

### THE EFFECT OF AQUEOUS EXTRACTS OF *MOMORDICA BALSAMINA* ON HAEMATOLOGICAL AND BIOCHEMICAL PARAMETERS IN RATS.

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The effect of the aqueous extract of the whole plant of *Momordica balsamina* (Linn), on haematological and biochemical indices in rats was studied. Two doses (1 and 1.5 g/kg<sup>-1</sup> body weight) of the extract were administered orally to four groups of rats for a period of 14 days. The extract did not significantly ( $P<0.5$ ) alter the level of haemoglobin (Hb), packed cell volume (PCV), red blood cells (RBC) and white blood cells (WBC), clotting and bleeding time. Aspirin (positive control) significantly ( $P<0.05$ ) altered the clotting and bleeding time, but not haemoglobin (Hb), packed cell volume (PCV), red blood cells (RBC) and white blood cells (WBC). The aqueous extract had no noticeable effects on the liver enzymes, alanine aminotransaminase (ALT), aspartate aminotransaminase (ASAT) and alkaline phosphatase (ALP). Histological studies showed that the extract had no effect on liver cells. There were clinical signs of abdominal contraction and general malaise during the experiment. These results explained the basis for the continual use of this plant by traditional medical practitioners.

**Keywords:** *Momordica balsamina*, Haematological parameters, Liver enzymes, Toxicity.

### INTRODUCTION

Herbal medicinal products are unlikely to pose a significant threat to human health, nonetheless, it is important to validate their safety. The confidence in herbal medicines is backed by their long term usage. Validation of their safety is necessary because crude herbal medicines are given in most cases without accurate dosage and over ingestion can result in toxicity. It is also possible for the plant to have silent toxic effect that may not be evident within a short time<sup>1</sup>. The use of herbal medicinal products may present potential risk to human health<sup>2</sup>, but some toxic herbal medicines has been proven to have beneficial effects at very low doses. Toxic and potent, useful herbal medicines from potentially hazardous plant species include; *Digitalis*, *Nux Vomica* (*Strychnos*), *Aconite*, *Croton Seed*, *Rauwolfia*, *Areca*, *rotalaria*, *Dryopteris* and *Strophanthus*.<sup>1</sup> To protect public health, it is necessary to ensure that all medicines, including unlicensed products, are safe for human consumption and of suitable quality. Herbal medicines are required to meet the same safety, quality and efficacy criteria as any other licensed medicine. Serious liver toxicity has been reported to be associated with the use of some herbal medicines (for example; *Kava-kava*, *Piper methysticum*<sup>1</sup>, *Crotalaria*, *Heliotropium* and *Senecio*). Recent research revealed that adverse reactions to herbal products are under-reported.<sup>3</sup> The extensive traditional use of plants as medicines has enabled herbal medicines with acute and obvious signs of toxicity to be well recognized and their use avoided. However, the premise that traditional use of a plant for perhaps many hundreds of years establishes its safety does not necessarily hold true.<sup>2,4</sup> The more subtle

and chronic forms of toxicity, such as carcinogenicity, mutagenicity, and hepatotoxicity, may well have been overlooked by previous generations and it is these types of toxicity that are of most concern when assessing the safety of herbal remedies<sup>5</sup>. Limited toxicological data are available on medicinal plants. It has been documented that Apiole and parsley are hepatotoxic.<sup>6</sup> Plants rich in Pyrrolizidine Alkaloids (PAs), notably *Crotalaria*, *Heliotropium*, *Aristolochia* and *Senecio* are known to injure the liver in humans giving rise to serious liver damage (hepatocellular veno-occlusive disease)<sup>7,9</sup>. Cases of human hepatotoxicity associated with the ingestion of comfrey have been documented.<sup>10,11</sup> Lectins, an active principle found in some plants possess haemagglutinating and potent mitogenic properties. Systemic exposure to pokeroot has resulted in haematological aberrations. Saponin in some plants causes severe gastrointestinal irritation involving intense abdominal cramping, haematemesis, hypotension and tachycardia. Many and more of these effects have been associated with unguarded ingestion of herbal medicine. *M. balsamina* (Linn) of the family Cucurbitaceae is a creeping plant commonly found in West Africa. In Northern Nigeria, it is used as an emetic and purgative<sup>12</sup>. It is also used in the management of pain, inflammation, hypertension and treatment of rheumatism in some African countries.<sup>12,13</sup>. The plant is known by many names including: "ejinrin" (in Yoruba speaking tribe), "Akban ndere" (by the Igbos) and "garahum" (in the Northern part of Nigeria).

In view of its many uses, especially in Nigeria and the fact that traditional medicine practitioners prescribe and

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administer concoctions of the whole plant of *M. balsamina* to patients regardless of its possible adverse effects. The present investigation was undertaken to assess the effect the crude extract on haematological and biochemical parameters, and histology of liver cells in treated rats. The earlier report by Otimenyin, and Uguru,<sup>14</sup> that this plant has potent analgesic and anti-inflammatory effects also necessitated this study and its comparison with Aspirin (an analgesic known to have effect on hematological parameters).

## **MATERIALS AND METHODS**

### **Collection and Identification of Plant Materials**

The fresh whole plant of the *M. balsamina* was collected from Babale, Jos South local government area of Plateau State, Nigeria in June 2005. The plant was identified and authenticated by Mr. Kareem of School of Forestry, Jos, Plateau State, Nigeria.

### **Experimental Animals**

Male and female healthy Wistar rats (0.20 Kg) bred in the animal house of Department of Pharmacology, Faculty of Pharmaceutical Sciences, University of Jos, were used for this study. They were fed with feeds (Vital feeds, Nigeria) and tap water throughout the period of the experiment.

### **Preparation of Extract**

Fresh whole plant of the *M. balsamina* was air dried at room temperature (28°C) in the laboratory. They were reduced into coarse powder with the aid of mortal and pestle. Two hundred (200) grams of the powdered plant parts was stirred in 400ml of distilled water and allowed to stay for 24 hours. The mixture was then filtered and the resultant concoction freeze dried. The freeze dried powder was kept in the desiccator till use.

### **Administration of the Extract**

Male rats were randomly distributed into four groups of six. Group 1 served as the control and received distilled water. Groups 2, and 3 received the aqueous extract of *M. balsamina* at doses of 1.0 and 1.5 gkg<sup>-1</sup> respectively and group 4 received Aspirin. Animals in the control group received the same volume of distilled.

Administration of the extract was done orally, by means of a polythene cannula. Animals received their doses once a day for 14 days<sup>15</sup>. They were observed daily for clinical signs of toxicity or pharmacological signs, throughout the period of study.

### **Biochemical Parameters**

At the end of treatment periods, 4 ml of blood was collected

from each rat. They were allowed to clot and then centrifuged at 1500g for 10min. Serum from blood so collected was used for assay of the maker enzymes namely alkaline phosphatase (ALP), alanine aminotransaminase (ALAT) and aspartate aminotransaminase (ASAT).

### **Haematological Parameters**

Blood samples were collected from the tail of the rats, at the end of the experiment into heparinized sample bottles. The bleeding and clotting time were noted.<sup>16</sup>. The haematological examinations performed were according to standard methods. Haematocrit was determined by the micro-haemotacrit method described by McGowen, et al.<sup>16</sup>. Erythrocytes and total leucocytes were counted using the improved Neubauer haemocytometer. The packed cell volume of each sample was determined by using a Hawksley microhaematocrit centrifuge at 1200 g for 5 min. Biochemical analysis of the serum enzymes for ALAT and ASAT was by the method of Reitman and Frankel<sup>17</sup>. ALP was assayed according to the method of REC<sup>18</sup>.

### **Histological examination of the liver**

Livers from treated animals were fixed in 10 percent neutral buffered-formalin prior to routine processing in paraffin-embedded blocks. Sections (4 µm thick) were cut and stained using hematoxylin-eosin stain<sup>15</sup> and photomicrographed.

### **Statistical Analysis**

Differences between control and treatment groups were analyzed by student t- test.<sup>19</sup>

## **RESULTS**

### **Haematological Parameters**

The mean ± standard deviation values of the bleeding time, clotting time and packed cell volume of rats treated with the extract of *M. balsamina* are shown in table 1. The mean PCV values in the treated animals and the animals that received aspirin were not significantly different at the end of the treatment from that of the control, thus showing that the extract of *M. balsamina* has no effect on hematological parameters. (Table 1 and 2). Bleeding time and clotting time were also not affected by the extract as compared to aspirin which significantly increased these times (Table 1).

The mean RBC, WBC and Platelet count were not significantly ( $P>0.05$ ) affected at the end of the treatment period when compared with the control, and positive control (Table 2). These results showed that the extract has no significant effect on haematological parameters

### **Biochemical Parameters**

The activities of the three most prominent maker enzymes,

**TABLE- 1 Effect of aqueous extract of *Momordica balsamina* on Bleeding time, Clothing time and Packed cell volume in rats.**

Grouping	Dose (g/kg)	Bleeding time (sec)	Clotting time (sec)	Packed cell volume
Control	....	99.0±9.10	84.0±3.89*	51.40±1.57
<i>M. balsamina</i>	1.50	112.5±5.30	82.5±15.91*	45.0±0.71
Aspirin	0.26	341.25*±28.25*	161.25*±14.40 +	49.75±0.65

\* Significantly different from the control at  $P < 0.05$    \*Significantly different from the negative control at  $P < 0.05$

**TABLE- 2 Effect of aqueous extract of *Momordica balsamina* on hematological parameters in rats.**

Groups	Dose (g/kg)	Heamoglobin (gHb/DL)	Red Blood Cell Count $\times 10^6$ cell/cm <sup>3</sup>	White Blood Cell Count $\times 10^4$ cell/cm <sup>3</sup>	Platelet Count $\times 10^5$ cell/cm <sup>3</sup>
Control	.....	12.84±1.56	7.27±0.14	1.25±0.14	1.04±0.04
<i>M. balsamina</i>	1.00	11.05 ± 0.43	7.94 ± 0.39	0.99 ± 0.15	1.46 ± 0.17
<i>M. balsamina</i>	1.50	9.02 ± 0.21	6.90 ± 0.96	1.20 ± 0.16	0.85 ± 0.01
Aspirin	0.26	14.71 ± 0.18	6.65 ± 0.15	1.41 ± 0.23	0.51 ± 0.04

\* Significantly different from the negative control (Aspirin) at  $P < 0.05$

alkaline phosphatase (ALP), alanine aminotransaminase, (ALAT) and aspartate aminotransaminase, (ASAT) were not affected after treatment of the animals with the extract of whole plant of *M. balsamina* (1.0 and 1.5 gkg<sup>-1</sup> body weight respectively). The activity of alkaline phosphatase insignificantly ( $P>0.05$ ) decreased, at a dose of 1.0gkg<sup>-1</sup> with activity level of 397.26 ± 0.59 u/l when compared with a control value of 403.35 ± 1.02 u/l. As the dose was increased to 1.5 gkg<sup>-1</sup>, the activity further decreased (395.19 ± 1.04 u/l). The decreases observed were not significantly different from that of the control animals (Table 3). Alanine transaminase activity decreased in the rats pretreated with the two doses of the extract. At a dose of 1.0 gkg<sup>-1</sup> the activity was insignificantly ( $P>0.05$ ) reduced to 26.24 ± 1.11 u/l as compared with a control value of 28.01 ± 0.94 u/l. The activity further reduced to 25.14 ± 0.99 u/l ( $P>0.05$ ) when the dose was increased to 1.5 gkg<sup>-1</sup>. The decreases observed were not significantly different from that of the control. (Table 3.). There was an insignificant ( $P>0.05$ ) decrease in the activities of aspartate aminotransaminase in all the rats treated with various doses (1.0 and 1.5 gkg<sup>-1</sup> respectively) of the extract when compared with control value. The overall results show that the extract has no effect on the liver cells and enzymes, (Table 3, Fig.- 1).

#### Histological examination of the Liver

Histological examination showed that *M. balsamina* extract had no effect on liver cells(Figures 1). These result supported the liver enzyme assay which also revealed that there was no difference in values of treated and control

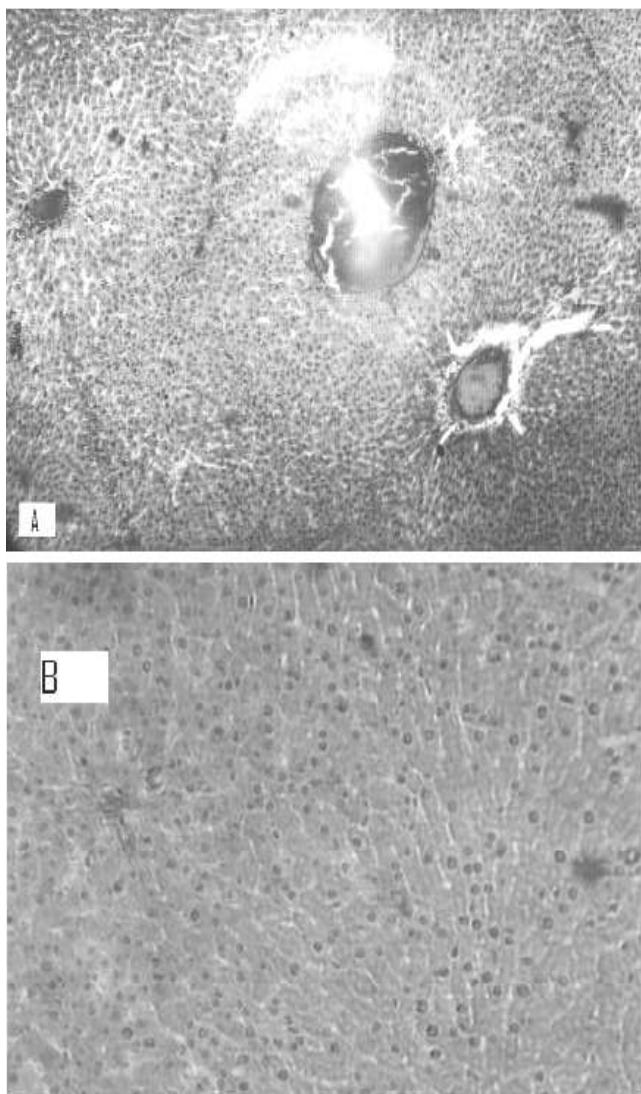
(Table 3).

#### DISCUSSION

Herbal Medicines has been in use since the existence of man. They were discovered by trial and error. Over the years the toxic plants were removed from the pharmacopeia when discovered to be Toxic. Only non-toxic plants are expected to be in the Pharmacopeia after a very long non-scientific screening of these herbs. The screening parameter used was in most cases death of animal or humans from the ingested toxic herbal medicine. The extensive traditional use of plants as medicines has enabled medicines with acute and obvious signs of toxicity to be well recognized and their use avoided. However, the premise that traditional use of a plant for perhaps many hundreds of years establishes its safety does not necessarily hold true.<sup>24</sup> It is therefore necessary for herbal medicines to be screened for effects on body organs and enzymes.

This present study revealed that, the aqueous extract of the whole plant *M. balsamina* had no effect on bleeding time and clotting time. Aspirin, the positive standard used was observed to prolong bleeding and clothing time. Aspirin is used in medicine for this property in the prevention of the formation of thrombosis in the myocardium coronary blood vessel<sup>20</sup>. Aspirin does this by discouraging/inhibiting platelet aggregation. This effect may also be one of the side effects of Aspirin as it can lead to excessive bleeding during cuts. The main mechanism of action of aspirin is the inhibition of Cyclo-Oxygenase enzyme<sup>21</sup>. The extract and aspirin had no effect on packed

**FIGURE 1. Effect of aqueous extract of *Momordica balsamina* on liver.**



**A Test Liver Showing normal cells (3.52)**

**B. Control Liver Showing normal cells (3.51)**

cell volume. Otimenyin, et al<sup>22</sup> earlier reported that the aqueous crude extract of *M. balsamina* possess analgesic and anti inflammatory properties which Aspirin does possess too, but this plant is devoid of the side effects associated with aspirin as verified by this study.

*M. Balsamina* insignificantly ( $P>0.05$ ) decreased the red blood cell, haemoglobin and white blood cell count. This shows that this plant had no effect on the haemopoietic system. This results supports Matawalli's<sup>23</sup> report on the hematological effects of the stem back of *M. balsamina*. He also reported that the extract increased the WBC count, but present findings revealed that the WBC was not significantly affected. The difference in these results may be due to the inclusion of other part of the plant (whole plant), the constituents from other part may have neutralized the effects of the constituents in the stem back. The slight insignificant ( $P>0.05$ ) decrease in the red blood cell and haemoglobin may have resulted from the suppression of circulating hormone, erythropoietin (a glycoprotein which stimulates the process of erythropoiesis)<sup>24</sup>. Reduction in blood concentration of erythropoietin may result in a normochromic, normocytic anaemia<sup>25</sup>.

The extract had no effect on the activity of the liver, as shown in both the liver enzymes assay and histology of the liver. Liver enzymes (ALAT and ASAT) are liberated into the blood whenever liver cells are damaged and enzyme activity in the plasma is increased<sup>26</sup>. Otimenyin et al<sup>22</sup> reported that the extract contain flavonoids. Flavonoids are reported to exhibit antioxidant activity<sup>27</sup> and are effective scavengers of superoxide anions (Robak, and Gryglewski, 1988). The reason why the extract did not affect the liver cell cannot be explained from these results, it may not be unrelated to the fact that some plants may have other constituents that are hepatoprotective. Interestingly, saponins (which is one of the constituents

**TABLE- 3 Effect of aqueous extract of *Momordica balsamina* on rats liver enzymes.**

<b>Grouping</b>	<b>Dose (g/kg )</b>	<b>Parameters Assayed</b>		
		<b>ALP(U/L)</b>	<b>ALAT (U/L)</b>	<b>ASAT(U/L)</b>
<b>Control</b>	.....	<b><math>403.35 \pm 1.02</math></b>	<b><math>28.01 \pm 0.94</math></b>	<b><math>56.14 \pm 0.43</math></b>
<b><i>M. balsamina</i></b>	<b>1.00</b>	<b><math>397.26 \pm 0.59</math></b>	<b><math>26.24 \pm 1.11</math></b>	<b><math>54.23 \pm 0.73</math></b>
<b><i>M. balsamina</i></b>	<b>1.50</b>	<b><math>395.19 \pm 1.04</math></b>	<b><math>25.14 \pm 0.99</math></b>	<b><math>53.96 \pm 0.95</math></b>

\* $P<0.05$ , when compared with the control

of the extract of *M. balsamina*) has been reported to enhance natural resistance and recuperative powers of the body<sup>28</sup>. There is the possibility that saponins might have contributed to the effect of the extract on the liver.

## **CONCLUSION**

The safety of the use of *M. balsamina* as herbal medicine is confirmed by the results obtained from these experiments. The extract was observed to have no significant effect on hematological and biochemical parameter and liver cells.

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