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EVALUATION OF THE EFFECTS OF *Mormodica balsamina Linn*. EXTRACTS ON SOME BIOCHEMICAL PARAMETERS IN MICE

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ABSTRACT

Medicinal plants can elicit changes in biochemical parameters and in the integrity of animal tissues on long term usage. Whole *Mormodica balsamina*, was extracted in ethyl acetate, methanol and hexane. The effects of these extracts on some biochemical parameters were investigated in mice. Eighty mice were divided into four groups (A, B, C and D) of twenty animals each. Group A mice were given normal saline (0.9% w/v NaCl) while group B, C and D were gavaged with the specific extracts each at 400mg/Kg/body weight for five weeks. Whole body weights, packed cell volume (PCV) and some biochemical parameters were analysed using standard methods. The results showed only minimal variations in PCV and total body weights of animals irrespective of the extracts. There were significant ($p<0.05$) variations in total proteins (5.14±2.16 mg/dL), triglycerides (126.55±2.61 mg/dL) and alkaline phosphatase (140.00±4.28 IU) in animals administered the ethyl acetate extract compared to the controls. The methanol extracts only caused an appreciable increase in total proteins (7.00±1.83 mg/dL) in week one of treatment but did not result in significant alterations of other parameters analysed. Similarly hexane extract significantly reduced triglyceride levels (130.68±1.23) in only week four of treatment. Histopatological examinations of kidney, liver and spleen in the ethyl acetate treated mice showed no associated lesions in these tissues. These results indicate that long term administration of the extracts of *Mormodica balsamina* at the safe dose of 400mg/Kg bw are not deleterious.

Keywords: *Mormodica balsamina*, Total Proteins, Triacylglycerides, Packed Cell Volume, Alkaline Phosphatase

INTRODUCTION

Mormodica balsamina Linn family *Cucurbitaceae* is a perennial herb found in tropical Africa, most of Asia and Australia. The plant is widely reputed and used in herbal medicaments for malaria fever, cutaneous lesions, emetics, as vermifuge, tranquilizer, anti-diabetic, abortifacient etc. **[1, 2]**

There are reports of the use of *M. balsamina* as vegetables. The Portuguese, Shangaan and Kanuri of Northern Nigeria relish the bitter taste of the leaves and fruits hence use them as culinary herb. Such leaves and green fruit are cooked and eaten as spinach, sometimes with groundnuts or mixed with porridge **[3]**.

M. balsamina contains two alkaloids including momordicin, volatile and fixed oils, carotene, a resin, vitamin A and calcium. The green fruit also contains toxic alkaloids and a saponic glycoside that causes vomiting and diarrhea. These metabolites are however denatured in the cooking process. The fruit has been implicated in the poisoning of dogs and pigs **[1, 4]**. Herbal Medicinal practices in most instances are speculative in terms of dosage, period of treatment and organ effects often with deleterious and even fatal outcomes.

Leaves of *Telferia occidentalis* Hook (fluted pumpkin) are common as vegetable in Nigeria but reportedly contain toxic alkaloids and saponins. *Symphytum officinale L.* (confrey) consumed as herbal tea in the USA is known to contain pyrolizidine alkaloids which are potent hepatotoxins **[5]**. *Khaya senegalensis* is used in the treatment of a variety of ailments especially malaria but its chronic administration has been implicated as nephrotoxic in rats. Also, *Moringa oleifera* is popular as medicine and vegetable but seed extracts at high doses elevated levels of serum transaminases and other biochemical parameters in rodents **[6, 7]**.

In view of the common usage of *M. balsamina* in herbal treatments and also as food, the present study has thus been necessitated to evaluate the effect of the plant on some biochemical parameters *in vivo*.

MATERIALS AND METHODS

Plant Materials

Fresh whole *M. balsamina* were collected between August – September in Minna, Northern Nigeria after due identification by a herbal medicinal practitioner. It was subsequently authenticated at the Department of Biological Sciences, Federal University of Technology, Minna.

Preparation of Extracts

40g of air dried whole plants were micronized and extracted exhaustively (48h) in the cold with 1.5L each of hexane, (Sigma-Aldrich

Europe), ethyl acetate and methanol in that order. The marc was dried after each extraction with a single solvent. Extracts were filtered with muslin cloth and solvents removed under reduced pressure in a rotary evaporator. Brown coloured pastes were obtained and weighed prior to further analysis.

Animals

Healthy Swiss albino mice of either sex, about 4 – 6 weeks old weighing between 20 – 30g each obtained from National Institute of Pharmaceutical Research and Development (NIPRD) Abuja were used for the experiments. The rodents were conveniently housed under standard environmental conditions: temperature, 27±2^{o}C; 70% relative humidity; had free access to commercial feed pellets and water and natural 12h daylight/night cycles. Experiments were conducted in strict compliance with relevant codes for animal use of the Federal university of Technology, Minna and also existing internationally accepted principles for laboratory animal use and care as contained in the Canadian Council on Animal Care Guidelines and Protocol Review **[8]**.

Safe Dose and Acute Toxicity (LD_{50})

Five groups of four mice each were used and the animals were given extracts intra peritoneally (i.p.) at doses of 200, 400, 600, 800, 1200mg/kg body weight (bw) respectively. Extracts were dissolved in dimethyl sulphoxide (Sigma Chemicals; St Louis, MO, USA).

A control group was given normal saline (0.9% w/v NaCl) at 20ml/kg bw. Mice were observed over 72h and clinical signs and mortality were recorded. LD_{50} was obtained as the intercept of % mortality (y-axis) and dosages (x-axis).

Evaluation of the Effects of Sub Chronic Administration of Crude Extract in Mice

Eighty mice were kept in four groups (A, B, C and D) of twenty each. Group A was used as control and were given 20ml/kg bw normal saline (0.9% NaCl) daily while B, C and D were used as test and gavaged 400mg/Kg bw extracts daily. All the animals were monitored for different biochemical parameters at weekly intervals for five weeks.

Weights of mice were taken with an Avery Balance (W and T) Avery Ltd, Birmingham, UK. Packed Cell Volume (PCV) was determined using the microhaematocrit method [**9]**. An uncaliberated capillary tube was filled 2/3 of the volume with blood by capillary action and one end was sealed with crystal seal. The tubes were transferred to the heamatocrit centrifuge and allowed to spin at 12,000rpm for 5 minutes. The PCV was then

obtained using the heamatocrit measuring guage.

Serum glucose was assayed using the Randox glucose diagnostic kit (Cat/Kat NR GL 2623) based on the glucose oxidase reaction. A red-violet quinineimine derivative was obtained from the series of reations and read at 500nm **[10]**.

Total proteins were also evaluated with the Randox Protein Diagnostic Kit (TP 245) based on the interaction of cupric ions in alkaline media with protein peptide bonds resulting in the formation of a coloured complex that was read against a protein standard at 530nm **[10]**.

The AGAPPE, Triglyceride Kit (Cat. 1121500, Kerala, India) was employed for serum triglycerides. This involved the reactions by lipases, glycerol kinase, glycerol – phosphate oxidase and peroxidase which produced a red quinone dye read at 630nm **[11]**.

Alanine Amino Transferase (ALT) and Aspartate Amino Transferase (AST) were analyzed using the DIALAB IVB standard diagnostic kits based on the method of **[12]**.

The test principle for the determination of ALT is based on the oxidation of NADH to NAD^+ in the conversion of Alanine to Pyruvate, the resulting decrease in absorbance at 340nm being proportional to the activity of the enzyme in the sample. This is a modified formulation for the assay of ALT, as recommended by the International Federal of Clinical Chemistry (IFCC). The IFCC reference method includes pyridoxal phosphate (PP) which functions as a co-factor in amino acid transamination. It increases ALT activity. It also avoids falsely low values in samples containing insufficient endogenous Pyridoxal Phosphate as in patients with myocardial infarction, liver disease and intensive care patients **[13]**.

The laboratory procedure involved taking a working reagent of (1000ul), serum (100ul), which were mixed and incubated at 37^oC for 1 minute. The change in absorbance (OD/min) was read at 340nm in a glass cuvette of 1cm path length within 3 minutes.

ALT activity (µ/l) = (OD/min) x 1768

The determination of AST is similar to that of ALT but only differs in the substrate aspartate. The oxidation of NADH to NAD^+ and resulting decrease in absorbance at 340nm is proportional to the activity of AST in the sample **[12]**.

Serum alkaline phosphatase (ALP) was however determined on the basis of the conversion of p-nitrophenol to its intensely yellow colored derivative, 4-nitrophenoxide **[10]**. The laboratory method involved setting up tubes for serum sample, serum blank,

standard and reagent blanks. 2mls of disodium phenyl phosphate carbonate bicarbonate buffer was added to all tubes and incubated for five minutes at 37^{o}C. 50ul of phenol (standard reagent) was added to the standard test tube only and 50ul of serum to serum samples, all tubes were incubated for exactly 15 minutes at 37^{o}C. After 15 minutes the blocking reagent (4-Amino antipyridine) was added to all the tubes in a quantity of 0.5ml each, after which 0.5ml of sodium arsenate buffer at pH 10 was added to all the tubes, mixed well and 50ul of distilled water was added to serum and reagent blank tubes only. They were mixed, allowed to stand for 10 minutes in the dark and the color intensity measured at 510nm.

This solution was stable for 45 minutes. Activity was calculated using the optical densities of serum blank and serum samples in relation to the optical density of the standard using a factor of 142.

Histopathology of Tissues

At the end of the treatment period, mice administered the ethyl acetate extract were sacrificed and liver, kidney and spleen were collected in sterile saline. Freshly dissected organs from each animal were cut rapidly and fixed in buffered neutral formalin (10%). The tissues were dehydrated in ascending grades of ethanol (70%, 80%, 90%, 95% and 100%), cleared in two changes of xylene, impregnated with 2 changes of molten paraffin wax, and finally embedded in wax. Tissue sections of 4-5µm in thickness were cut with a microtome and stained with hematoxylin and eosin **[14]**.

Statistical Analysis

Results are expressed as mean ± standard error of the mean. While student's t-test was used to test for differences between groups using Statistical Package for Social Sciences (SPSS) version 16. A value of $p<0.05$ was accepted as significant and the data compared using Analysis of variance (ANOVA)

RESULTS

Table 1 contains the results for the safe dose determination of crude *M. balsamina*. No apparent changes were observed with the test animals up to 400mg/Kg bw which was hence chosen as the safe dose. LD_{50} was calculated to be 1200mg/Kg bw. A variety of reactions were observed at higher doses with some mortalities at 1000 and 1200mg/Kg bw. Results for the analysis of serum glucose, total proteins and triglycerides in mice administered different *M. balsamina* extracts for 5 weeks are in **Table 2**. There were no significant ($p>0.05$) differences in glucose levels between test and control animals in the three treatments. Total protein only varied significantly ($p<0.05$) in week three with

ethylacetate extract and week one with methanol extract but these levels were not significant ($p>0.05$) by week five. Ethyl acetate extract treatment significantly ($p<0.05$) decreased triacylglycerides in mice. A similar effect was obtained in week four only with hexane extracts. Methanol extracts had no significant ($p>0.05$) effect on triglyceride levels.

Only ethylacetate extracts significantly ($p<0.05$) elevated ALP levels in treated animals **(Table 3)** ALT and AST levels were not significantly ($p>0.05$) different between test and control mice irrespective of the extracts used.

Figure 1 depicts the changes in total body weight of mice administered different extracts of *M. balsamina* over a period of five weeks. It shows only a slight decrease in animals given methanol extracts. Also, **Figure 2** shows only minimal variations in the packed cell volume between test animals and controls over the period of study. **Figures 3, 4** and **5** are micrographs of the kidney, liver and spleen of mice administered ethylacetate extracts of *M. balsamina* over a period of 5 weeks. These tissues were not damaged and exhibited normal histological patterns compared to the controls.

Table 1: Safe Dose (Pre LD_{50}) of *M. balsamina* Extract in Mice

Dose (mg/kg / bw)	Observations	Mortality
100	No Observable Changes	0/4
200	Apparently No Changes	0/4
400	No Physical Activity or Alterations in the Animals	0/4
600	Restless Irritable but Normal	0/4
800	Drowsy, Slow Breathing	0/4
1000	Erythrema, Salivation, Laboured Breathing	1/4
1200	Very Weak, Bradycardia	2/4

NOTE: Safe Dose=400mg/kg bw; LD_{50}=1200mg/kg bw

Table 2: Serum Glucose, Total Proteins and Triacylglyceride Levels in Mice Administered *M. balsamina* Fractions

Parameters (mg/dL)	1	Period (Weeks)		4	5
		2	3		
Glucose					
Control	94.22± 1.13	96.18± 3.12	100.02± 1.15	98.45± 2.86	102.17± 5.28
ME	96.15± 3.35	96.00± 1.23	98.59± 3.59	102.66± 4.00	102.88± 3.79
MM	91.15± 1.10	95.99± 1.01	95.08± 3.58	97.62± 2.22	98.00± 3.60
MH	100.00± 4.56	98.83± 2.36	99.82±2.51	97.85± 3.11	98.00± 2.68
Total Protein					
Control	5.21± 0.02	6.15± 1.00	7.36± 2.25	6.10± 1.15	7.00± 1.50
ME	4.89± 0.25	5.00± 0.88	5.14± 2.16*	7.08± 1.11	6.94± 1.84
MM	7.00± 1.83*	7.04± 1.06	8.03± 1.05	6.67± 0.85	6.88± 0.50
MH	6.28± 1.11	6.24± 0.01	6.08± 2.00	7.01± 1.20	8.08±2.54
Triacylglycerides					
Control	135.08± 5.53	138.16± 3.15	139.10± 2.11	140.88±3.12	136.51±2.16
ME	130.00±1.35	126.26±3.00*	125.01±2.23*	128.88±3.24*	126.55±2.61*
MM	136.84± 3.35	136.66± 3.25	135.01±1.12	138.84±2.10	135.18± 2.55
MH	140.00±4.80	138.85±2.53	138.88±1.85	130.68±1.23*	131.52± 2.62

NOTE: * = Significant (P<0.05)

Table 3: Serum Levels of Alkaline Phosphatase(ALP), Alanine Aminotransferase(ALT) and Aspartate Aminotransferase(AST) in Mice Administered *M. balsamina* Fractions

Serum enzymes (I.U)	1	Period (weeks)		4	5
		2	3		
Alkaline Phosphatase					
Control	102.00± 2.29	110.30± 2.35	108.84± 5.03	120.00± 4.79	110.08± 4.34
ME	105.02± 1.13	108.00± 3.44	115.61± 3.55	138.06±4.88*	140.00±4.28*
MM	110.30± 2.93	115.04± 3.99	110.60± 3.03	120.66± 5.18	115.00± 5.60
MH	98.88± 1.38	112.05± 5.55	113.60± 5.45	125.00± 4.88	120.06± 3.51
Alanine Aminotransferase					
Control	32.56± 1.65	31.84± 3.18	29.99± 1.00	33.15± 3.21	31.98± 1.22
ME	31.58± 2.23	33.54± 2.11	32.58± 1.54	30.00± 1.28	29.75± 1.34
MM	35.34± 4.43	35.99± 4.63	32.80± 2.18	34.77± 2.15	34.88± 5.63
MH	30.11± 1.04	32.00± 3.73	34.89± 3.33	35.60± 3.25	36.58± 4.99
Aspartate Aminotransferase					
Control	42.00± 3.84	43.15± 6.00	43.85± 2.23	40.83± 2.56	45.01± 4.35
ME	41.04± 2.48	48.16± 6.51	40.51± 3.20	43.70± 3.11	43.08± 2.22
MM	43.70± 5.00	40.28± 3.11	46.00± 5.91	42.08± 3.11	44.44± 4.18
MH	43.00± 1.08	45.65± 3.19	42.18± 3.22	44.65± 3.85	43.99± 3.01

NOTE: ME= Ethyl Acetate Fraction; MM=Methanolic Fraction; MH=Hexane Fraction; *=Significant (P<0.05)

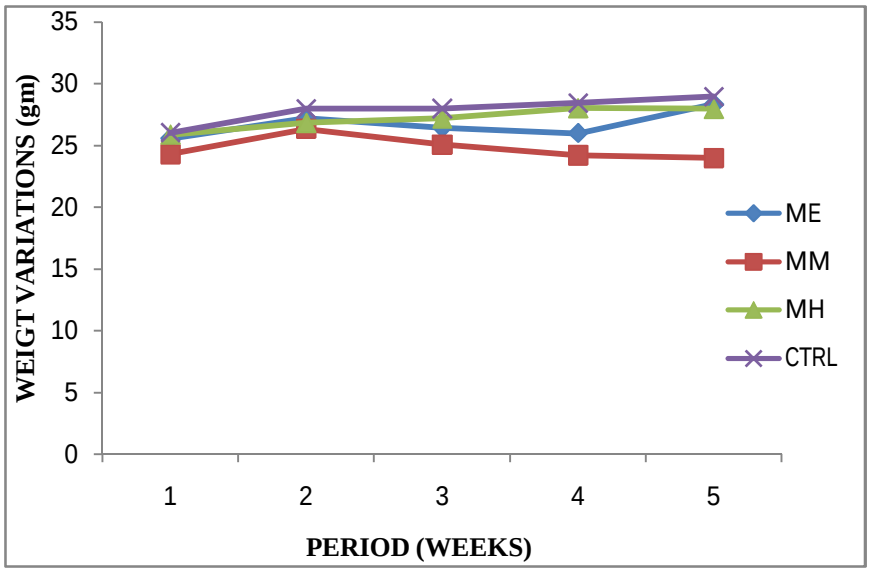

Figure 1: Weight Variations in Mice Administered Fractions of *M. balsamina*

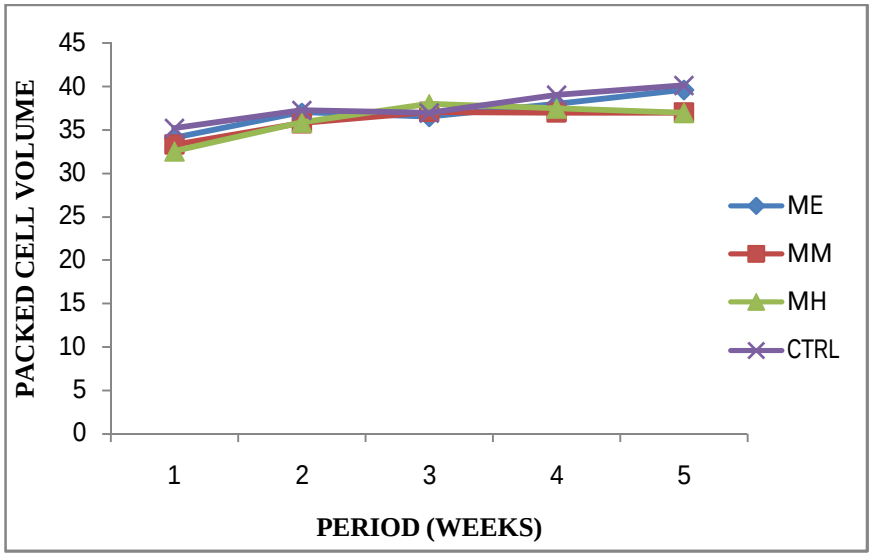

Figure 2: Variations in Packed Cell Volume of Mice Administered Fractions of *M. balsamina*

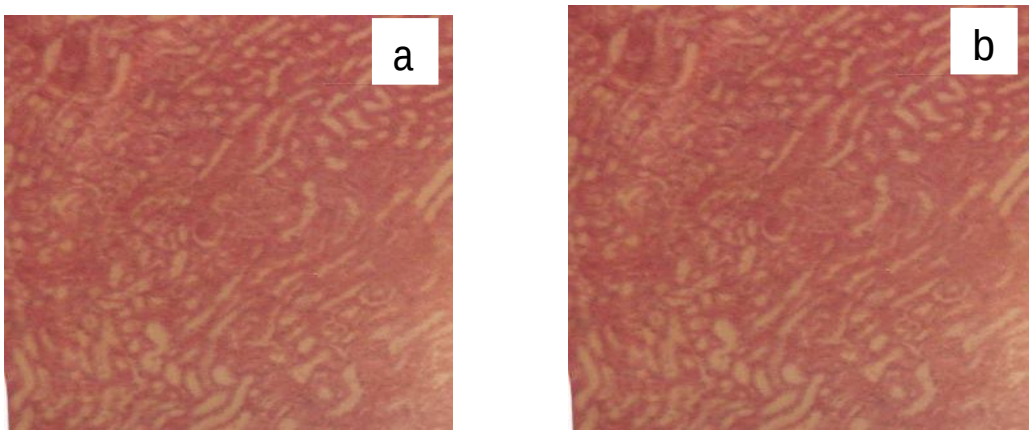

Figure 3: (a) Kidney (H.E. X400) Section of Control Mice Showing Normal Cortical Distribution of Glomeruli; Tubules and Vascular Channels are Unremarkable
(b) Kidney (H.E. X400) Section of Treated Mice; Similar Morphology to (a) Showing Normal Histology

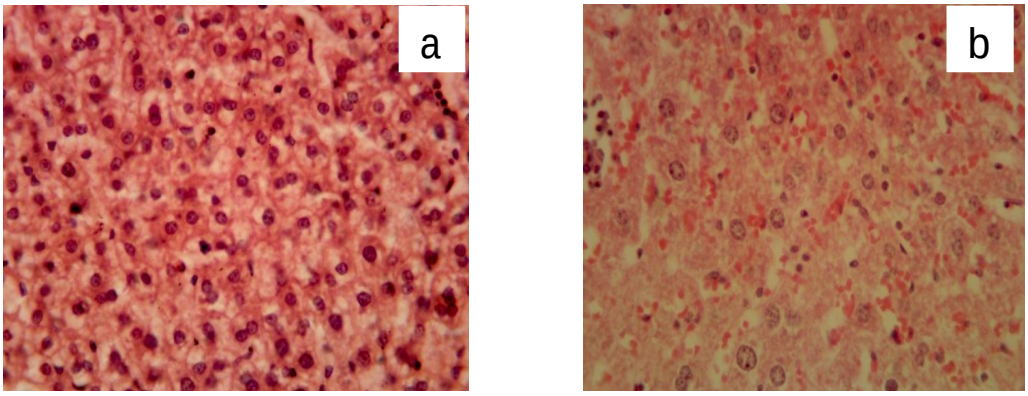

Figure 4: (a) Liver (H.E. x 400) Section of Control Mice With Normal Portal Triad and Sinus; Normal and Hexagonal Hepatocyte Architecture
(b) Liver (H.E. X400) Section of Treated Mice Similar and Normal Compared to Control (a)

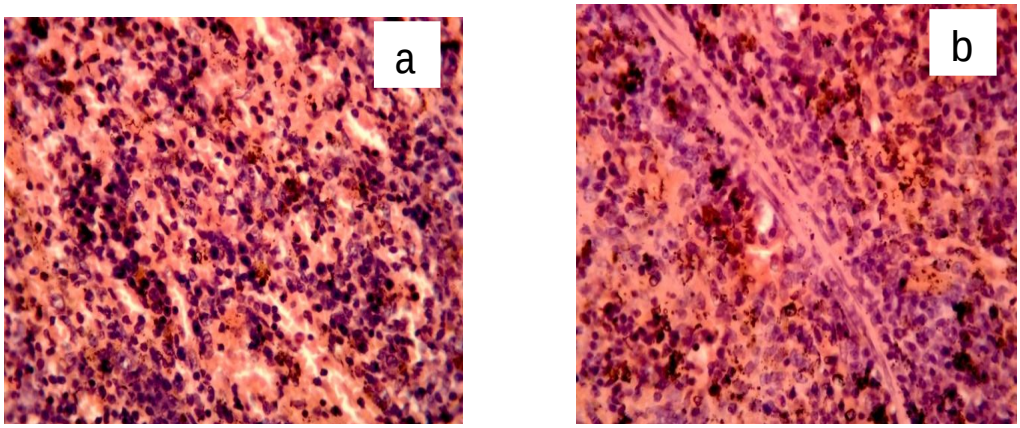

Figure 5: (a) Spleen (H.E X400) Section of Control Mice with Normal Cortical Arrangements of the Lymphoid Follicles and Parafollicular Proportion of T-Lymphocytes
(b) Spleen (H.E. X400) Section of Treated Mice; Appears Normal Despite Slight Discoloration

DISCUSSION

The present study has shown only a minimal body weight variation in total body weight of mice administered methanol extract of *M. balsamina*. Assessment of weight variations in experimental animals on repeated xenobiotic administration provides a preliminary but important insight about the safety of materials under investigation. Feed utilization and body weights are reported to be the only significant changes obtained with materials of low toxicity. Dehydration, diarrhea and stress are other factors that influence weight change **[15]**. Similar to weight, the Packed Cell Volume (PCV) of the animals were unaffected by prolonged administration of the extracts. This could be due to low concentration or the absence of anti-nutritional factors in the extracts. Such compounds chelate minerals e.g. iron, rendering them unavailable and ultimately affecting erythropoiesis. It is also feasible that the hematopoietic organ, spleen was unaffected **[16]**.

Serum glucose levels were unaffected by the extract throughout the duration of the study. Fluctuations in the blood sugar levels are associated with the liver as the principal glycogen depot and also glucostatic hormones obtained from the pancreas. A closer scrutiny of the results indicate that glucose values in animals given methanol or hexane extracts were consistently lower than values obtained for controls during weeks 3- 5. The finding necessitates further investigation to confirm whether the extracts can actually lower sugar level in diabetes induced animal models. This is moreso that diabetes was mentioned among the plethora of diseases acclaimed by herbal practitioners to be responsive to *M. balsamina* medication **[4]**.

Variability in total serum proteins between the control and test animals were observed in ethylacetate and methanol extract treatments in weeks 3 and 1 respectively. These effects however reverted to normal in the final weeks of experimentation. Depreciation in total proteins in the serum is an early indication of hepatic or renal injury. Liver tissues synthesize and secrete 50% of the total protein produced by the body on a daily basis. These include albumins, α and β- globulins, fibrinogen, prothrombin, transport proteins etc. The kidney is responsible for filtering out lower molecular weight and reabsorption of threshold metabolites hence renal failure compromises these vital processes **[10]**.

Triacylglyceride levels were consistently and significantly ($p<0.05$) lowered by ethyl acetate fractions of *M. balsamina.* A similar effect was obtained in week 4 for hexane extracts. Triacyglycerides are rich alternative

sources of metabolic energy and can readily be metabolized on demand such as stress. The hypolipidemic potential of *M. balsamina* is noteworthy and could be exploited in the management of some metabolic disorders **[17]**.

Alkaline phosphatase levels were significantly ($p<0.05$) elevated in animals administered ethylacetate extracts of *M. balsamina*. Raised serum ALP is indicative of bone, intestinal and bililiary disorders such as obstructive jaundice and liver cirrhosis **[15]**. Histopathology especially of the liver is required as confirmatory evidence to establish such injuries.

The transaminases (ALT and AST) did not vary significantly ($p>0.05$) between test and control animals. These enzymes are markers of hepatocytes. Serum ALT level is of greater diagonistic specificity in respect to the liver than AST. This is because the latter exists in high levels in other tissues such as kidney, heart, pancreas and skeletal muscles **[18]**.

The histopathological assessment of tissues is confirmatory to diagnostic, clinical or biochemical findings, although the latter is most sensitive. Enzymes can be elevated and serve as early warning of diseases before clinical parameters are significantly disturbed **[19].** The kidneys of mice in the present study have normal cortical distribution of glomeruli with unremarkable tubules and vascular channels. Liver sections exhibited normal portal triad and sinus with hexagonal hepatocyte architecture. The treated animals also showed normal cortical arrangement of lymphoid follicles and parafollicular proportions of T-Lymphocytes in the spleen. These findings indicate that despite the alterations in some parameters noted especially with the ethylacetate fractions, no damage occurred to the liver, kidney or spleen.

CONCLUSION

Mormodica balsamina extracts are generally safe and can be consumed repeatedly over a reasonable period without adverse physiological effects or tissue damage. The need for caution is still paramount pending a more comprehensive study to include other haematological, biochemical and histopathological examinations.

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