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ORIGINAL CONTRIBUTION

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Acute and sub-chronic toxicity assessment and evaluation of the gastro-protective activity of polyherbal formulation "Mystomate4®" against gastric ulcer in experimental laboratory animal

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Abstract

Background: Ulcer remains a health challenge worldwide with antibiotics and proton pump inhibitors as major management therapy. The study investigated the acute, sub-chronic toxicity and gastrointestinal protective activity of a polyherbal formulation (Mystomate4®) used locally in Nigeria.

Methods: Oral LD_{50} and the sub-chronic toxicity test were determined in mice and rats. Mice were grouped into 8 groups of 8 mice each. They were dosed a graded concentration of the formulation (1.28, 2.56; 5.12; 10.24; 20.48; 40.96; 81.92; 163.84 g/kg body weight). The graded dose used was arrived at after an initial pilot study. Thereafter doses were chosen around the dose obtained from the pilot study. Animal were dosed orally and observed for sign of toxicity and number of death recorded after 24 h. The sub-chronic toxicity study was carried out for 3 months in rats at a dose of 2.5 and 5.0 g/kg body weight arrived at by titrating down the LD_{50} value after which some vital tissues were harvested and assessed for toxicity using relevant biomarkers. Anti-ulcer activity was evaluated in rats using ethanol, indomethacin and pylorus ligation induced ulcer models. Data were analysed with Graph Pad Prism version 5.0 using appropriate statistical method and significant level placed at $p \leq 0.05$.

Results: The acute toxicity study showed an LD_{50} result of 22,837.21 g/kg. The sub-chronic toxicity study resulted in a significant reduction in body weight due to significant decrease ($p \leq 0.05$) in feed consumption. Biochemical analyses of the blood samples showed a significant increase ($p \leq 0.05$) in creatinine and albumin level in the 2.5 mg/kg female group. ALT was significantly increased in all the treated rats except in 2 mg/kg female rats. Alkaline phosphatase significantly increased in high dosed male (HM) group while blood urea:creatinine ratio was significantly lowered in all the treated groups. There was a significant increase in serum TGL in all rats while LDL was significantly increased and decreased in HM and high dosed female (HF) respectively.

Conclusion: Mystomate4® showed significant protection against ethanol and indomethacin-induced ulcer models but did not modify the gastric parameters in pylorus ligation-induced ulcer model. The polyherbal formulation is nontoxic with promising potentials for treating experimental peptic ulcer.

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Keywords: Mystomate4®, LD_{50}, Acute toxicity, sub-chronic toxicity, Bioactivity, Anti-ulcer, Peptic ulcer

Background

Peptic ulcer is a clinical defect in the gastrointestinal mucosa lining, characterized with sores that develop along the epithelial lining of the stomach, lower esophagus, or small intestine. The prevalence has been attributed to the changing prevalence of *Helicobacter pylori* (*H. pylori*) infection, the increasing use of non-steroidal anti-inflammatory drugs, and the aging population [1–3]. Peptic ulcer was reported as one of the world's major gastrointestinal disorders. It was reported to affect as high as 10% of the world population with a death rate of as high as 15,000 yearly [1]. Although, the true prevalence of peptic ulcer in Nigeria is unknown, report showed that peptic ulcer disease is very common in Africa with an estimated prevalent rate of 70 to 90% especially among young adults contrary to what was obtainable in Europe where prevalence is higher among adults above 40 years of age [2–5].

The medical intervention in the treatment of peptic ulcer is predicated on the prognosis. However, treatment includes the use of antibiotics, antiacids and discontinuation of the ue of non steroidal anti inflammatory drugs (NSAIDs) which could provoke its development. Disease causing microbes are fast developing resistance to the lines of antibiotics commonly prscribed [6], and thus the quest for new and more effective drugs for use. Research work towards the development of new drugs from medicinal plants is thus attracting increasing interest. Many medicinal plants and other naturally products are continuously been screened for their potential in developing new and more effective drugs. Although, medicinal plants and other natural products may possess biological activities that are beneficial to humans, many are used in folk medicine without taking into account their toxicity and other related adverse effects [7]. There is therefore the need to evaluate these products for possible toxic effect on the body systems when consumed by man or his animals.

Mystomate4® is a polyherbal formulation with claims of effectiveness in the treatment of ulcer. According to the manufacturer; Egflorence Biosciences Ltd., Lagos, the formulation contains grounded charcoal, *Brassica juncea* (L.) Czern seed and sodium chloride suspended in edible oil. It is a formulation locally used in the treatment of peptic ulcer. There are few reports on possible anti-ulceractive activities of the constituents. Olive oil an edible oil was reported to express bacteriocidal effect against *H. pylori* a major peptic ulcer causative agent [8] while the *Brassica juncea* seed has been reported to possess antioxidant and anti-colon cancer activity [9, 10]. The objective of this study therefore, was to investigate the acute and sub-chronic toxicity effect of Mystomate4® as well as the gastroprotective activity or anti ulcerative activities in laboratory animals.

Materials and methods

Herbal formulation

The herbal formulation (Mystomate4®) contains ground charcoal (4000 mg), *Brassica juncea* seed (2500 mg) and sodium chloride (550 mg) all suspended in 15 ml of edible oil. Mystomate4® was obtained from Egflorence Biosciences Ltd., Lagos-Nigeria. The preparation has a stock concentration of 0.47 g/ml of edible oil.

Animal

Healthy male *Swiss* mice (20–21 g) were used for the acute toxicity study while the male and female *Sprague Dawley* rats weighing between 120 and 140 g were used for the sub-chronic and the gastro protective study. They were maintained under standard laboratory conditions in temperature-controlled rooms (24–28 °C), under a 12:12 h light–dark cycle, with access to water and food *ad libitum*. Animal care and handling procedures were in accordance with the International Association for the Study of Pain guidelines for the use of animals in pain research [11]. All experimental animals used in this study were obtained from the Animal house of the College of Medicicne, University of Lagos.

Toxicity study

Acute toxicity study

The acute toxicity study was performed according to the Organization of Economic Co-operation and Development (OECD) guideline for testing of chemicals (OECD, 2001). All methods used also comply with the ethical regulations as provided by College of Medicine, University of Lagos. The male mice were weighed and divided into eight groups containing eight mice each. The mice were given a single dose from graded doses that ranged from 1.28, 2.56, 5.12, 10.24, 20.48, 40.96, 81.92 to 163.84 g/kg of the formulation. The doses used were arrived at after an initial pilot study to determine an average dose at which lethality was first recorded. Doses used during the pilot study rangeds from 0.1 to 200 g/kg and thereafter doses were chosen around the dose obtained from the pilot study. The formulation was administered orally using a mice-size oral dosing needle after an overnight fast. The

mice were thereafter monitored for 72 h for sign and symptoms of toxicity and till 7 days for delayed toxicity symptoms. Water and feed was provided *ad libitum* and number of death and life mice was recorded for each group within the observation period. All doses used were dispensed from the stock solution of 0.47 g/ml of edible oil.

Sub-chronic toxicity study

The sub-chronic toxicity study was designed to evaluate the system toxicity of doses of the Mystomate4® administered orally over a period of 3 months. The test was carried out in line with OECD guideline for testing of chemicals [12]. The doses used (2.5 g/kg and 5.0 g/kg body weight) were arrived at by titrating down the LD_{50} value obtained from the acute toxicity study [13]. There were five groups of 6 rats each; control (CN), 2.5 g/kg male (LM), 2.5 g/kg female (LF), 5.0 g/kg male (HM) and 5.0 g/kg female (HF) groups. All doses used were dispensed from the stock solution of 0.47 g/ml of edible oil. All the laboratory rats used in the sub-chronic study were gavage the Mystomate4® for a period of 3 months (90 days), while the control received equal volume of the vehicle (edible oil). Weekly weight and daily feed consumption per rat was measured. At the end of the 3 months treatment, the rats were sacrificed by cervical dislocation followed by decapitation and blood samples collected into heparinised sample bottles. Blood samples were centrifuged at 3500 revolutions per min at a temperature of 25 °C for 20 min. The plasma was carefully separated with the aid of a sterile pasteur pipette and stored at − 80 °C.

Blood analysis for toxicity confirmation

The plasma collected was used for assessment of toxicity test and the plasma lipid profile respectively. The following toxicity biomarkers were analyzed from the blood samples collected from the different group of animals after the 3 months period; Blood Urea, Total Protein (TP), Albumin, Aspartate aminotransferase (AST), Alanine aminotransferase (ALT), Serum Alkaline Phosphatase (SAP), blood urea nitrogen (BUN) and blood and urine creatinine assay using ELISA assay kits produced by Sigma-Aldrich, Cayman Chemical USA and Elabscience China. The toxicity effects of the oral administration of the Mystomate4® on the liver and the kidney was evaluated in male and female *Sprague-Dawley* rats. Serum lipid profile level was also assessed using the appropriate ELISA lipid profile assay kits from Elabscience, China. Assay was carried out according to the manufacturer protocol.

Histopathologic studies of vital organs involved with body detoxification

The following organs were carefully harvested over ice and their wet weight taken; the liver, the kidney and the spleen. The kidney and the liver samples were placed in Boin solution for 2 days after which the Boin solution was replaced with 70% alcohol. The 70% alcohol was changed twice after every 3 three days. The tissue was passed through graded concentration of alcohol as follows; 80% alcohol for 1 h, (2x), 90% alcohol for 1 h (2x), 95% alcohol for 1 h, 100% alcohol for 1 h (2x), 50% + 50% xylene for 1 h, xylene for 45 min. The tissue was removed and placed in the metal tissue mould with molten wax in the oven (50 °C) for 45 min. After 45 min, the mould was removed and the mould basket placed on it and more molten wax added. The mold containing the waxed tissue was placed in a fridge at 0 °C to for 15 min to allow it to solidify. Five micrometer size of the tissue was prepared with a rotary microtome (LEICA RM 2235 rotary microtome) and placed on a glass slide pre-coated with lysine. The slides were thereafter stained with haematoxylin and eosin (H&E) and viewed under the microscope at a magnification of × 400.

Ulcer study

Ethanol-induced gastric ulcer model

The gastrointestinal protective potential of the formulation against ethanol-induced gastric ulcer model was investigated in rats. Prior to the experiment, male rats weighing between 110 and 130 g were fasted for 24 h. Thirty-five rats were divided randomly into 5 groups of 7 rats each and pre-treated orally with the vehicle (edible oil, 10 ml/kg), misoprostol (0.1 mg//kg) a standard anti-ulcer drug as control or Mystomate4® (1.14, 2.28 and 3.43 mg/kg,). After 1 h of pre-treatment, all the rats were gavage with 80% ethanol solution (1 mL/animal). The rats were sacrificed 1 h later by cervical dislocation and their stomachs were immediately excised, cut along greater curvature, rinsed with normal saline and ulcer scored according to the method of Galati et al. (2001) [14]. Ethanol is a gastric acid secretagogue stimulating increase gastric acid secretion from the gastric gland.

Indomethacin-induced gastric ulcer model

Rats fasted for 36 h were treated with vehicle (edible oil, 10 ml/kg or Mystomate4® (1.14, 2.28 and 3.43 mg/kg,). One hour later, indomethacin was given to rats orally at the dose of 80 mg/kg [15]. The animals were sacrificed after 6 h. The stomachs were removed, washed gently with saline (0.9%) and scored as referenced in ethanol-induced model. Indomethacin is a non steroidal anti

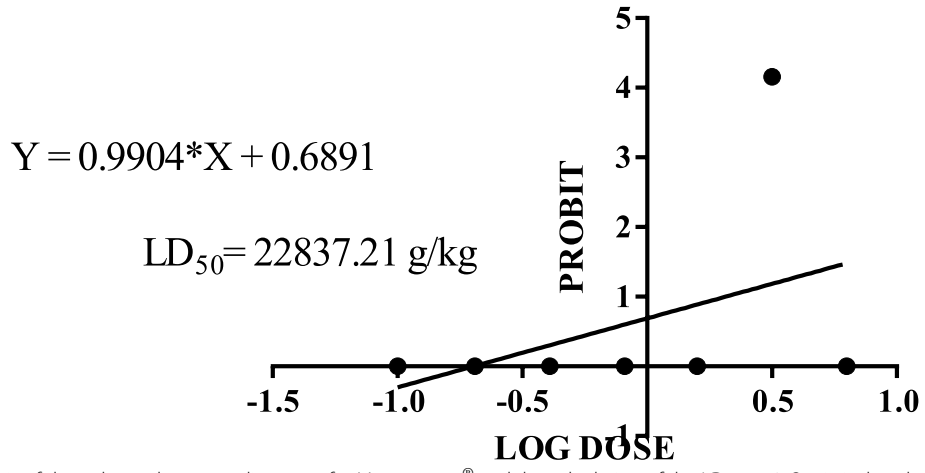

Fig. 1 Probit analysis of dose-dependent mortality curve for Mystomate4® and the calculation of the LD_{50} n=8, Statistical analysis was carried out using probit analysis in Graph pad prism5

inflammatory drugs (NSAIDs), induced gastric ulcer via inhibiting the release of cyclooxygenase-1 (COX-1), prostaglandin E2 (PGE2) [16].

Pylorus ligation-induced gastric ulcer model

The animals were divided into 5 groups, each consisting of six rats. Group 1 serves as the control and received edible oil, 10 ml/kg only. Group II, III and IV received the Mystomate4® at the doses of 1.14, 2.28 and 3.43 mg/kg, while group V received cimetidine at the dose of 40 mg/kg. After 1 h of the treatment pylorus ligation was done. Ligation was done without causing any damage to the blood supply of the stomach. Six hours after the ligation, the animals were sacrificed and the stomach removed. The gastric contents were collected, centrifuged and the supernatant measured. The ulcer formed in the gastric mucosa were measured and scored as described earlier [17].

Analysis of data

Data are expressed as mean ± SEM. Statistical analysis was performed with one-way analysis of variance followed by Tukey's test as post hoc test using Graph Pad Prism version 5.0 for Windows (San Diego, CA, USA). The level of data significance was set at $p \leq 0.05$. LD50 result was arrived at by plotting the nonlinear regression graph of percentage number of deaths with increasing doses of Mystomate4®using Graph Pad Prism version 5.0 for Windows (San Diego, CA, USA).

Compliance with ethical standards

All methods and procedures used in this study including the care and the use of laboratory animal for biomedical research were approved by the Health Research and Ethical Committee of the College of Medicine, University of Lagos, Nigeria (CM/HREC/12/16/094), and they are in accordance with International best practices in the use of laboratory animal in Biomedical research.

Results

Acute oral toxicity study of Mystomate4®

Highest number of deaths was recorded at very high doses. The mice showed signs of drowsiness but no stomach cramp before death. Probit analysis of the acute oral toxicity study of Mystomate4® revealed an LD_{50} value of 22,837.21 g/kg (Fig. 1).

Sub-chronic oral toxicity

General behavior of the animals, feed consumption and weight gain in rats treated for 12 weeks with Mystomate4® during the sub-chronic study

All the animals showed signs of hypoactivity as they stayed cuddled up most of the time in their cages but with no diarrhea during the first 2 weeks of administration of Mystomate4®. There was a significant decrease in body weight in all the Mystomate4®treated groups from 2nd week till 12th week of treatment. However, the HM-5 mg/kg group eventually recorded no significant difference in body weight at 12th week compared with control (Fig. 2). There was a significant decrease in the feed consumption in HF, LM and LF group from the 3rd week as recorded in this table all through the 12th weeks of treatment compared with control. Both HM and LM group daily feed consumption per rat was not significantly different from control towards the 9th to 12th week of treatment compared to control (Table 1).

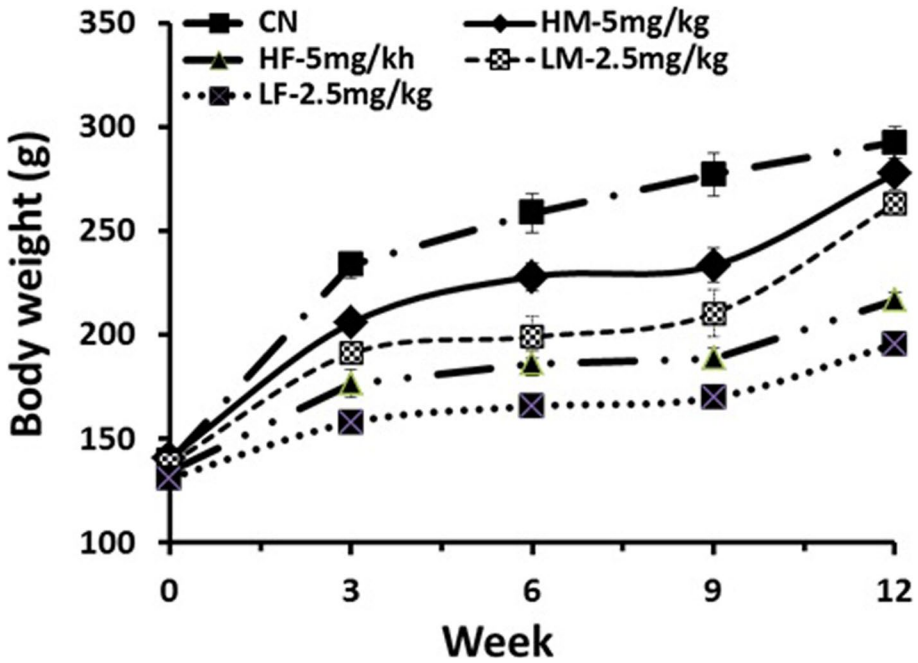

Fig. 2 Body weight changes in rats exposed orally to different doses of Mystomate4® for 12 weeks compared to control. CN=Control, LM=2.5 g/kg male, LF=2.5 g/kg female, HM= 5.0 g/kg male, and HF=5.0 g/kg female, n=8 rats per group.

Percentage organ coefficient in rats administered Mystomate4® at different doses for 3 months and control

The liver, the kidney and the spleen relative weights all remain unchanged compared with the control results (Table 2).

Serum biomarkers of sub-chronic toxicity from male and female rats treated at 2.5 mg and 5 mg/kg body with (Mystomate4®)

The result of the sub-chronic toxicity study also revealed no significant difference in the AST, uric acid and total protein level in all treated groups compared to control. There was however, a significant decrease in the level of creatinine (5 mg/kg and 2.5 mg/kg body weight female groups), urea (2.5 mg and 5 mg/kg body weight male groups) and bilirubin (2.5 mg female and male groups) and the blood urea-creatinine ratio (Table 3). There was a significant increase in the level of ALT (2.5 mg /kg, 5 mg/kg female and 5 mg/kg male groups), Albumin (2.5 mg/kg female group) and Alkaline phosphatase (5 mg/kg male group) (Table 3.)

Table 1 Average daily feed consumption per rat in the various groups treated for 3 months with Mystomate4®compared with control treated with the vehicle

Week	Control	Daily Feed consumption/rat (g)			
		HM-5 mg/kg	HF-5 mg/kg	LM-2.5 mg/kg	LF-2.5 mg/kg
0	19.28 ± 2.21	18.10 ± 2.10	19.35 ± 3.23	14.06 ± 1.16	19.59 ± 5.76
3	21.59 ± 2.21	15.23 ± 2.12*	12.22 ± 2.23*	12.34 ± 0.21*	13.12 ± 2.11*
6	21.24 ± 5.03	16.45 ± 2.20*	11.23 ± 2.24	13.55 ± 3.54*	10.21 ± 1.12*
9	22.12 ± 3.43	20.38 ± 1.12	10.21 ± 2.22*	18.23 ± 2.25*	10.24 ± 0.12*
12	20.22 ± 2.25	20.23 ± 2.21	10.22 ± 2.21*	20.58 ± 2.13	11.55 ± 0.45*

CN Control, *HM* 5 g/kg male, *HF* 5 g/kg female, *LM* 2.5 g/kg male and LF = 2.5 g/kg female; *n* = 8 rats per group

Statistical comparison was determined by one way ANOVA followed by Tukey's multiple comparison tests. *$P \leq 0.05$, statistically significant compared to control

Serum lipid profile in male and female rats administered Mystomate4® at graded dose of 5 g/kg body weight, L = 2.5 g/kg body weight)

The results showed a significant decrease in the serum level of LDL (HF) and a significant increase in the serum level of LDL in high dose male rats (HM). There was also a significant increase in triglyceride level in all the Mystomate4® treated male and female rats at all the doses used compared to control (Table 4).

Ulcer study

Effect of polyherbal formulation (Mystomate4®) on ethanol-induced gastric ulcers

Pre-treatment with the Mystomate4® at the doses of 1.14, 2.28 and 3.43 mg/kg significantly ($P \leq 0.01$) reduced ulcer lesions by 40.43%, 65.74% and 70.21%, respectively. Standard drug, misoprostol showed a percentage protection of 67.73% compared to control (Table 5). The effect of the Mystomate4® at the dose of 3.43 mg/kg was comparable to that of misoprostol.

Table 2 Percentage liver, kidney and spleen coefficient in rats administered Mystomate4® at different doses for 3 months and control administered the vehicle

Group	CN	HM-5 mg/kg	HF-5 mg/kg	LM- 2.5 mg/kg	LF-2.5 mg/kg
Liver (%)	1.3409 ± 0.0075	1.3497 ± 0.0075	1.3507 ± 0.0073	1.3401 ± 0.0074	1.3576 ± 0.0074
Kidney (%)	0.7963 ± 0.0071	0.8006 ± 0.0076	0.7834 ± 0.0072	0.7891 ± 0.0070	0.8038 ± 0.0069
Spleen (%)	0.1946 ± 0.0040	0.2060 ± 0.0089	0.2019 ± 0.0071	0.2007 ± 0.0081	0.2015 ± 0.0081

CN Control, *HM* 5 g/kg male, *HF* 5 g/kg female, *LM* 2.5 g/kg male and LF = 2.5 g/kg female; *n* = 8 rats per group

Statistical comparison was determined by one way ANOVA followed by Tukey's multiple comparison tests. *$P \leq 0.05$, statistically significant compared to control

Table 3 Sub-chronic toxicity study from the plasma using AST, ALT, Total protein, bilirubin, albumin, ALP, creatinine, urea and uric acid in male and female rats treated at 2.5 mg/kg and 5 mg/kg dose of Mystomate4® compared with control administered the vehicle

Group	Control	HM-5 mg/kg	HF-5 mg/kg	LM-2.5 mg/kg	LF-2.5 mg/kg
AST(U/L)	308.10 ± 2.11	298.14 ± 8.06	307.62 ± 20.14	318.32 ± 11.17	270.12 ± 8.06
ALT (U/L)	60.12 ± 1.22	71.22 ± 2.12*	86.53 ± 7.25*	70.56 ± 2.28*	56.25 ± 2.23
Total protein (g/dL)	76.56 ± 4.01	69.87 ± 4.50	74.12 ± 2.52	67.10 ± 4.37	76.30 ± 1.52
Total Bilirubin (umol/L)	2.37 ± 0.22	2.12 ± 0.76	1.75 ± 0.24	1.65 ± 0.12*	1.63 ± 0.31*
Albumin (g/L)	38.15 ± 5.10	43.84 ± 3.25	44.12 ± 1.20	38.22 ± 4.22	46.53 ± 2.45*
Alkaline phosphatase (ALP) (U/L)	160.23 ± 42.12	250.53 ± 8.24*	155.25 ± 10.12	192.25 ± 45.54	175.56 ± 15.25
Creatinine (umol/L	69.22 ± 4.21	66.20 ± 1.25	57.35 ± 5.21*	68.67 ± 8.52	61.22 ± 1.24*
Urea (umol/L)	5.98 ± 1.10	1.24 ± 1.46*	4.54 ± 1.52	4.56 ± 1.21*	4.87 ± 0.82
Uric acid (mmol/L	122.14 ± 2.40	110.58 ± 1.20	140.25 ± 20.54	116.34 ± 18.24	102.01 ± 5.54
Blood Urea: Creatinine	0.086 ± 0.001	0.019 ± 0.000*	0.079 ± 0.001*	0.066 ± 0.000*	0.080 ± 0.002*

CN Control, *HM* 5 g/kg male, *HF* 5 g/kg female, *LM* 2.5 g/kg male and LF = 2.5 g/kg female. n = 8 rats per group. Statistical comparison was determined by one way ANOVA followed by Tukey's multiple comparison tests. *$P \leq 0.05$, statistically significant compared to control

Table 4 Serum lipid profile in male and female rats treated with 2.5 mg/kg and 5 mg/kg dose of Mystomate4® for 3 months compared with control rats administered the vehicle

Group	Control	HM-5 mg/kg	HF-5 mg/kg	LM-2.5 mg/kg	LF-2.5 mg/kg
HDL (mmol/L)	1.30 ± 0.31	1.22 ± 0.09	1.23 ± 0.07	1.51 ± 0.09	1.27 ± 0.09
LDL (mmmol/L)	0.36 ± 0.01	0.48 ± 0.10*	0.21 ± 0.04*	0.39 ± 0.09	0.38 ± 0.09
Cholesterol (mmol/L)	1.85 ± 0.13	1.90 ± 0.09	1.91 ± 0.08	1.57 ± 0.01	1.86 ± 0.05
Tryglyceride (mmmol/L)	0.06 ± 0.00	1.83 ± 0.41*	1.48 ± 0.25*	1.55 ± 0.21*	1.38 0.20*

CN Control, *HM* 5 g/kg male, *HF* 5 g/kg female, *LM* 2.5 g/kg male and LF = 2.5 g/kg female, n = 8 rats per group

Statistical comparison was determined by one way ANOVA followed by Tukey's multiple comparison tests. *$P \leq 0.05$, statistically significant compared to control

Table 5 Effect of Mystomate4® on ethanol-induced ulcer in *Sprague Dawley* rats showing the ulcer index and percentage inhibition

Treatment	Dose (mg/kg)	Ulcer index	% Inhibition
Control		6.71 ± 1.11	–
Mystomate4®	1.14	4.00 ± 0.77*	40.43
Mystomate4®	2.28	2.30 ± 0.52***	65.74
Mystomate4®	3.43	2.00 ± 0.46***	70.21
Misoprostol	0.10	2.17 ± 0.62***	67.73

Results are mean ± SEM, $n = 7$. Statistical comparison was determined by one way ANOVA followed by Tukey's multiple comparison tests. *$P < 0.05$, ***$P < 0.001$, statistically significant compared to control

Table 6 Effect of Mystomate4® on indomethacin-induced ulcer in *Sprague Dawley* rats showing the mean ulcer score and percentage inhibition

Treatment	Dose (mg/kg)	Mean ulcer score	% Inhibition
Control		1.00 ± 0.22	–
Mystomate4®	1.14	0.40 ± 0.19	33.33
Mystomate4®	2.28	0.10 ± 0.10**	50.00
Mystomate4®	3.43	0.10 ± 0.10**	50.00
Omeprazole	100.00	0.10 ± 0.10**	50.00

Results are mean ± SEM, $n = 5$. Statistical comparison was determined by one way ANOVA followed by Tukey's multiple comparison tests. **$P < 0.01$, statistically significant compared to control

Effect of polyherbal formulation (Mystomate4®) on indomethacin- induced gastric ulcers

The results obtained with the Mystomate4® in this model are presented in Table 6. The Mystomate4® administration showed significant protection (50.00%, $p \leq 0.05$) at the doses of 2.28 and 3.43 mg/kg compared to control. The effect of the formulation at these doses was not significantly different from that of the standard drug, omeprazole.

Effect of polyherbal formulation (Mystomate4®) on pylorus-ligated induced gastric ulcers

The ulcer index and percent protection against ulcers in the pylorus-induced ulcer model are shown in Table 7. In this assay, Mystomate4® reduced ulcer index, producing a protection index of 54.89, 65.71 and 81.9% at doses of 1.14, 2.28 and 3.43 mg/kg body weight, respectively. The effect was only significant at 3.43 mg/kg. On the other hand, the formulation did not significantly ($P \leq 0.05$) affect the gastric secretion volume, pH and H^+ concentration. These findings suggest that the gastrointestinal protective action of the, Mystomate4® may not involve anti secretory activity.

Histological evaluation of the kidney and liver tissues dissected from control and rats gave Mystomate4® during the sub-chronic toxicity study

Photomicrograph of the kidney tissue slices showed normal kidney tissue architectural layout in all the samples collected from control and treated rats. There were normal and adequate nephron tubules (T), and glomeruli (G) (Fig. 3). Photomicrograph of the liver tissue slices taken from control and treated rats also revealed a normal liver tissue architectural layout with the presence of an intact portal tract "PT", central vein "CV" and hepatocyte "H" (Fig. 4).

Discussion

Acute toxicity test is aimed at determining toxic manifestations of any test substance in animals that are exposed to graded doses of the test substance within a single 24-h period. Acute oral toxicity study of Mystomate4® revealed an oral LD_{50} value of 22,837.21 g/kg body weight. The LD_{50} result for Mystomate4® was far above the proposed 5000–15,000 mg/kg body weight value at which any substance can be said to be practically nontoxic [18, 19]. The Mystomate4® was further subjected to a sub-chronic toxicity test with determination of serum level of some indicators of organ and tissue toxicity such as alanine amino transferase (ALT), alkaline phosphatase (Alk-Phos), creatinine, bilirubin, urea, uric acid and total protein. The weekly weight

Table 7 Effect of Mystomate4® on pylorus ligation- induced ulcer in *Sprague Dawley* rats showing the ulcer index and percentage inhibition, gastric juice pH and its acidity level

Treatment	Dose (mg/kg)	Ulcer index	% Inhibition	Gastric juice (ml)	Gastric pH	Gastric acidity (meq/L)
Control	–	3.17 ± 0.60	–	0.55 ± 0.18	6.28 ± 0.39	6.67 ± 0.14
Mystomate4®	1.14	2.50 ± 0.62	54.89	0.55 ± 0.08	6.32 ± 0.31	7.83 ± 0.04
Mystomate4®	2.28	1.58 ± 0.71	65.71	0.40 ± 0.06	6.30 ± 0.17	9.00 ± 0.02
Mystomate4®	3.43	0.67 ± 0.21**	81.95	0.72 ± 0.09	5.98 ± 0.11	7.00 ± 0.08
Cimetidine	40	0.67 ± 0.33**	75.94	0.17 ± 0.09	7.12 ± 0.30	3.33 ± 0.08*

The results are expressed as mean ± SEM; $n = 6$. * $P < 0.05$, ** $P < 0.01$, compared to control group followed by Dunnett post test

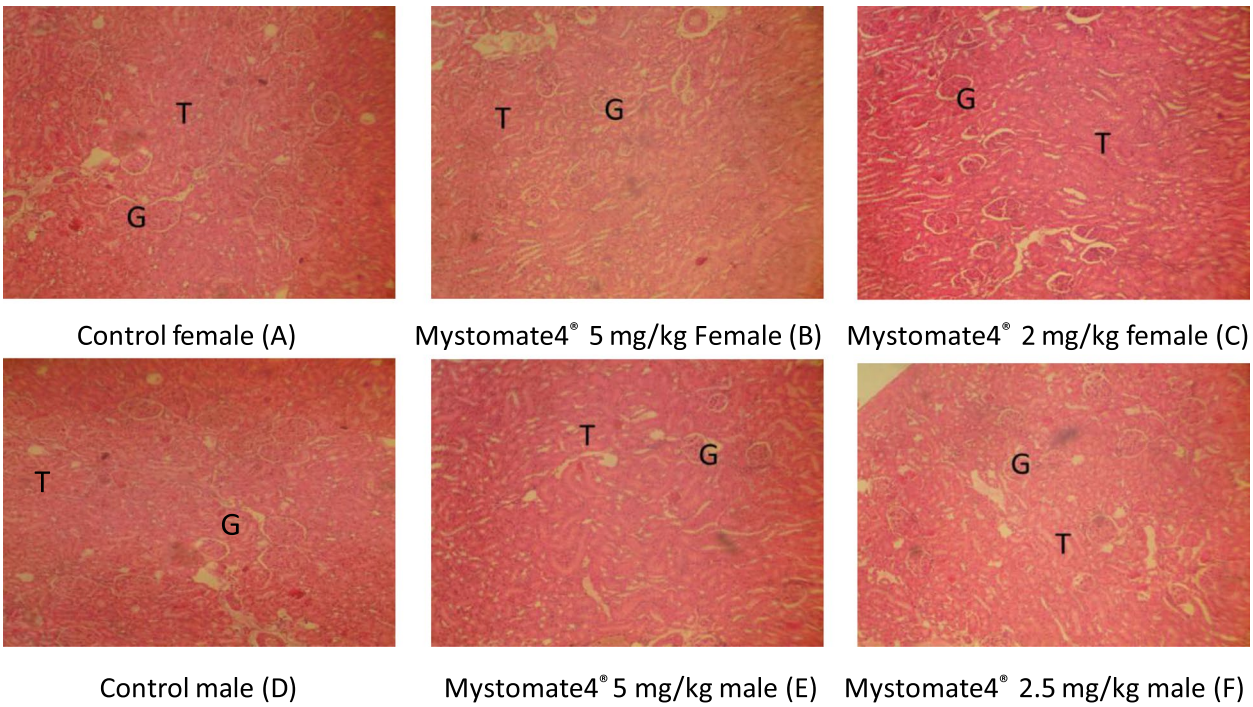

Fig. 3 Photomicrograph of the kidney tissue slides in control, 5 mg/kg-male, 5 mg/kg-female, 2.5 mg/kg-male and 2.5 mg/kg-female. Mystomate4® treated rats (H&E stained and x 400 magnification, T= nephron tubules, G = glomeruli)

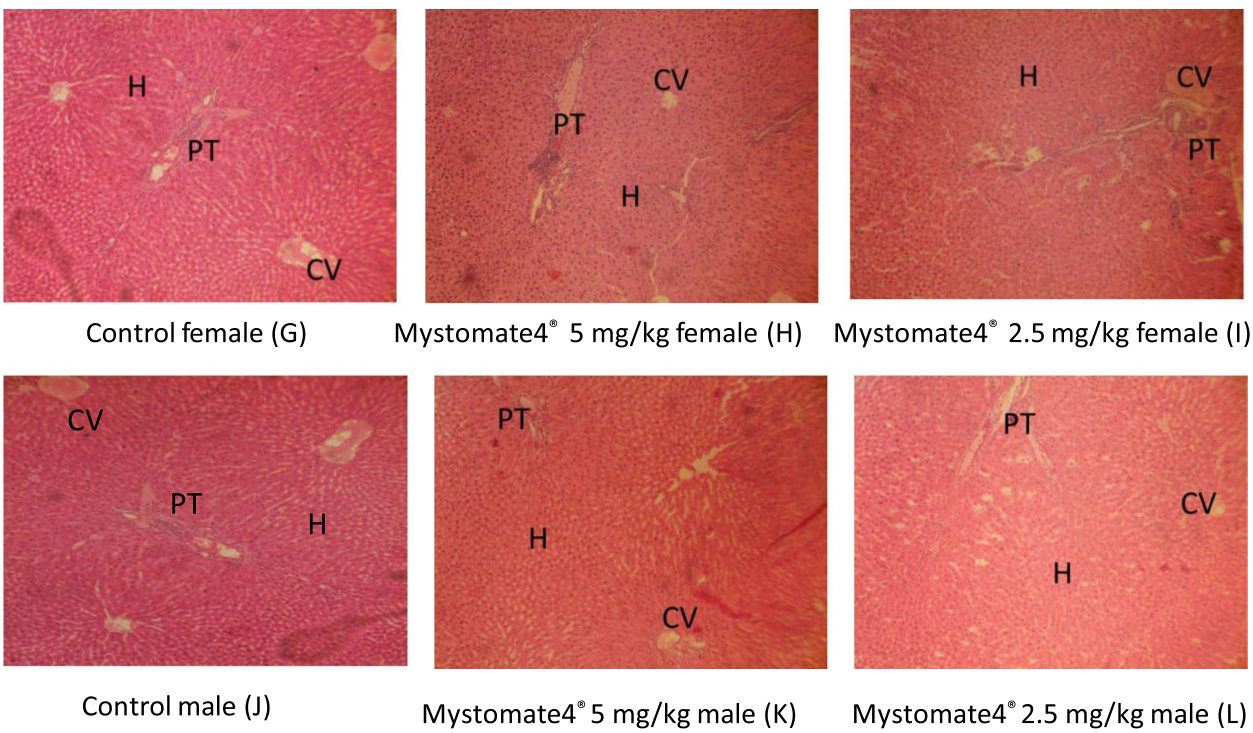

Fig. 4 Photomicrograph of the liver tissue slides in control, 5 mg/kg-male, 5 mg/kg-female, 2.5 mg/kg-male and 2.5 mg/kg-female Mystomate4® treated rats (H&E stained, x400 magnification, Portal tract=PT, central vein=CV and hepatocyte=H)

of all the treated rats showed a significant decrease in weight compared to control group all through the period of the experiment except for the HM-5 mg group whose body weight was not significantly different from control at the end of the 12 weeks of treatment (Fig. 2). The decreased weight gain was due to a decrease in feed consumption in all the affected group of rats (Tab. 1) as a result of decreased feed acceptability due to the effect of the polyherbral mixture on the animal taste taste bud. The coefficient of organ weight revelaed no significant changes in the weights of neither the liver, the spleen nor the kidney in all the treated groups. Liver, kidney and spleen are important detoxification organs of the body and they usually record hyperthrophy due to excessive detoxification activities whenever the body is under toxicity assult [20].

The serum level of ALT was significantly elevated in the 5 mg/kg treated male and female rats as well as the 2.5 mg/kg treated male rats (Tab 3). ALT is an enzyme contained in the fluid found in the liver cells, with resultant increase in the blood level depending on the degree of damage to the liver tissue [21]. Thus, the elevated level of ALT especially at 5 mg/kg showed a possible liver toxicity effect as a result of long period (12 weeks) of exposure despite the non significant change recorded in the weight of the liver from Mystomate4® treated rats. The alkaline phosphatase level was also significantly elevated in the serum samples from male rats exposed at 5 mg/kg body weight. The alkaline phosphatase enzyme is found primarily in the liver and bones but also in other tissues of the body. The significant increase in alkaline phosphatase level has been used as a biomarker of inflammation and possible damage to the tissue [22, 23]. Further analysis of the serum level of AST and uric acid revealed no significant change in any of the treated groups compared to control. However, there was a significant decrease in the level of creatinine in the female groups treated with 2.5 mg/kg and 5 mg/kg, while the serum urea level was also signizficantly lowered in 2.5 mg/kg and 5 mg/ kg treated male rats.

Creatinine is a bye product of the breakdown of creatinine phosphate by the activities of creatinine phosphokinase [24]. Blood creatinine level is a very useful biomarker of kidney functions [25]. The decreased serum level of creatinine showed no adverse effect on the kidney function. The serum bilirubin level was significantly lowered compared to control group among the 2.5 mg/kg treated male and female rats. Bilirubin is a breakdown product of heme obtained from red blood cells. High concentration of bilirubin in the blood is an indication of possible increased destruction of red blood cells or compromise of the red cell membrane integrity [26]. It could also give an indication of early liver deficiency in the conjugation process leading to removal of bilirubin from circulation for onward deposit into the gall bladder [27].

Furthermore, there was a significant increase in the serum albumin level in the 2.5 mg/kg group. This was despite the non significant change recorded in the result of the total serum protein in the same group. The total blood protein includes clotting factors, enzymes, antibodies, transport substances, albumin and most of the alpha and beta globulins. The urea assay result however, was significantly reduced in the blood samples from male and female rats dosed with 5 mg/kg of the Mystomate4®. Urea is a by-product of the breakdown of protein in the liver. High level of blood urea indicates that the kidney is unable to properly clear urea from circulation [28]. The significant decrease in the blood urea recorded in all the 5 mg/kg treated rats in this study portent no adverse effect on the liver function. An analysis of the blood urea, creatinine and the blood urea-creatinine ratio when considered together gives a good indicator useful in differentiating kidney disease from dietary protein metabolism problems as well as possible degree of bodily hydration. The blood urea-creatinine ratio results in this study were all significantly reduced compared with the control group again showing that the functions of the kidney was not compromised [29].

The serum triglyceride level was significantly increased in all the Mystomate4® treated rats compared to the control. This was in addition to the significant decrease and increase recorded in the serum level of LDL in 2.5 mg/kg and 5 mg/kg treated male rats respectively. Triglyceride is a known independent indicator and risk factor for coronary heart diseases when LDL:HDL ratio is elevated [30]. The results of the histological studies of the liver and kidney tissues (Figs. 3 and 4) from all treated animals and control revealed a normal architecture with intact cellular components. The results of the histological studies further buttress the results of the relative organ weight, biochemical analysis and the sub chronic toxicity studies in which the Mystomate4® showed no significant toxicity on the liver and kidney in all the exposed rats compared with the control.

The gastro protective activity of the Mystomate4® was investigated in three models of antiulcer assays; ethanol, indomethacin and pylorus ligated-induced models. Ethanol induced gastric ulcers have been widely used for evaluation of gastrointestinal protective activity [31]. Ethanol directly induces injury to the mucosa of gastric, declining the bicarbonates secretion and the generation of mucus [32]. In this model, the formulation showed a significant reduction in the mean ulcer score at all doses administered compared to control. These results indicate that Mystomate4® possess an antiulcer activity which could be related to cytoprotective activity.

Indomethacin like other NSAIDs such as aspirin, adversely affect the gastroduodenal mucus causing ulcerative lesions on the mucus membrane. One of the main factors of indomethacin-induced gastric damage is the inhibition of prostaglandin synthesis through the cyclooxygenase pathway. Other mechanisms include decreased blood supply to the gastric mucosa, increased gastric acid secretion, as well as inactivation of the growth factors involved in mucosa defense and repair [33, 34]. The formulation significantly reduced mucosal damage in the indomethacin–induced ulcer model, suggesting the possible involvement of prostaglandin in the gastrointestinal protective effect of the polyherbal formulation.

The effect of the treatment of the polyherbal formulation on the parameters of gastric secretion was also determined using the pylorus ligation-induced antiulcer model. Ulcers induced by this model are due to accumulation of gastric acid and pepsin which leads to digestion of gastric mucosa [35]. The formulation reduced ulcer index but did not significantly ($P > 0.05$) affect the gastric secretion volume, pH and H^+ concentration. These findings suggest that the gastrointestinal protective action of the formulation may not involve anti gastric acid secretory activity. The major components of the formulation are *Brassica juncea* seed and edible oil . *Brassica juncea* seeds have been shown to possess antioxidant activity [11]. Olive oil is an example of edible oil and has been reported to contain high amount of phenolic compounds with antioxidant properties and other potent biological activities including anti-ulcer activity [8, 36–40], that could partially account for the observed effect. However, further studies will be required to identify other biological activities of edible oil and *Brassica juncea* seed and to confirm their possible roles in the gastroprotective activities of Mystomate4® as recorded in the present study. Further work will be needed to confirm the adverse effect or otherwise of Mystomate4® on heamatological parameters, the lungs and the heart which are other vital visceral organs of the body.

Conclusion

In summary, the results presented in this study indicated that the Mystomate4® has a very high oral LD_{50} value and showed no significant toxicity at both acute and subchronic test stage. It also possessed gastro protective properties against gastric ulceration in rats. It can be subjected to further clinical trial for further studies.

Abbreviations

LD_{50}: Lethal dose at 50%; HM: High dosed male; LM: Low dosed male; HF: High dosed female; LF: Low dosed female; LDL: Low density lipoprotein; TGL: Tryglyceride; NSAID: Non-steroidal anti-inflammatory drugs; OECD: Organization of Economic Co-operation and Development; CN: Control; TP: Total Protein; AST: Albumin, Aspartate aminotransferase; ALT: Alanine aminotransferase; SAP: Serum Alkaline Phosphatase; BUN: Blood urea nitrogen; H&E: Hhaematoxylin and eosin; COX-1: Cyclooxygenase-; PGE2: Prostaglandin-E2; CM/HREC: Health Research and Ethical Committee of the College of Medicine, University of Lagos; T: Nephron tubules; G: Glomeruli; PT: Intact portal tract; CV: Central vein; H: Hepatocyte.

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Authors' contributions

FOA took part in the design of the work, the bench work, data analysis of data on acute and sub-chronic toxicity and manuscript writing. MOS took part in the design of the work, the bench work, analysis of anti-ulcer study data, and manuscript writing. OTA was part of the deisgn, data all the dat analysis and interpretation and writing of the manuscript. BOF was part of the deisgn, data all the dat analysis and interpretation and writing of the manuscript. The authors read and approved the final manuscript.

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Declarations

Ethics approval and consent to participate

All procedures used in this study including animal welfare, humane treatment of laboratory animals and euthanasia were approved by the institutional HREC College of Medicine, University of Lagos. (Ref. No: CM/HREC/12/16/094).

Consent for publication

Not applicable.

Competing interests

The authors of this manuscript declared that they have no competing interests.

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