf ashes dissolved in water are given to patients to relieve acidity, indigestion and flatulence. *M. acuminata* Cavendish has been found to be rich in carotenoids (lutein and beta-carotene), organic acids such as malic acids, several active amines and sugars (Schiota, 1993). Chemical analysis of *M. acuminata* Cavendish inflorescences (male bud and bracts) revealed considerable nutritional value, with high potassium and fibre and low calories (Fingolo *et al.*, 2012).

Indomethacin is a non-steroidal anti-inflammatory drug (NSAID), commonly used in the treatment of pain, fever, and inflammation (Ozbakis *et al.,* 2007). Although the gastric mucosa may protect itself by the secretion of substances stimulated by prostaglandins, however it has been reported that NSAIDs block the function of cyclooxygenase 1 (COX-1), which is essential for the production of these prostaglandins (Wallace, 2008). As found by Schwartz (2009), patients with rheumatoid arthritis and osteoarthritis who take indomethacin have 15-20% annual incidence of gastric lesions. More than half of patients who present with gastric lesion hemorrhage or perforation report the recurrent use of NSAIDs, including aspirin and Indomethacin (Schwartz, 2009). Various therapeutic agents utilized in management of/and regimens for treating NSAID-induced gastric lesions include H_2 blockers (ranitidine), proton pump inhibitors (pantoprazole), antacids and prostaglandins analogues such as misoprostol (Dharmani and Palit, 2006). However, reports of adverse effects and relapse in the long run has been the rationale for the development of newer, non-toxic drugs (Akinwumi and Mubo, 2019). Herbal medicine from plant origin is fast emerging as a substitute treatment to available synthetic drugs for gastric disorder management possibly due to lower costs, fewer adverse effects, availability and perceived effectiveness. Hence, this study was designed to assess the ameliorative potentials of dichloromethane extract of *Musa acuminata* Cavendish bract in indomethacin administered Wistar rats.

2. MATERIALS AND METHODS

2.1 Chemicals / Reagents

Indomethacin and dichloromethane were procured from Joe Chemicals Limited, Port Harcourt, Nigeria. Hematoxylene and Eosin Stains were products of Abbey colors, Philadelphia, USA. One step faecal occult blood rapid test kit was a product of Atlas Medical, United Kingdom. Reagent Assay test kits were all products of Randox Laboratories Limited, Ardmore diamond road, United Kingdom.

2.2 Sample Collection and Preparation

Fresh bracts of *Musa acuminata* Cavendish were harvested in Eleme, Rivers State, Nigeria. After sample collection, the fresh bracts were properly washed using tap water and ground using an electric blender (BL-460 kenwood, England). A measured amount (250ml) of bract sample was macerated in 2.5 litres of dichloromethane for seventy-two hours. The resulting solution was filtered and the filtrate was concentrated in a rotary evaporator (XMTE-7000 Genser Scientific Instruments, Germany) at 40^0C. Total recovery of dichloromethane was however achieved at 42^0C. The extract was again further concentrated in a water bath (KW-1000DB HH-6 Wincom Company Ltd, China) at 40^0C for 72hrs yielding 17g extract.

2.3 Gas Chromatography/ Mass Spectrometry Analysis

The milled sample of *Musa acuminata* Cavendish was extracted in dichloromethane after soaking for five days. Up to 10g of sample was weighed into well stoppered bottles and 20mls of the organic solvent (dichloromethane) added. The mixture was vigorously agitated and left to stand for five days. The crude extract was collected by filtering into quartz beakers. The process was repeatedly carried out for two more consecutive times. The combined aliquots collected were concentrated on a steam bath to 5ml. This was purified by passing through a Pasteur pipette packed with silica gel and anhydrous sodium sulphate on a membrane and air dried to about 2ml for gas chromatographic analysis.

The extract of the sample was subjected to GC/MS analysis, this group of powerful instruments interface helped to characterize the various compositions. The gas chromatographic Model: 7890A (GC) analysis was performed on an Agilent Technologies interfaced with Mass Selective Detector model: 5975C (MSD). The electron ionization was at a 70v with an ion source temperature at 250 ^{o}C. Highly pure helium gas (99.9% purity) was used as carrier gas, while HP-5 (30mm X 0.25mm X 0.320µm) was used as the stationary phase. The oven temperature was at 60 ^{o}C held for 0.5 minute and ramped to 140 ^{o}C at the rate of 4 ^{o}C/minutes holding for a minute, then ramped to 280 degrees while holding for 5 minutes at the rate of 8 ^{o}C /minutes. Up to 1µ/l was auto injected into Agilent Tech. model 5973 mass spectrophotometer. The constituent compounds were then identified using the Chem-Office software attached to the MS-library. The names and structures of the component organic acids were confirmed using the database of National Institute of Standard and Technology (NIST).

2.4 Animal Studies and Treatments

Forty five male Wistar rats weighing between 120-180g were obtained from the animal house of the Department of Pharmacology, University of Port Harcourt using standard cages. The rats were acclimatized for seven days with water and feed *ad libitum.* Thereafter, the animals were fasted for twenty-four hours, divided into five groups of nine rats each and a single oral dose of indomethacin 40mg/kg bodyweight was administered to all groups except group 1 (normal control) using the method of Adinortney *et al.,* (2013). Faecal occult blood tests were carried out in all groups administered indomethacin and positive tests were obtained after eight hours before commencing treatment. Group 1 (normal control) received only distilled water. Group 2 (negative control) received only indomethacin 40mg/kg bodyweight. Groups 3, 4 and 5 were treated with 100mg, 150mg and 200mg/kg bodyweight of extract respectively for three weeks. After one week of extract administration, the animals were weighed and three animals sacrificed from each group. Faecal occult blood

tests were carried out and blood samples were collected by cardiac puncture in heparin and EDTA bottles. Determination of hematological and biochemical parameters were carried out. These procedures were repeated for week two and week three. Thereafter, the abdomen of the sacrificed animals were opened and the stomach, kidneys and liver excised and immediately fixed in 10% formaldehyde for histological examinations.

2.5 Determination of Faecal Occult Blood

The faecal sample was collected by dipping the sample collection stick into three different places of the stool sample. The stick was then returned to the sample collection device, screwed tightly and vigorously agitated. The test device was removed from its foil pouch by tearing along its notch. Holding the sample collection device upright, the tip was carefully broken off and 2-3 drops of the sample solution was squeezed onto the test sample pad. The result was read within 5-10 minutes where presence of lines at both control and test areas indicated positive while a single line in control area indicated negative.

2.6 Hematological Indices

Packed Cell Volume (PCV), Hemoglobin (Hb), Red Blood Count (RBC), Platelet count and White Blood Count (WBC) were determined using an auto-analyzer machine (Mindray BC-6800, China).

2.7 Biochemical Analysis

Liver and kidney function markers; Alanine aminotransferase (ALT), Aspartate aminotranferase (ALT), Alkaline phosphatase (ALP), Creatinine and Urea were determined using commercial prepared Kits by Randox Laboratories Ltd, United Kingdom.

2.8 Histopathology of the Kidney, Stomach and Liver

The liver, kidney and stomach tissues were immediately fixed to prevent any degenerative or autolytic changes by fixing in 10% formaldehyde.. Thereafter, dehydration was carried out by immersing tissues in ascending grades of alcohol (50%, 70%, 90%) and lastly 100% (absolute alcohol). The alcohol in tissues was cleared out by immersing in a clearing agent (xylene). Tissues were then embedded by placing in molten paraffin wax at a constant temperature of 56-60^{0}C in a paraffin bath, after which, fresh paraffin wax and embedding mould was used to form a tissue block. For microtomy/sectioning, the tissue block was first cast on a wooden block before taking to the microtome for sectioning using the microtome knife or blade. The sectioned tissues were then fixed on the slides and lastly stained using hematoxylene and eosin stains. The slides were examined under a light microscope (Olympus CX-31, Japan) using x400 magnification.

2.9 Statistical Analyses

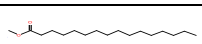
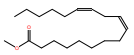
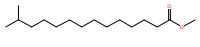
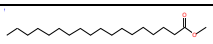
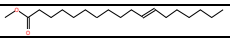
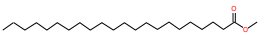
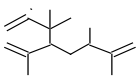
All statistical analyses were carried out using IBM SPSS Version 23. Data were expressed as Mean $\pm$ Standard deviation of three replicate determinations. Experimental groups were compared using a one-way analysis of variance (ANOVA) as mean values in each column with same superscript letters were considered not significant at $p>0.05$.

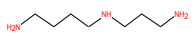
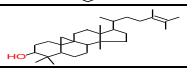
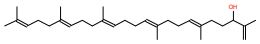
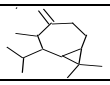
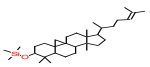
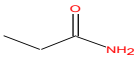
3.0 RESULT AND DISCUSSION

3.1 Gas Chromatography / Mass Spectrometry Analysis

The GC-MS analysis of dichloromethane extract of *M. acuminata* Cavendish bract revealed the names, retention time, percentage concentration, molecular weight, molecular formula and structures of eighteen bioactive compounds present in the sample analysed as shown in Table 1.

Table 1. Bioactive components of dichloromethane extract of *Musa acuminata* Cavendish bract.

S/N	Compound	Retention Time (min)	Percentage of the total	Molecular Formula	Molecular Weight	Structure
1	Hexadecanoic acid, methyl ester	14.563	19.83	$C_{17}H_{34}O_2$	256.420	
2	9,12-Octadecadienoic acid, (Z,Z) methyl ester	15.930	9.71	$C_{19}H_{34}O_2$	294.472	
3	Methyl-13-Methy tetradecanoate	13.411	1.74	$C_{16}H_{32}O_2$	296.500	
4	9-Octadecanoic acid (Z)-, methyl ester	16.001	8.35	$C_{19}H_{36}O_2$	296.500	
5	Methyl Stearate	16.230	4.56	$C_{19}H_{38}O_2$	298.504	
6	11-octadecanoic acid	16.044	6.95	$C_{19}H_{36}O_2$	296.488	
7	Docosanoic acid, methyl ester	18.982	2.35	$C_{23}H_{46}O_2$	354.610	
8	1-Methylene-2b-hydroxymethyl-3,3-dimethyl-4b-(3-	19.582	3.52	$C_{15}H_{26}O$	222.366	

	methybut-2-enyl) cyclohexane					
9	p-Hydroxy-norephedrine	14.411	1.74	$C_{16}H_{13}NO_2$	167.200	
10	3-Propoxy amphetamine	19.778	1.85	$C_{14}H_{23}O_3$	253.340	
11	1,4-Butanediamine, N-(3-aminopropyl)	19.916	1.09	$C_{14}H_{47}N_6O_{12}$	584.480	
12	Norpseudoephedrine	20.068	2.76	$C_{19}H_{13}NO$	151.210	
13	9,19-Cyclolanost-24-en-3-ol, (3.beta.)-	20.240	4.96	$C_{30}H_{50}O$	426.700	
14	1,6,10,14,18,22-Tetracosa hexaen-3-ol, 2,6,10, 15,19, 23-hexamethyl-, (all-E)-(. +/-.)-	20.320	6.05	$C_{30}H_{50}O$	426.717	
15	Cyclopropane-1,1-dimethyl-2-(2-methyl-2-propenyl)-	20.420	5.87	C_9H_{16}	124.223	
16	Alloaromadendrene	20.506	7.97	$C_{15}H_{24}$	204.361	
17	9,19-Cyclolanost -24-en-3-ol, (3.beta.)-, TMS derivative	20.620	4.49	$C_{31}H_{52}O$	440.700	
18	Propanamide	20.887	1.21	C_3H_7NO	73.0390	

Ethno-pharmacological knowledge has contributed greatly to scientific investigations into herbal medicine and their mechanism of action. Various medicinal plants and dietary nutrients have been shown to possess gastro-protective properties (Kath and Gupta, 2006). GC-MS analysis of sample revealed the presence of fatty acids that vary in chain length and position of double bonds. Fatty acids have been known to possess antifungal and antibacterial properties (Wallace 2008). Saturated fatty acids such as hexadecanoic acid, inhibit the formation of potent inflammatory mediators through its inhibition of the enzyme phospholipase A_2 thus exerting anti-inflammatory effects (Vasudevan *et al.,* 2012). The essential fatty acid 9,12-octadecadienoic acid is a precursor to arachidonic acid which give rise to three groups of eicosanoids; prostaglandins (PG), thromboxanes, leukotriene and lipoxins which are physiologically and pharmacologically active compounds (Bhagavan, 2002). Physiologically, they are considered to act as local hormones functioning through G-protein-linked receptors to elicit their biochemical effects. Thromboxanes cause vasoconstriction and platelet aggregation. Prostacyclins (PGI2) are produced by blood vessel walls and are potent inhibitors of platelet aggregation. Prostaglandins on the other hand are known to play important roles in mucosal cytoprotection by promoting mucus and bicarbonate secretion, on surface epithelial cells, mucosal circulation and prevention of hemorrhagic lesions by aggregation of platelets when required thus having a protective effect on the gastric mucosal (Wallace, 2008). Other bioactive compounds present have also been reportedly found to play roles in both therapeutics and nutrition, exerting anti-inflammatory, anti-oxidant, anti-arthritis, anti-rheumatoid and anti-allergy (Choudhury *et al.,* 2019).

3.2 Faecal Occult Blood Test (FOB)

Faecal occult blood test result for both control and study groups are presented in Table 2. Faecal occult blood test was positive in all treated groups administered 40mg/kg bodyweight indomethacin at week zero and week one. However, at weeks two and three, FOB tests were negative in treated groups administered 200mg, 150mg and 100mg/kg bodyweight dichloromethane bract extract of *Musa acuminata* Cavendish, compared to untreated control. This shows the gradual ability of the extract to ameliorate the effect of indomethacin-induced gastric mucosal injury, in agreement with Mahadeva *et al.* (2016) that *Musa* specie has significant gastro protective effect. This finding is not in agreement with Pannangpetch *et al.* (2001) who reported that only *Musa paradisiaca* promotes gastric mucosal healing by a similar mechanism as prostaglandins.

Table 2: Faecal occult blood test of indomethacin administered Wistar rats treated with dichloromethane bract extract of *Musa acuminata* Cavendish.

Group	FOB Test	Week 0	Week 1	Week 2	Week 3
1	Rat chow and water (Normal Control)	-	-	-	-
2	40mg/kg b.w Indomethacin (Negative Control)	+	+	+	+
3	40mg/kg b.w Indomethacin + 200mg/kg b.w *M. acuminata* Cavendish	+	+	-	-
4	40mg/kg b.w Indomethacin + 150mg/kg b.w *M. acuminata* Cavendish	+	+	-	-
5	40mg/kg b.w Indomethacin + 100mg/kg b.w *M. acuminata* Cavendish	+	+	-	-

3.3 Effect of Dichloromethane Bract Extract of Sample on Haematological Parameters

Hematological parameters of the control and study groups for weeks one, two and three are shown in Tables 3-5.

At week one of treatment, a significant increase ($p \leq 0.05$) in packed cell volume (PCV), haemoglobin (Hb) and red blood count (RBC) in treated groups 4 and 5 administered 150mg and 100mg/kg bodyweight of extract respectively, was observed compared to the control. PCV and Hb of treated group 3 administered 200mg/kg of extract had no significant difference compared to control (group 2). A significant decrease ($p \leq 0.05$) in white blood count (WBC) and platelet count in treated groups 3, 4 and 5 administered 200mg, 150mg and 100mg/kg bodyweight of extract respectively, was observed when compared to untreated control (group 2). The most significant increase ($p \leq 0.05$) was observed in group administered 150mg/kg bodyweight of the extract (group 4) when compared to the untreated control (group 2) and to treated groups 3 and 5 (Table 3).

After two weeks of extract administration, a gradual steady trend was observed as the values of PCV, Hb and RBC continued to increase significantly at ($p \leq 0.05$) in treated groups 3, 4 and 5 while decreases were observed significantly ($p \leq 0.05$) in WBC and platelets values in treated groups compared to untreated control group 2 (Table 4).

Three weeks of extract administration reveal significant increases ($p \leq 0.05$) in hematological parameters; PCV, HB and RBC, in treated groups 3, 4 and 5 administered 200mg, 150mg and 100mg/kg bodyweight of extract respectively compared to untreated control group 2. Within treated groups, the most significant increases were observed in groups 4 and 5 administered 150mg and 100mg/kg bodyweight of extract respectively when compared to treated group 3 administered 200mg/kg bodyweight of extract. Similarly, WBC and Platelet counts decreased significantly ($p \leq 0.05$) in treated groups compared to untreated control group 2 (Table 5).

Table 3: Hematological parameters of rats after one week treatment with dichloromethane bract extract of *Musa acuminata* Cavendish.

Group	PCV (%)	Hb (g/dl)	WBC ($x10^9$/L)	RBC ($x10^9$/L)	Platelets ($x10^9$/L)
1. Rat chow and water (Normal Control)	40.70 ± 0.17^{a}	13.57 ± 0.06^{a}	8.04 ± 0.02^{a}	6.23 ± 0.25^{a}	281.33 ± 0.58^{a}
2. 40mg/kg b.w Indomethacin (Negative Control)	21.90 ± 0.03^{bc}	7.30 ± 0.01^{bc}	10.00 ± 0.01^{b}	3.87 ± 0.12^{b}	304.00 ± 2.00^{b}
3. 200mg/kg b.w Extract	22.60 ± 0.62^{bc}	7.53 ± 0.21^{bc}	8.70 ± 0.12^{c}	4.38 ± 0.03^{ce}	297.69 ± 1.15^{c}
4. 150mg/kg b.w Extract	34.00 ± 0.23^{d}	11.33 ± 0.08^{d}	8.30 ± 0.02^{d}	5.16 ± 0.17^{d}	284.67 ± 0.48^{d}
100mg/kg b.w extract	24.80 ± 1.00^{e}	8.27 ± 0.33^{e}	8.61 ± 0.01^{e}	4.43 ± 0.03^{ce}	288.00 ± 1.00^{e}

Values are means ± SD of triplicate determinations. Mean values with similar superscript letters along the column are not statistically significant at p>0.05. PCV= Packed Cell Volume, Hb= Hemoglobin, WBC= White Blood Count, RBC=Red Blood Count, b.w=Bodyweight.

Table 4: Hematological parameters of rats after two weeks treatment with dichloromethane bract extract of *Musa acuminata* Cavendish.

Group	PCV (%)	Hb (g/dl)	WBC ($x10^9$/L)	RBC ($x10^9$/L)	Platelets ($x10^9$/L)
1. Rat chow and water (Normal Control)	41.16 ± 0.30^{a}	13.72 ± 0.01^{a}	8.07 ± 0.01^{ad}	6.50 ± 0.02^{a}	281.67 ± 0.01^{a}
2. 40mg/kg b.w Indomethacin (Negative Control)	21.80 ± 0.04^{b}	7.27 ± 0.01^{b}	10.43 ± 0.02^{b}	3.81 ± 0.01^{b}	306.67 ± 0.15^{b}

3. 200mg/kg b.w Extract	27.53 ± 0.02^{c}	9.18 ± 0.01^{c}	8.47 ± 0.02^{ce}	4.63 ± 0.02^{ce}	293.00 ± 0.02^{c}
4. 150mg/kg b.w Extract	35.39 ± 0.02^{d}	11.80 ± 0.01^{d}	8.03 ± 0.04^{ad}	5.43 ± 0.01^{d}	286.67 ± 0.01^{de}
100mg/kg b.w extract	29.53 ± 0.02^{e}	9.84 ± 0.03^{e}	8.43 ± 0.05^{ce}	4.67 ± 0.03^{ce}	286.16 ± 0.01^{de}

Values are means ± SD of triplicate determinations. Mean values with similar superscript letters along the column are not statistically significant at p>0.05. PCV= Packed Cell Volume, Hb= Hemoglobin, WBC= White Blood Count, RBC=Red Blood Count, b.w=Bodyweight

Table 5: Hematological parameters of rats after three weeks treatment with dichloromethane bract extract of *Musa acuminata* Cavendish.

Group	PCV (%)	Hb (g/dl)	WBC (x10^{9}/L)	RBC (x10^{9}/L)	Platelets (x10^{9}/L)
1. Rat chow and water (Normal Control)	41.50 ± 1.21^{a}	13.83 ± 0.40^{a}	8.00 ± 0.02^{ad}	6.77 ± 0.12^{a}	281.67 ± 1.16^{a}
2. 40mg/kg b.w Indomethacin (Negative Control)	21.70 ± 0.12^{b}	7.24 ± 0.04^{b}	10.90 ± 0.10^{b}	3.75 ± 0.05^{b}	308.33 ± 0.15^{b}
3. 200mg/kg b.w Extract	32.50 ± 0.50^{c}	10.83 ± 0.15^{c}	8.33 ± 0.04^{ce}	4.97 ± 0.03^{ce}	288.67 ± 1.16^{c}
4. 150mg/kg b.w Extract	36.80 ± 0.17^{d}	12.27 ± 0.06^{d}	7.96 ± 0.07^{ad}	5.70 ± 0.11^{d}	283.33 ± 0.62^{de}
100mg/kg b.w extract	34.20 ± 0.15^{e}	11.40 ± 0.05^{e}	8.38 ± 0.03^{ce}	4.88 ± 0.21^{ce}	284.33 ± 0.52^{de}

Values are means ± SD of triplicate determinations. Mean values with similar superscript letters along the column are not statistically significant at p>0.05. PCV= Packed Cell Volume, Hb= Hemoglobin, WBC= White Blood Count, RBC=Red Blood Count, b.w=Bodyweight

Analysing the hematological parameters is an important tool in monitoring the effect of a test substance on a test subject. Result from this study revealed that administration of indomethacin induced a significant decrease in PCV, Hb and RBC in the untreated negative control group, indicative of anaemia possibly due to hemorrhaging in the gastro-intestinal (GI) tract. However, there was a gradual significant increase ($p \leq 0.05$) in PCV, Hb and RBC after one, two and three weeks of extract administration in all treated groups, when compared to untreated control, confirming the gastric mucosal healing ability of *M. acuminata* Cavendish as well as its ability to promote hematopoietic activity. This report corroborates with Goel and Saram, (2002) who reported on the gastroprotective effects of *M. acuminata* Cavendish pulp. The study also observed the dose dependent mucosal healing potential even after three weeks of treatment as most significant increases ($p \leq 0.05$) in PCV, RBC and haemoglobin were observed in the 150mg and 100 mg/kg b.w of extract compared to the group administered 200mg/kg b.w of extract, in line with Pannangpetch *et al.* (2001) that the gastro-protective effect of *Musa* specie may vary depending on different varieties and doses.

Platelet and WBC values were significantly reduced ($p \leq 0.05$) in treated groups when compared to the untreated control, confirming the extract possesses anti-inflammatory properties, resolving inflammation resulting from possible stimulation of the immune defense system. This report agrees with okon *et al.*, (2013) on the hematopoietic properties of *Musa* species.

3.4 Effect of dichloromethane bract extract of sample on biochemical parameters

The biochemical parameters of the control and study groups for weeks one, two and three are shown in Tables 6-8.

A significant increase ($p \leq 0.05$) in aspartate aminotransferase (AST), alanine aminotransferase (ALT), alkaline phosphatase (ALP), was observed in untreated negative control when compared to the normal control at one, two and three weeks. This is indicative of hepatic damage as AST, ALT which are intra-cellular enzymes and ALP, a membrane bound enzyme, leak into circulation and their activities increase in serum when there is a damage to the hepatocytes (Nirmala, 2012). Treated groups however showed significant decreases ($p \leq 0.05$) when compared to untreated negative control indicating no toxic effect on hepatocytes. The most significant decrease ($p \leq 0.05$) was observed in the treated group administered 150mg/kg bodyweight of extract compared to the group administered 200mg/kg bodyweight of the extract, indicating a dose dependent potential of the extract. Result from the study showed that dichloromethane extract of *M. acuminata* Cavendish bract is non-hepatoxic in agreement with Nirmala *et al*., (2012) who also reported on the hepatoprotective effect of *Musa acuminata*.

Creatinine and urea concentrations in serum are common biomarkers of kidney function. Though urea is mostly influenced by the diet, creatinine is more definitive as a product of muscle metabolism (Edmund and David, 2006). Findings from this study revealed significantly reduced ($p \leq 0.05$) levels of creatinine and urea when

compared to untreated negative control indicating no adverse effect on renal function, further corroborated by the normal histology of kidney tissues in plate 1.

Table 6: Biochemical parameters of rats after one week treatment with dichloromethane bract extract of *Musa acuminata* Cavendish.

Group	AST (IU/L)	ALT (IU/L)	ALP (IU/L)	Creatinine (µmol/l)	Urea (mmol/l)
1. Rat chow and water (Normal Control)	58.00 ± 1.00^{a}	30.67 ± 1.15^{a}	60.67 ± 2.08^{a}	167.00 ±1.00^{a}	6.57 ± 0.15^{a}
2. 40mg/kg b.w Indomethacin (Negative Control)	75.33 ± 2.08^{b}	59.00 ± 1.00^{b}	80.67 ± 3.06^{b}	198.33 ± 1.53^{b}	10.47 ± 0.15^{b}
3. 200mg/kg b.w Extract	64.67 ± 1.15^{cde}	51.00 ± 2.00^{c}	77.00 ± 1.00^{c}	180.00 ± 1.00^{c}	9.28 ± 0.03^{c}
4. 150mg/kg b.w Extract	64.33 ± 0.57^{cde}	41.33 ± 1.53^{d}	66.33 ± 1.54^{d}	173.66±0.58^{d}	7.80 ± 0.17^{d}
100mg/kg b.w extract	65.66 ± 0.48^{e}	46.33 ± 1.15^{e}	73.67 ± 1.15^{e}	175.33 ± 1.12^{e}	8.27 ± 0.12^{e}

Values are means ± SD of triplicate determinations. Mean values with similar superscript letters along the column are not statistically significant at p>0.05. AST=Aspartate aminotransferase, ALT=Alanine aminotransferase, ALP=Alkaline phosphatase, b.w=Bodyweight.

Table 7: Biochemical parameters of rats after two weeks treatment with dichloromethane bract extract of *Musa acuminata* Cavendish.

Group	AST (IU/L)	ALT (IU/L)	ALP (IU/L)	Creatinine (µmol/l)	Urea (mmol/l)
1. Rat chow and water (Normal Control)	58.67 ± 0.01^{a}	31.02 ± 0.02^{a}	60.67 ± 0.01^{a}	167.17 ±0.01^{a}	6.53 ± 0.02^{a}
2. 40mg/kg b.w Indomethacin (Negative Control)	74.07 ± 0.06^{b}	57.67 ± 0.01^{b}	80.67 ± 3.06^{b}	195.33 ± 0.02^{b}	10.27 ± 0.02^{b}
3. 200mg/kg b.w Extract	65.97 ± 0.06^{c}	48.52 ± 0.02^{c}	76.33 ± 0.02^{c}	178.15 ± 0.03^{c}	9.03 ± 0.03^{c}
4. 150mg/kg b.w Extract	64.93 ± 0.06^{de}	39.83 ± 0.01^{d}	67.35 ± 0.03^{d}	171.13 ± 0.04^{d}	7.63 ± 0.03^{d}
100mg/kg b.w extract	64.30 ± 0.35^{de}	44.17 ± 0.01^{e}	73.67 ± 0.03^{e}	175.34 ± 0.01^{e}	8.06 ± 0.01^{e}

Values are means ± SD of triplicate determinations. Mean values with similar superscript letters along the column are not statistically significant at p>0.05. AST=Aspartate aminotransferase, ALT=Alanine aminotransferase, ALP=Alkaline phosphatase, b.w=Bodyweight.

Table 8: Biochemical parameters of rats after three weeks treatment with dichloromethane bract extract of *Musa acuminata* Cavendish.

Group	AST (IU/L)	ALT (IU/L)	ALP (IU/L)	Creatinine (µmol/l)	Urea (mmol/l)
1. Rat chow and water (Normal Control)	59.33 ± 0.60^{a}	31.00 ± 1.00^{a}	61.00 ± 1.00^{a}	167.34 ± 0.58^{ad}	6.50 ± 0.05^{a}
2. 40mg/kg b.w Indomethacin (Negative Control)	72.33 ± 0.42^{b}	56.33 ± 0.60^{b}	75.33 ± 1.16^{b}	194.33 ± 0.60^{b}	9.97 ± 0.15^{b}
3. 200mg/kg b.w Extract	64.33 ± 1.16^{ce}	45.00 ± 1.00^{c}	73.00 ± 1.00^{c}	175.67 ± 1.05^{c}	8.75 ± 0.09^{c}
4. 150mg/kg b.w Extract	63.32 ± 0.57^{d}	38.66 ± 0.43^{d}	63.34 ± 1.37^{d}	168.66 ± 0.67^{ad}	7.42 ± 0.14^{de}
100mg/kg b.w extract	64.34 ± 1.16^{ce}	41.33 ± 0.60^{e}	68.67 ± 1.30^{e}	173.00 ± 1.00^{e}	7.77 ± 0.12^{de}

Values are means ± SD of triplicate determinations. Mean values with similar superscript letters along the column are not statistically significant at p>0.05. AST=Aspartate aminotransferase, ALT=Alanine aminotransferase, ALP=Alkaline phosphatase, b.w=Bodyweight.

3.5 Histological Result

Plates 1-3 represent results of histological examinations carried out on rat kidney, stomach and liver tissues of control and study groups after three weeks of extract administration. Histological findings from untreated negative control revealed glomeruli containing messengial cells with an occluded Bowman's capsule which explains the high concentrations of urea and creatinine in serum as the filtration rate of the kidney was compromised. The stomach tissues of untreated

negative control equally revealed a distorted stomach with a broken epithelium showing induced lesions resulting from indomethacin administration. Histology result also showed a distorted liver tissue with symptoms of microvessicular steatosis. Treated groups however, after three weeks of treatment with dichloromethane extract of *M. acuminata* Cavendish, revealed no major degenerative damage to the kidneys, stomach and liver tissues when compared to the untreated control, further confirming the ability of the extract to ameliorate the effects caused by indomethacin administration.

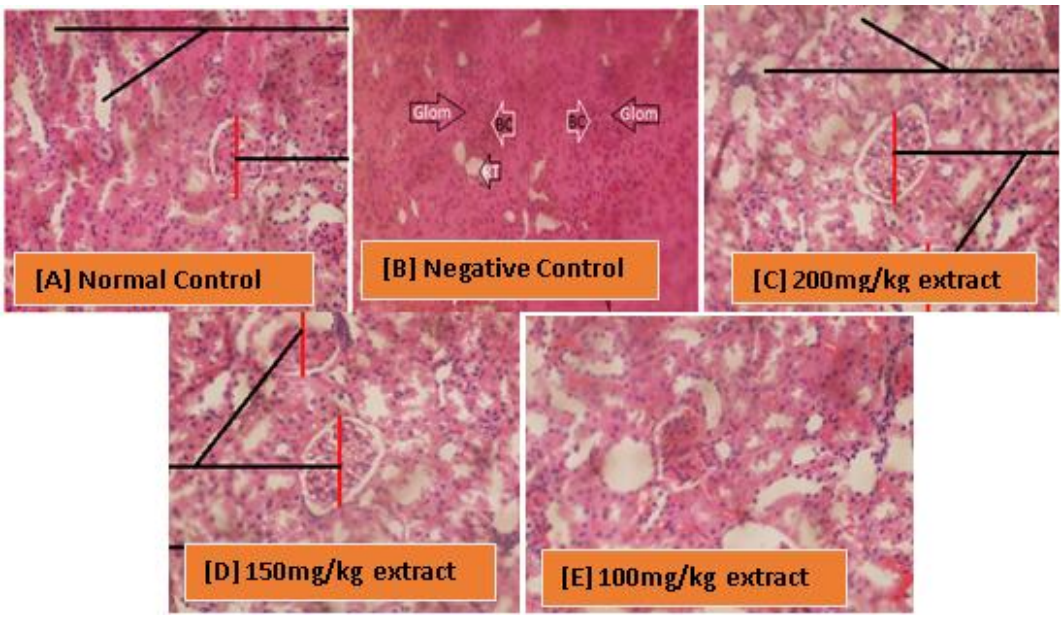


Plate 1: Photomicrograph of x400 H&E-stained rat kidney after three weeks treatment with extract.

[A] Normal control; kidney showed good glomeruli tuffs with patent capsular spaces.
[B] Negative control; glomeruli containing messengial cells with an occluded Bowman's capsule
[C] 200mg/kg extract; showed histologically normal kidney.
[D] 150mg/kg extract, renal tubules, glomeruli and Bowman's capsule are normal.
[E] 100mg/kg extract, renal tubules and glomeruli appear normal with patent capsular spaces.

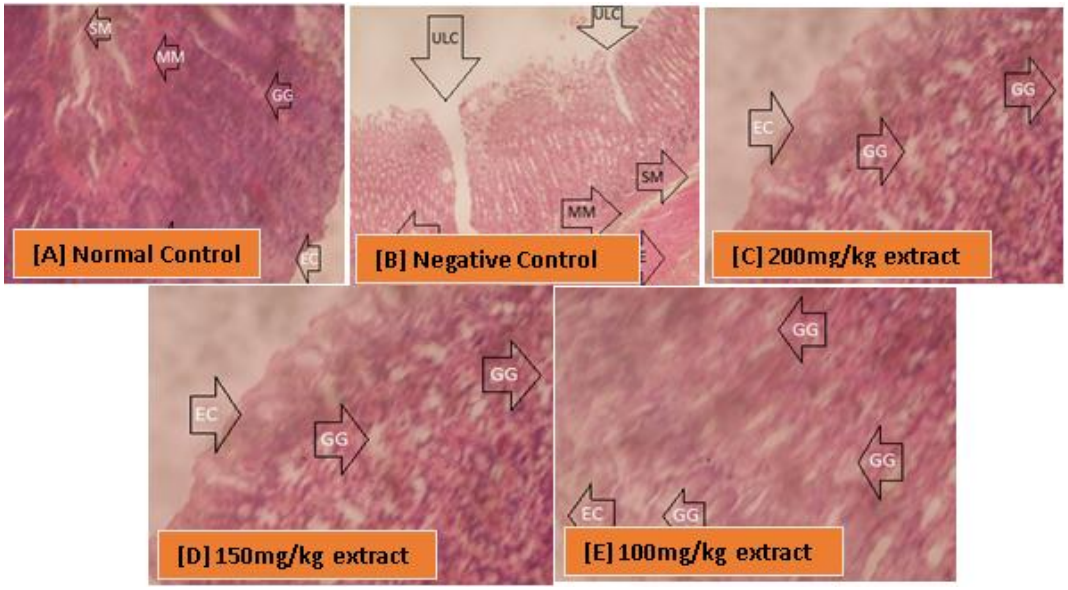


Plate 2. Photomicrograph of x400 H&E-stained rat stomach after three weeks treatment with extract.

[A] Normal control; Stomach shows patent epithelial cells and gastric glands.
[B] Negative control; distorted stomach showing broken epithelium indicating lesions.
[C] 200mg/kg extract; histologically normal stomach showing gastric glands.
[D] 150mg/kg extract; histologically normal stomach showing intact epithelial cells and gastric glands.
[E] 100mg/kg extract; histologically normal stomach with gastric glands and epithelial cells.

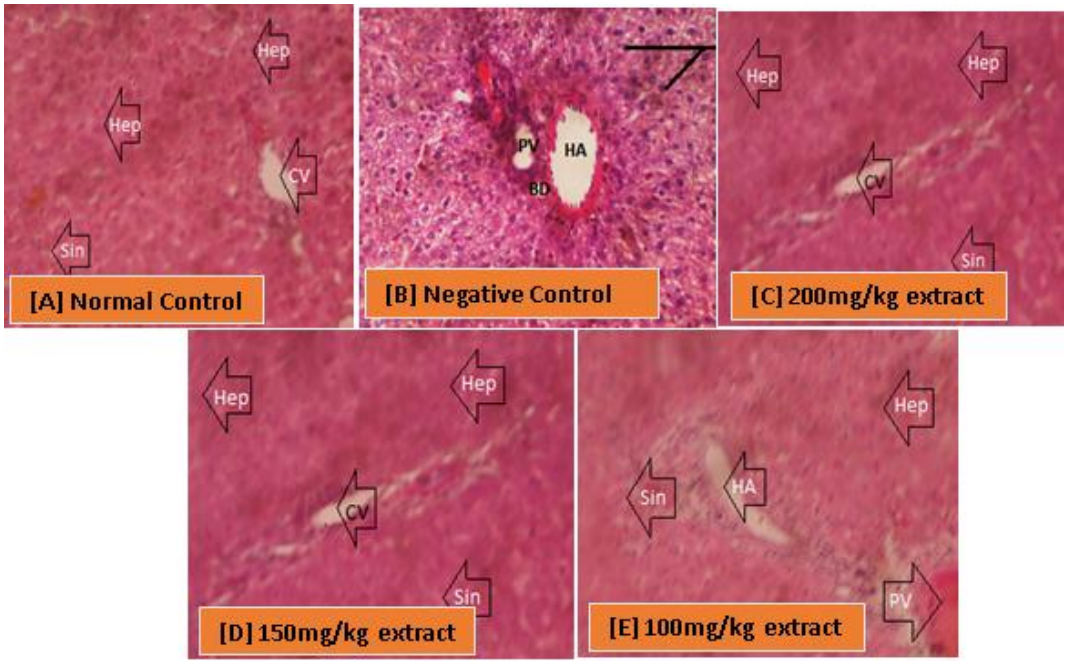


Plate 3: Photomicrograph of x400 H&E-stained rat liver after three weeks treatment with extract.

[A] Normal control; liver revealed a patent central vein, sinusoids indicating normal hepatocytes.
[B] Negative control; showed portal triads, mildly distorted liver tissue with microvessicular steatosis.
[C] 200mg/kg extract; Liver shows normal hepatocytes and sinusoids with patent central vein.
[D] 150mg/kg extract; histologically normal liver showing normal hepatocytes.
[E] 100mg/kg extract; normal liver showing normal hepatocytes, sinusoids and congested central vein.

5. CONCLUSION

Findings from this study revealed that dichloromethane bract extract of *Musa acuminata* Cavendish greatly ameliorated indomethacin-induced gastric mucosal injury and had no deleterious effects on hepatorenal function in Wistar rats administered indomethacin. However, dichloromethane bract extract of *Musa acuminata* Cavendish had the highest ameliorative effect at 150mg/kg of the extract as evidenced by the hematological and biochemical parameters. Thus caution must be taken when administering higher doses of the extract.

COMPETING INTERESTS

The authors have declared no competing interests exist.

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