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ORIGINAL RESEARCH

EFFECT OF ETHANOLIC EXTRACT OF *MOMORDICA CHARANTIA* ON ANTIOXIDANT AND INFLAMMATORY MARKERS IN MALE BALB/c MICE ADMINISTERED ACYCOR-PLUS

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Abstract:

Introduction: Acycor-plus (ACY) is a single dose combined analgesic and anti-inflammatory drug. Prolonged usage and overdose often lead to gastro-intestinal ulcerations, inflammations and oxidative stress which increases the use of synthetic H2 antagonists such as cimetidine (CMT).

Aims: This study was carried out to monitor the antioxidant and inflammatory effects of ethanolic extract of *Momordica charantia* (MC) in ACY-administered male Balb/c mice.

Materials and Methods: Twenty-five mice weighing between 15-20g were equally divided into five groups and received normal saline (Control), 2.5mg/kg ACY alone (ACY), ACY combined with either 50mg/kg cimetidine (CMT) or 100mg/kg and 200mg/kg ethanolic extract of *Momordica charantia* (MC100 and MC200, respectively). The animals were sacrificed after three days by cervical dislocation and post-mitochondrial fractions of their livers, kidneys, small intestines and colons were used to assess the activities of antioxidant and inflammatory markers.

Results: MC100 and MC200 significantly reduced antioxidant markers except catalase (CAT) when compared with ACY while they increased hepatic myeloperoxidase (MPO) and nitric oxide (NO) ($p<0.05$). Kidney and colonic MPO activities were significantly reduced by MC100. However, MC200 significantly increased renal CAT, superoxide dismutase (SOD) and SOD/(GPx+CAT) ratio with increased oxidative stress.

Conclusion: It can be concluded that MC100 had comparable effect with CMT while MC200 might induce renal toxicity. However, further research is needed using other routes of administration.

Keywords: ulceration, inflammation, oxidative stress, *Momordica charantia*.

All co-authors agreed to have their names listed as authors.

1. INTRODUCTION

ACYCOR-Plus is a fixed dose combination of 100mg aceclofenac and 500mg paracetamol. Aceclofenac belongs to the group of Non-Steroidal Anti-Inflammatory Drugs (NSAIDs) with potent analgesic, antipyretic, anti-inflammatory and platelet inhibitory actions [1], [2]. Acycor was proven to be efficient in proffering relief from several painful and inflammatory conditions, ranging from osteoarthritis to rheumatoid arthritis, ankylosing spondylitis, low back pain, dental pain, gynaecological pain and other painful inflammatory conditions of ear, nose & throat. Aceclofenac has an outstanding anti-inflammatory profile mediated primarily through inhibition of cyclooxygenase-2 (COX-2) activity which is a key enzyme in the inflammation cascade [3]. The inhibition of COX-2 leads to the suppression of pro-inflammatory prostaglandins and cytokines. However, studies have shown that aceclofenac is biotransformed into chemically reactive metabolites capable of binding covalently to certain protein targets in the hepatocytes of animals administrated the drug [4],[5]. In addition, *in vitro* studies also revealed that the metabolic activation in hepatocytes produced 4-hydroxyaceclofenac and 5-hydroxyaceclofenac which was mediated by UDP-glucuronosyltransferase and cytochrome P450 isozymes (CYP1, CYP2B, CYP2C, CYP2C11 and CYP3A) [6, 7],[8].

Some studies have also shown that the administration of NSAIDs decreases the level of GSH and GPx in tissues [9, 10] while paracetamol has been reported as an hepatoxic, nephrotoxic and carcinogenic agent after prolonged usage or overdose. Although the adverse effects associated with acycor are minor and reversible with treatment discontinuation, one of such that is of germane significance is the gastrointestinal disorder (including duodenal and peptic ulcers), which can be treated with anti-ulcer drugs such as cimetidine.

Cimetidine is a substituted imidazole, a basic drug with high water solubility and specific antagonistic effect on histamine H2 receptors but without significant interaction with catecholamine-, β-2-adrenergic-, histamine H1- or muscarinic- receptors. [11]. It inhibits both daytime and nocturnal basal gastric acid secretions as well as gastric acid secretions stimulated by histamine, pentagastrin, acetylcholine, insulin, food and other secretagogues at the histamine H2- receptor site of the parietal cells [12]. Cimetidine is metabolized in the liver by oxidative hydroxylation and conjugation with up to 80% of a single dose of the drug being excreted in the urine and about 70% in an unchanged form. Its potency in terms of administered dose and, more meaningfully, in terms of blood concentrations achieved, is reportedly very comparable in man and in experimental animals.

There is currently a globally increasing trend in the use of herbs and other natural agents for prophylaxis and in the treatment of various disorders. In most African countries, many people prefer natural means of therapy to synthetic drugs majorly because they live in an environment rich in diverse medicinal plants which are affordable. There are scientifically proven claims of beneficial effects of these natural agents in comparison with their synthetic counterparts. However, one must also be cautious of abuse of these agents, as toxicity is also reported due to overdose or uncontrolled usage, while adverse drug interactions are also inevitable [13]. *Momordica charantia* (ejìnrìnweere in Yoruba) is one of nature's most bountiful vegetable in Africa, but often discarded by people, just because of its bitter taste. This plant belonging to the family Cucurbitaceae, is commonly known as bitter gourd or bitter melon in English and it is grown mainly in the tropical regions of the world. This plant is rich in essential vitamins and minerals, especially vitamin A, B_1, B_2, C, iron and flavonoids which are contributory to its immense nutritional and medicinal benefits [14]. Among its traditional usage are as antidiabetic, abortifacient, anthelmintic, contraceptive, antimalarial and laxative. It is equally employed for treatment of dysmenorrhea, eczema, emmenagogue, galactagogue, gout, jaundice, kidney (stone), leprosy, leucorrhea, piles, pneumonia, psoriasis, rheumatism and scabies. Many scientific studies have proven its medicinal uses as antiviral, antitumor, antileukemic, antibacterial, antimutagenic, antimycobacterial, antioxidant, antiulcer, anti-inflammatory, hypocholesterolemic, hypotri-glyceridemic, hypotensive, immunostimulant, and insecticidal agent [15]. The isolated compounds of *Momordica charantia*(MC) have been reported to increase the activity of adenosine-5-monophosphate kinase, an enzyme that facilitates cellular glucose uptake and fatty acid oxidation. Hypoglycemic agents in *M. charantia* promote efficient oxidation of glucose into fuel, and conversion into glycogen. During glucose shortages, fats/fatty acids are burnt as fuel. Continued demand for energy in the absence or shortage of glucose causes fat cells to release their fat contents to maintain energy balance [15]. This increased fatty acid oxidation eventually leads to weight loss which is one of the mechanisms involved in its anti-obesity effects. Total protein in the serum and tissues is the summation of the albumin, globulin, inflammatory markers, regulatory proteins, structural and enzyme classes of proteins. Assessment of total protein is quite important in diagnosing for an infection, and the concentration of globulins is extrapolated from the total protein concentration [16].

Oxidative stress which results from an imbalance in the levels of antioxidant markers and free radicals in an organ or cell can be measured by assessment of lipid peroxidation and hydrogen peroxide. Oxidative stress is not age-bound, and is capable of causing deleterious increase in the concentrations of reactive oxygen species and other tissue-damaging radicals. Reduced glutathione (GSH) is an endogenous dipeptide which plays vital role as a non-enzymatic antioxidant that scavenges free radicals by abstracting excess electrons while it becomes oxidized (GSSG). It can be converted to its reduced form (GSH) by abstracting protons from the system to enable it undergo other oxidation-

reduction cycles. Apart from acting as an antioxidant, GSH is also an important coenzyme for other antioxidant enzymes such as glutathione peroxidase (GPx) and glutathione-s-transferase (GST). Therefore, reduction in the activities of these two enzymes may be linked to low level of reduced Glutathione. Other antioxidant enzymes such as superoxide dismutase (SOD) and catalase (CAT) protect aerobic cells from oxidative damage by respectfully dismutating superoxide radicals and detoxifying excess hydrogen peroxide produced during respiration[17]

Nitric oxide (NO_2^-), is an important mediator of vascular tone and renal function, it regulates glomerular, vascular and tubular function in the kidney [18]. The discovery that NO_2^- can influence many aspects of the inflammatory cascade, ranging from its expression to recruitment of leukocytes to an infected tissue is now well appreciated (Larouxet *al*., 2001). This makes it an anti-inflammatory marker, but cytotoxicity is associated with the inducible NO_2^- formed by inducible nitric oxide synthase (iNOS).

Myeloperoxidase (MPO) is a unique peroxidase that, in addition to its peroxidation activity, also catalyzes the conversion of H_2O_2 and chloride to hypochlorous acid (HOCl). MPO which is an inflammatory marker is used in detecting risks of different ailments. An earlier study reported, that elevated MPO levels more than doubled the risk for cardiovascular mortality over a certain period [19].

Combined usage of herbs with synthetic drugs have been reported to impair the activities of certain enzymes in the body and this may either increase the efficacy of such drugs or cause adverse reactions which may be toxic depending on the organ. This study was therefore designed to determine acute effects of two different doses of *Momordic acharantia* co-administered with acycor-plus on some biochemical parameters in selected organs of juvenile Balb/c mice while cimetidine was used as standard for comparison. It is hoped that data obtained from this experiment would serve as a useful guide concerning herbal-drug combination therapy in order to avoid adverse drug-herbal interactions, toxicity and other undesirable side effects.

2. MATERIALS AND METHODS

2.1 Chemicals

Thiobarbituric acid(TBA),Trichloro-acetic acid(TCA), iron iii chloride($FeCl_3$), aluminum chloride, sodium hydroxide(NaOH),$NaHPO_4$,Na_2HPO_4, Distilled water, sulphosalicyclic acid, carbonate buffer,potassium buffer, sodium azide(NaN_3), hydrogen peroxide (H_2O_2),tris potassium chloride, 1 chloro-2,4-dinitrobenzene – CDNB (Sigma), hydrochloric acid, epinephrine (Sigma), stock bovine serum albumin, potassium iodide,5,5-dithiobis-[2-nitrobenzoic acid] – DTNB (Sigma), normal saline.

2.2 Drugs

Acycor-Plus, manufactured by Stallion Laboratories PVT. Ltd. C-1B, 30512 & 3, G.I.D.C., Kerala (Bavla), District, Ahmedabad; Cimebios (Cimetidine 400), manufactured by Jiangsu RuinianQianjin Pharmaceutical Co., LTD, Chuanbu village, Yixing Economic Development Zone, Jiangsu Province, China.

2.3 Plant Collection

Fresh plants of *Momordica charantia* collected from a house in Oke Odan, Iyana Isasi, Lagos, were identified and authenticated by the Department of Botany, Lagos State University, Ojo. The leaves were plucked and dried under room temperature at the laboratory of Biochemistry Department, Lagos State University, Ojo Campus.

2.4 Preparation of Crude Ethanolic Extract

The dried leavess of *Momordica charantia* were ground into powder, using a mechanical grinder, then 500g of the powder was soaked in 1.5L of absolute ethanol for 48 hours, filtered and extracted using rotary evaporator. The extract was dried under reduced pressure at a temperature of 40^0C, in a vacuum oven to remove residual solvent. The final weight of the crude ethanolic extract of *Momordica charantia* was 9.136g, making a percentage yield of 1.83%.

2.5 *IN VIVO* ANIMAL STUDY

2.5.1 Experimental animals

Juvenile Wistar mice (weighing between 15g-20g) of both sexes were used for this study, they were housed under astandard condition in the animal house of the Department of Biochemistry, Lagos state University, Ojo, they were acclimatized for a week and allowed water and standard pellets *ad libitum.*

2.5.2 Experimental design

Twenty-five (25) juvenile *Balb/c* mice were divided into five groups, each group consisting of five mice, their weights were recorded daily and they were subjected to the following intraperitoneal treatments once a day for three days. Control group (normal saline); ACY (2.5mg/kg body weight of Acycor-plus); CMT group (2.5mg/kg Acycor-plus and 50mg/kg body weight of Cimetidine 400); MC100 group (2.5mg/kg Acycor-plus and 100mg/kg body weight of crude ethanolic extract of *Momordica charantia*); MC200 (2.5mg/kg Acycor-plus and 200mg/kg body weight of crude ethanolic extract of *Momordica charantia*).

N.B. Intraperitoneal administration was chosen to enhance absorption, bypass first pass effect and also prevent loss of appetite due to bitter taste of the plant extract.

2.5.3 Body weight measurement

Body weights of the mice in each group were taken before the commencement of the intraperitoneal administration using electronic weighing scale. These

were considered to be the initial body weight. The body weights of all groups were alsorecorded on the last day of oral administration, and these were considered to be the final bodyweight.

2.6 Animal Organ Preparation and Biochemical Assessment

2.6.1 Animal dissection and organ weight measurement

Animals of each group were sacrificed at the end of three treatment days by cervical dislocation. A vertical midline incision was made with scissors from the neck to pubis to open the peritoneum. The liver, kidneys was quickly removed, mopped free of blood and weighed with 0.01precision balance, washed in a phosphate buffered saline of pH 7.4 and they were transferred by blunt forceps to a plain tube, homogenized in storage phosphate buffer and thereafter kept frozen. The small intestines and colons were also removed, opened longitudinally and rinsed in cold phosphate buffered saline of pH 7.4, weighed and kept frozen to be used for biochemical assays.

2.6.2 Preparation of post-mitochondrial fractions

The organs were homogenized using in 0.1M cold potassium phosphate buffer of pH 7.4 Teflon Evelghem homogenizer and centrifuged for 10 minutes at 1000g, to pellet the nuclei. The supernatants were further centrifuged (in TG16 cold centrifuge) at 10,000g for 15 minutes to separate the post-mitochondrial fractions, which were used for biochemical analysis.

2.6.3 Assessment of biochemical parameters

The following biochemical parameters were determined following the protocols indicated in parentheses: total protein concentration (Biuret method by Gornall and Nardawill, 1949), catalase -CAT activity[20], superoxide dismutase -SOD activity [21], glutathione peroxidase (GPx) activity[22], reduced glutathione-GSH [23], glutathione-s-transferase-GST[24], nitrite ion concentration–NO using Griess reaction protocol [25] and myeloperoxidase (MPO) activity [26].

2.6.4 Statistical analysis

Statistical analysis was performed using GraphPad Prism 6.0 statistical package (GraphPad Software, USA). The data were analysed by one or two way analysis of variance (ANOVA), followed by Tukey's multiple comparison test. All values were expressed as mean ± standard error of the mean (mean ± SEM), and all results were considered to be statistically significant at an α-level of $P < 0.05$. Superscripts a, b, c and d indicate groups that were significantly different from negative control (Control – vehicle alone), positive control (ACY only), CMT and MC100.

3. RESULTS

3.1 Co-administration made no significant impact on change in body weight

To determine whether crude ethanolic extract of MC could lead to reduction in body mass within a short period of administration, change in body weight of the mice in all the groups were determined and result of Figure1 shows that MC100 group gained more weight than MC200 when compared with CMT and ACY groups. Control group however, gained the least weight but the differences in body weight gain were not statistically significant.

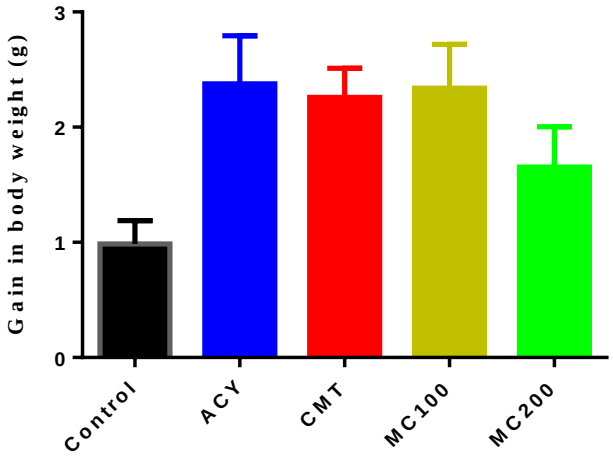

Figure 1: Effect of drug-extract co-administration on change in body weight.
No significant difference was observed. Control (Vehicle only), ACY (acycor), CMT (acycor + cimetidine), MC100 (acycor +Momordica charantia extract at 100mg/kg body weight), MC200 (acycor +Momordica charantia extract at 200mg/kg body weight)

Table 1: Percentages of Liver and Kidney Weight in Relation to Body Weight of Mice

	Con-trol	ACY	CMT	MC 100	MC 200
Percentage relative organ weight	0.99±0.20	2.38±0.42	2.26±0.25	2.34±0.38	1.66±0.35
LIVER	4.64±0.25	4.74±0.22	4.53±0.12	5.09±0.16	5.22±0.18^{bc}
KID-NEY	1.22±0.11	1.12±0.12	1.20±0.09	1.29±0.05	1.22±0.05

Values are represented as mean ± SEM. There was no significant difference among the groups and in all the organs.

3.2 MC200 caused elevated percentage relative liver weight compared with ACY and CMT

In this study MC200 had significantly higher percentage relative liver weight when compared with ACY and CMT groups (Table1). The result however, was not significantly different from Control and MC100 groups.

3.3 Total Protein Concentration, SOD and CAT Activities

The results obtained from this study show no significant difference in the total protein concentrations across all the groups and in all the organs (Table 2).
Specific SOD activity was significantly reduced in the liver by MC100 and MC200 and in the colon by CMT but in the kidney, significant increase in specific activity of SOD resulted from MC200 co-administration (Figure 2). Similarly, catalase activity was significantly increased in the liver and reduced in the kidney by MC200 co-administration (Figure2).

Table 2: Total Protein Concentrations of Liver, Small Intestine, Kidney and Colon

	Con-trol	ACY	CMT	MC 100	MC 200
Total Protein Concentration (mg BSA Equivalent/ml)	0.99±0.20	2.38±0.42	2.26±0.25	2.34±0.38	1.66±0.35
LIVER	14.13±0.31	14.10±0.35	17.07±0.52	17.89±0.47	18.88±0.28
Small Intestine	16.48±0.62	16.50±0.39	15.80±0.38	16.47±0.58	15.52±0.27
KIDNEY	14.82±0.58	14.92±0.41	15.12±0.39	14.21±0.49	15.62±0.35
Colon	11.27±0.18	10.12±0.05	10.01±0.18	10.66±0.13	10.07±0.12

Values are represented as mean ± SEM. There was no significant difference among the groups and in all the organs.

3.4 Concentration of Reduced Glutathione (GSH) and Specific Activities of Glutathione-s-Transferase (GST) and Glutathione Peroxidise (GPx)

As shown in Table 3, CMT co-administration caused significant reduction in the level of reduced glutathione (GSH) in the liver compared with acycor administered alone. MC100 caused significant reduction in GSH level in both liver and small intestine while MC200 caused significant reduction in GSH levels three organs: liver, small intestine and kidney (*P <0.05*). All

the three groups had significantly lower hepatic, renal and small intestinal GSH levels than negative Control but an opposite trend was observed in the colon (p<0.0001). In addition, MC100 group had significantly lower colonic GSH concentration than CMT group (*P <0.05*) while MC200 had significantly lower hepatic and renal GSH concentrations than CMT group (*P <0.05*).

Specific activities of hepatic GST (Table 4) were significantly reduced in CMT, MC100 and MC200 groups when compared with ACY group while in the kidney, CMT group had the highest activity.
In Table 5, specific activities of GPx were significantly reduced in CMT, MC100 and MC200 groups when compared with Control and ACY. MC200 had the least specific activity of GPx in the kidney, while MC100 had reduced colonic GPx activity when compared with ACY, CMT and MC200.

Table 3: Effect of Drug-Extract Co-Administration on Reduced Glutathione (GSH) Concentration expresses as (µg GSH/mg protein)

GSHConc.	Con-trol	ACY	CMT	MC100	MC200
Liver	14.37±0.17	14.57±0.16	11.98±0.21^{a,b}	11.53±0.15 ^{a,b}	10.73±0.15 ^{a,b,c,d}
S.I.	15.02±0.36	12.97±0.24^{a}	12.23±0.23 ^{a}	11.58±0.15^{a,b}	12.00±0.15^{a,b}
Kid-ney	14.73±0.23	13.59±0.16 ^{a}	13.69±0.16 ^{a}	13.89±0.26 ^{a}	12.20±0.13^{a,b,c,d}
Co-lon	16.49±0.32	18.33±0.10 ^{a}	18.70±0.17 ^{a}	17.89±0.12^{a,c}	18.61±0.22 ^{a}

Values are represented as mean ± SEM; Groups with letters a, b, c, d are significantly different from Control, ACY, CMT and MC100, respectively (P <0.05). S.I. – small intestine

3.5 Equilibrium of H_2O_2 Generation and Breakdown Using SOD/ (GPx + CAT) Ratio

The ratio of SOD activity to the sum of the activities of CAT and GPx were also assessed and the results were expressed as bar chart (Figure 3). SOD/(GPx+CAT) values for CMT, MC100 and MC200 did not differ significantly from ACY group in liver, small intestine and colon. Contrarily in the kidney, MC200 had significantly higher SOD/(GPx+CAT) ratio than other groups but this value did not reach unit (1) which makes it insignificant on the long run.

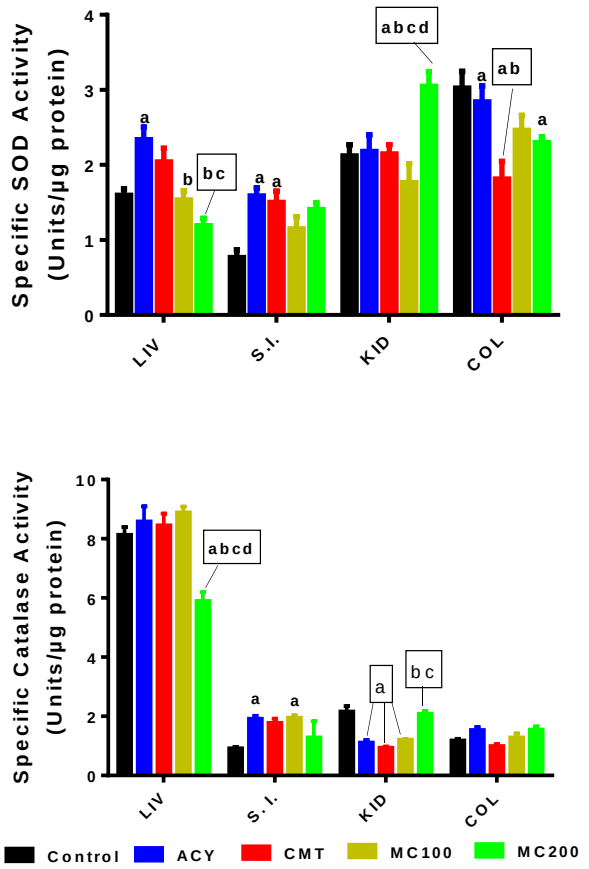

Figure 2: **Effect of Drug-Extract Co-Administration on Specific SOD and Catalase Activities**

Each bar represents mean±SEM. Groups with letters a,b,c,d were significantly different from Control, ACY, CMT and MC100, respectively (P < 0.05)

LIV (liver), KID (Kidney), S.I. (Small intestine), COL (Colon), SOD (superoxide dismutase)

Table 4:Effect of Drug-Extract Co-Administration on Glutathione-s-Transferase (GST) Specific Activity (Expressed in terms of µmolCDNB/mg Protein)

Speci-fic GST activity	Con-trol	ACY	CMT	MC 100	MC 200
Liver	31.21 ±1.22	43.51±3 .28 [a]	28.69± 3.24 [b]	26.69± 5.25 [b]	20.91± 2.79 [a,b]
S.I.	8.91± 1.90	12.11±1 .37	10.40± 0.65	9.02±0. 91	9.41±0. 69
Kidney	16.97 ±0.71	10.80±0 .77	25.61± 1.90 [a,b]	15.72± 2.73 [c]	14.00± 0.64 [c]
Colon	9.62± 1.02	8.97±1. 34	8.34±1. 71	10.03± 1.85	7.23±0. 73

Values are represented as Mean±SEM; Groups with letters a, b, c, d are significantly different from Control, ACY, CMT and MC100, respectively (P <0.05). S.I. – Small intestine

Table 5: Effect of Drug-Extract Co-Administration on Glutathione Peroxidase (GPx) Specific Activity (Expressed as µg GSH Consumed/mg Protein)

Specific GPx Activity	Contr ol	ACY	CMT	MC100	MC200
Liver	15.39 ±0.23	15.48± 0.38	12.99± 0.39 [a,b]	12.43±0 .33 [a,b]	11.68±0. 20 [a,b]
Small Intestine	13.25 ±0.41	13.21± 0.26	13.71± 0.31	13.08±0 .57	13.08±0. 25
Kidney	14.22 ±0.38	14.17± 0.40	13.77± 0.38	15.18±0 .48	12.21±0. 28 [a,b,c,d]
Colon	19.45 ±0.36	21.98± 0.36 [a]	22.08± 0.45 [a]	19.51±0 .29 [b,c,d]	21.11±0. 24 [a]

Values are represented as mean ± SEM; Groups with letters a, b, c, d were significantly different from Control, ACY, CMT and MC100, respectively (*P <0.05*).

3.6 Assessment of Inflammatory Markers (Nitrite Ion Concentration-NO_2^- and Myeloperoxidase Activity-MPO)

The anti-inflammatory effects of MC have been reported in previous studies. Results obtained from this study (Figure 4) show that ethanolic extract of *Momordica charantia* (MC) displayed different inflammatory effects in the four organs examined in relation to NO^{2-} concentration and MPO activity. Hepatic and renal NO^{2-} of MC100 and MC200 were significantly higher than both ACY and CMT groups but were significantly lower than them in the small intestine. CMT co-adminmistration with ACY reduced NO^{2-} concentration in the liver and colon but significantly increased it in the kidney compared to ACY administered alone. In the colon also, MC100 was significantly higher than CMT while MC200 was significantly lower than ACY.

As shown in the present study, hepatic MPO activity increased significantly after co-administering ACY with the two doses of *Momordica charantia* leaf extract, and was also significantly elevated in the small intestinal post-mitochondrial fractions of CMT and MC200 compared with ACY. In the kidney however, MC100 co-administration caused a significant reduction in the enzyme activity when compared with Control and ACY groups.

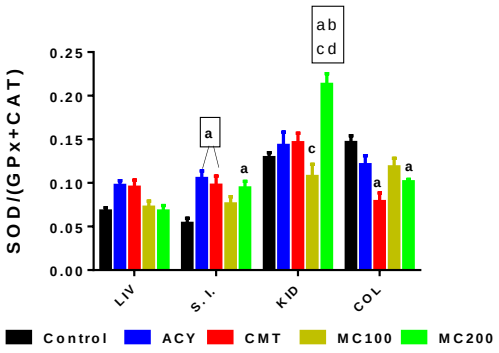

FIGURE 3: Effect of Acycor Co-administration with Cimetidine and MC extract on SOD/(GPx+CAT) Ratio.

Values are represented as Mean±SEM; Groups with letters a, b, c, d were significantly different from Control, ACY, CMT and MC100, respectively (P <0.05).
LIV (liver), KID (Kidney), S.I. (Small intestine), COL (Colon)

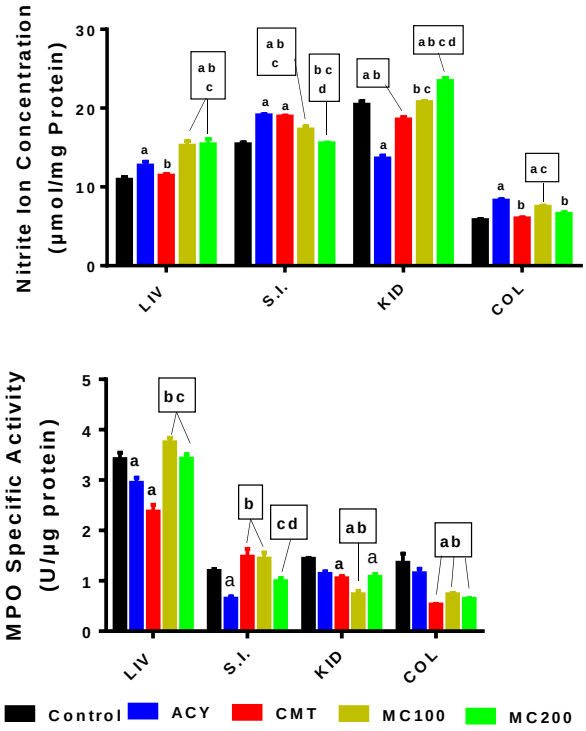

FIGURE 4: Effect of Acycor Co-administration with Cimetidine and MC extract on Nitrite ion Concentration and Myeloperoxidase (MPO) Activity
Values are represented as Mean±SEM; Groups with letters a, b, c, d were significantly different from Control, ACY, CMT and MC100, respectively (P <0.05)
LIV (liver), KID (Kidney), S.I. (Small intestine), COL (Colon)

4.0 DISCUSSION

Due to the short duration of this study, intra-peritoneal method of drug and extract administration was adopted to enhance absorption of administered agents from GIT lumen and avoid first pass metabolism. No significant change in body weight was observed among the five groups of mice used in this study. Acycor singly administered and co-administered with cimetidine and *Momordica charantia* plant extracts caused slight increases in body weight compared with Control (which received only the vehicle). Although studies have shown that MC extract can capably prevent excessive gain in body mass possibly due to the presence of some essential vitamins which are vital for healthy growth and development [27], significant effect on body weight was not observed in this study The reason for this might be due to the short duration of the experiment.

Some studies have reported renal toxicity due to excess use of *Momordica charantia* fruit extract up to 4,000mg/kg body weight while lower doses were not found to be toxic[28] Similarly in this experiment, no significant effect was observed on percentage relative kidney weights, their colon lengths and intestinal weights (results not shown) in the mice that received 100mg/kg and 200mg/kg body weight doses of MC extract. However, significant increase in percentage relative liver weight of the group administered a higher dose of the plant extract (200mg/kg body weight) might indicate a trend towards hepatotoxicity at higher dosage and prolonged duration of the extract. Probable mechanism could be an interaction between acycor and some phytochemical constituents in the plant extract that possibly altered metabolic activities of the animals.

Protective antioxidant enzymes such as SOD and CAT are the first line of defence against reactive oxygen species-ROS [19]. Many normal biochemical processes that occur in eukaryotes involve generation of controlled amounts of reactive oxygen and nitrogen species most often to serve as self-defence mechanism against antigenic substances. These reactive species are normally removed from the system by specialized antioxidant defensive mechanisms which work in a concerted manner to prevent oxidative damage to cells and tissues. However, when the levels of these radicals exceed the capacity of antioxidant molecules, oxidative stress results [17]. The changes in the equilibrium between the formation of hydrogen peroxide by superoxide dismutation and its decomposition by the GPx and CAT in the organs are represented by the Ratio (R) = SOD/(CAT+GPx) which is the ratio of SOD specific activity to the sum of CAT and GPx specific activities. Ratio less than one (1) is an indication of high antioxidant status, ratio of exactly 1 indicates an equilibrium (i.e. a balance between the rate of ROS production and their breakdown), while a ratio above 1 indicates excessive ROS production with depleted levels or activities of antioxidant enzymes. Significant depletion in hepatic SOD activity caused by co-administration of the plant extract with acycor possibly indicated increased superoxide anion generation. From the result obtained for superoxide dismutase, components of MC extract possibly inhibited superoxide anions ($O_2^{\cdot-}$) generation in the liver which led to

reduction of SOD activity. The significant increase in renal SOD activity in the MC200 group might however mean that high levels of superoxide anions were generated in that group.

In order to determine antioxidant state of an organism, ratio of SOD/(CAT+GPx) was determined in all the organs. Although renal SOD/(CAT+GPx) was significantly higher in the group administered higher dose of *Momordica charantia* leaf extract (MC200) than other groups ($p<0.0001$), the ratio was not up to 1 which means none of the agents administered elicited significant effect during the period of the experiment. However, oxidative stress might likely occur over prolonged administration of high dose of MC leaf extract which may possibly lead to renal damage, following this route of administration. In other organs however, the extracts displayed good antioxidant activity by lowering the ratio of SOD/(CAT+GPx) which was comparable with CMT group. Conversely, the lower dose of the plant extract (MC100) had consistently lower SOD/(CAT+GPx) ratio than ACY which suggests that the ethanolic extract of MC at low dosage (e.g. 100mg/kg body weight or less) is relatively save, being able to reduce reactive oxygen free radicals and improve the activities of antioxidant enzymes in all the four organs assessed [29].

The reduced activities of hepatic CAT of MC200 mice were proportional to the hepatic reduced glutathione (GSH) concentration. *Momordica charantia* was not effective in improving the activity of glutathione peroxidase (GPx) in the liver of the animals, the same trend was observed with GSH concentration. Since GSH is a co-substrate for GPx and Glutathione-S-transferase (GST), the reduction in the activity of these enzymes may be due to the inhibition in the synthesis of GSH or increased ROS generation in MC200 group. Certain bioactive components present in *Momordica charantia* might have inhibited the activities of γ-glutamyl cysteine synthetase and GSH synthetase involved in GSH synthesis. Equally, most studies on the medicinal effects of MC usually involve the oral route of administration. Therefore, the reduced activities of GPx and GST in MC groups may be associated with the route and duration of administration.

Recently, more insight has been gained on the NSAIDs-induced mucosal injuries through clinical research. Increase in the generation of ROS and lipid peroxidation has been reported to be involved in the pathogenesis of many diseases of known and unknown aetiology and in the toxic actions of many compounds [3]. Ratio of SOD/(CAT+GPx) greater than 1 is an indication of excessive generation of superoxide anions ($O2^{\cdot-}$) above the rate at which they are being mopped off from circulation and vice versa. When the rate equals to 1, it is an indication of a balance between the rate of $O2^{\cdot-}$ production by SOD and their conversion by CAT or GPx to less reactive agents such as H_2O_2 or their complete breakdown to oxygen and water. Although ACY groups had higher SOD, CAT and GPx activities than MC groups, the elevated ratios of SOD/(CAT+GPx) in ACY groups in the liver, kidney and the bowels show that the rate of superoxide anions generation by SOD far exceeds the rate of their elimination from these organs, thus confirming side effects experienced by patients after prolonged usage or over dose of NSAIDS. However, this elevated ratio was reduced by co-administration of 100mg/kg body weight of MC extract and the results were comparable with control group. The 200mg/kg dose of MC may pose oxidative stress in the kidney as revealed by the significantly elevated ratio of these antioxidant markers compared to other groups. Nitric oxide (NO), is an important mediator of vascular tone and renal function, it regulates glomerular, vascular and tubular function in the kidney [18]. The anti-inflammatory effects of MC have been reported in previous studies, and this property may be connected to the production of healthy concentration of hepatic NO_2^- (i.e. endothelial nitric oxide). In a study, it was shown that an increase in renal and vascular oxidative stress can be effectively counteracted by NO via its rapid inactivation of superoxide [30]. Since nitrite ion concentration is both beneficial and cytotoxic, one cannot conclusively say that the high levels observed in the MC groups were beneficial or not until a clear distinction is made between the endothelial and inducible forms of NO_2^-. However, the significantly raised values compared to cimetidine-administered group (CMT) should caution one concerning the dosage to use when administering the extracts for chronic studies.

An earlier study reported, that elevated Myeloperoxidase (MPO) levels more than doubled the risk for cardiovascular mortality over a certain period [19]. Myeloperoxidase, a pro-inflammatory marker, is capable of using nitric oxide as a physiologic substrate, thereby catalytically sinking the therapeutic concentration of NO_2^-, which might contribute to cytotoxicity of the endothelial cells. MPO has been associated with oxidative damage of tissues at the site of inflammation, and an increase in their activity is often used as a marker for inflammation. The reduced MPO activities of ACY group compared to other groups is not surprising due to the therapeutic dose of the drug administered, itself also, an anti-inflammatory agent. 100mg/kg dose of *Momordica charantia* extract however, showed some better anti-inflammatory properties in the kidney and colon than acycor-plus administered alone. Similarly, cimetidine synergistically increased anti-inflammatory properties of ACY in the liver and colon compared with the two doses of the extract.

4. CONCLUSION

The results obtained from this study reveals that co-administration of cimetidine with acycor-plus, an NSAID, at low dosage did not significantly alter the antioxidant effect of the drug but instead synergized its anti-inflammatory properties in the liver, small intestine and colon. The 100mg/kg dose of ethanolic extract of

Momordica charantia had comparable antioxidant effects with CMT and also increased the anti-inflammatory effects of acycor-plus in all the organs. However, caution should be exercised in the dosage administered as the 200mg/kg dose may cause renal damage due to increased activity of MPO in the kidney and elevated ratio of SOD to the sum of CAT and GPx. However, further research is necessary using the oral route of administration and longer duration to further ascertain therapeutic and toxic effects of this plant extract.

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COMPETING INTERESTS

No competing interest as far as the authors are concerned.

AUTHORS' CONTRIBUTIONS

BO designed the study, and wrote the protocol; **BO, MA, OE** and **SH** took part in the biochemical assays; **BO, MA OI** and **GA** performed the statistical analysis, **BO** wrote the first draft of the manuscript; All authors managed the literature searches, read and approved the final manuscript.

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