

Biochemical And Hematological Study On Butanol Fraction Of Leaves Of *Moringa Stenopetala* In Experimental Rats.

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Abstract: - Objective: The objective of the present study was to investigate the sub-chronic toxic effects of butanol fraction of leaves of *M. stenopetala* on blood parameters of experimental rats.

Methods: For this study the rats were divided into three groups. Group I, the control group received distilled water, while the experimental groups: group II received 500 and group III received 1000mg/kg for 63 days by oral gavage. At the end of the experiment blood samples were collected and examined for hematological and biochemical parameters.

Results: Treated rats showed no significant changes on hematological and biochemical parameters except blood glucose level ($p < 0.05$) as compared with the controls.

Conclusion: Although, butanol fraction of leaves of *M. stenopetala* did not produce adverse effects on hematological & biochemical parameters, further detailed studies should be carried out on other vital organs to recommend its therapeutic use.

KEYWORDS: Hematological, Biochemical, parameters, *Moringa stenopetala*, Experimental rats.

I. INTRODUCTION

Blood is defined as a special connective tissue; its hematological & biochemical parameters play significant roles in toxicological studies due to their response to chemical compounds found in medicinal plant materials. The leaves of medicinal plants such as *Moringa stenopetala* are traditionally used for the treatment of various ailments such as malaria, hypertension, asthma, diabetes, stomach pain and also consumed as vegetables regularly. Potentially useful chemical compounds (such as alkaloids, flavonoids, glycosides, polyphenols etc) have been isolated from the fresh leaves of *M. stenopetala*. These chemical compounds are used directly as therapeutic agents, and also as starting materials for the synthesis of drugs (WHO, 2000; Debella, 2002; Abuye *et al.*, 2003; Mengisitu, 2007; Sileshi, 2010).

Despite their curative effects, toxicity studies of leaves of *M. stenopetala* are limited. Therefore, the present study was aimed to investigate the effect of butanol fraction of the leaves of *M. stenopetala* on biochemical and hematological parameters in experimental rats.

II. MATERIALS AND METHODS

The present study was carried out in the laboratories of Ethiopian public health Institute (EPHI). The leaves were collected in Arba-Minich, Southern Ethiopia, about 502 kilometers south of Addis Ababa. The leaves were identified and authenticated by a taxonomist, cleansed from extraneous materials and dried under shade and powdered. The powdered leaves were extracted with 70% ethanol using percolator and fractionated in a separatory funnel with n-hexane (3x50), dichloromethane (3x50) and n-butanol (5x50) successively. The procedure for plant material preparation was adopted from Debella (2002), Ranjan and Reeba (2002) with some modification.

The study was conducted, for nine weeks (63 days), based on the OECD 408 guideline (OECD, 1998). For this study eighteen healthy adult rats were randomly distributed into three groups (I, II, & III) each consisting of six rats (three females and three males). The two sexes were kept in separate cages until the end of the study. Group-I served as control group and received distilled water or vehicle, whereas group II & III rats were treated with butanol fraction of the leaves at doses of 500 and 1000mg/kg body weight per day respectively. The doses were prepared based on their body weight and both the vehicle and test substance were administered via oral gavage.

At the end of the experiment (by 64th day) they were anesthetized under diethyl ether and blood samples were collected by cardiac puncture. Hematological parameters such as white blood cell count, red blood cell count, platelet count, hematocrit, hemoglobin, mean corpuscular hemoglobin, and mean corpuscular hemoglobin concentration (WBCs, RBCs, Platelet, HCT, HGB, MCH & MCHC respectively) were analyzed using Automated Hematology Analyzer (Symex-XT, 1800i, Japan). Glucose, urea, creatinine, total protein, alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (ALP) were measured using Automated Clinical Chemistry Analyzer (Huma Star 80, Germany).

III. STATISTICAL ANALYSIS

The values of hematological and biochemical parameters are presented as mean and standard error of the mean (mean \pm SEM). Statistical differences between the means of different groups were evaluated by a one-way analysis of variance (ANOVA) using the SPSS version 20 program followed by a Dunnett's t-test. P-values less than 0.05 were considered statistically significant.

IV. RESULTS

Effects of the fraction on hematological parameters

In the present sub-chronic toxicity study, the hematological parameters, such as WBC, RBC, PLT, HGB, HCT, MCH, and MCHC, of the treated groups (500 mg/kg and 1000mg/kg) were within the reference range for rats and were not significantly different from the control group (Table-1). The mean amount of WBCs in rats treated with doses of 500mg/kg and 1000mg/kg were $7.69 \times 10^3/\mu\text{L}$ and $7.19 \times 10^3/\mu\text{L}$ respectively, while it was $8.41 \times 10^3/\mu\text{L}$ in the controls. Treatments with the fraction decreased the mean values of WBC by 8.56% at 500mg/kg and by 14.5% at 1000mg/kg, as compared with the controls. The mean values of RBCs in rats treated with doses of 500mg/kg and 1000mg/kg were $7.98 \times 10^6/\mu\text{L}$ and $7.49 \times 10^6/\mu\text{L}$ respectively, while $8.21 \times 10^6/\mu\text{L}$ with the controls. RBCs count showed decrement by 2.8% at 500mg/kg and by 8.76% at 1000mg/kg, as compared with the controls. The mean amounts of HGB were 15.16 ± 0.27 g/dL at 500mg/kg and 14.83 ± 0.38 g/dL at 1000mg/kg, while that of the control was 15.72 ± 0.31 g/dL. The mean values of HGB decreased by 3.56% at 500mg/kg and by 5.66% at 1000mg/kg, as compared with the controls. The mean values of HCT in rats treated with doses of 500mg/kg and 1000mg/kg were 46.5% and 43.23%, while 47.25% with the controls.

The mean values of HCT exhibited decrement by 1.58% at 500mg/kg and by 8.50% at 1000mg/kg, as compared to the controls. The mean values PLTs were $700.53 \times 10^3/\mu\text{L}$ at 500mg/kg and $639.2 \times 10^3/\mu\text{L}$ at 1000mg/kg, while $798.5 \times 10^3/\mu\text{L}$ with the controls. The mean amounts of PLTs showed decrement by 12.27% at 500mg/kg and by 19.95% at 1000mg/kg, as compared with the controls. The mean amount of MCH was 19.19 ± 0.58 pg at 500mg/kg and 19.18 ± 0.27 pg at 1000mg/kg, while 19.50 ± 0.19 pg with the controls. The mean values of MCH decreased by 1.59% at 500mg/kg and by 1.64% at 1000mg/kg, as compared with the controls. The mean amount of MCHC was 32.93 ± 0.21 g/dL at 500mg/kg and 32.58 ± 0.41 g/dL at 1000mg/kg, while 33.27 ± 0.22 with the controls. The mean values of MCHC also showed decrement by 1.02% at 500mg/kg and by 2.07% at 1000mg/kg, as compared to the controls. As summarized in table 6, most of the hematology parameters decreased at the higher dose of (1000mg/kg) the butanol fraction of the leaves of *M. stenopetala* than those treated at the lower dose (500mg/kg).

Table: 1. Comparison of the effect of butanol fraction on hematological parameters.

Hematological parameters	Control	500 mg/kg	% of mean difference	1000 mg/kg	% of mean difference
WBC($\times 10^3/\mu\text{L}$)	8.41 ± 0.83	7.69 ± 0.95	- 8.56	7.19 ± 0.62	- 14.5
RBC($\times 10^6/\mu\text{L}$)	8.21 ± 0.25	7.98 ± 0.10	- 2.8	7.49 ± 0.45	- 8.76
HGB(g/dL)	15.72 ± 0.31	15.16 ± 0.27	- 3.56	14.83 ± 0.38	- 5.66
HCT(%)	47.25 ± 0.92	46.5 ± 0.62	- 1.58	43.23 ± 1.73	- 8.5
MCH (pg)	19.50 ± 0.19	19.19 ± 0.58	- 1.59	19.18 ± 0.27	- 1.64
MCHC (g/dL)	33.27 ± 0.22	32.93 ± 0.21	- 1.02	32.58 ± 0.41	- 2.07
PLT($\times 10^3/\mu\text{L}$)	798.5 ± 77.98	700.53 ± 48.95	- 12.27	639.2 ± 88.82	- 19.95

Values are mean \pm SEM, n= 6/group.

Effects of the fraction on biochemical parameters

In the present sub-chronic toxicity study, the biochemical parameters (except glucose) of the treated groups (500mg/kg and 1000mg/kg) were within the reference range for rats and were not significantly different from

the control group (Table 2). The mean values of serum glucose were 127.5 ± 4.14 mg/dl at 500mg/kg and 129.83 ± 3.24 mg/dl at 1000mg/kg, while 166.00 ± 14.92 mg/dl with the controls. The mean values of glucose decreased by 23.19% at 500mg/kg and by 21.78% at 1000mg/kg. The mean serum glucose level showed significant change/decrease ($p < 0.05$) at both doses as compared with the controls. The mean values of plasma urea were 44.74 ± 1.97 mg/dl at 500mg/kg and 41.83 ± 0.79 mg/dl at 1000mg/kg, while 41.00 ± 0.97 mg/dl with the controls. The mean values of urea showed increment by 9.12% at 500mg/kg and by 2.02% at 1000mg/kg of the fraction, as compared to the controls.

The mean amounts of creatinine in rats treated with doses of 500mg/kg and 1000mg/kg were 0.56 ± 0.013 mg/dl and 0.54 ± 0.014 mg/dl respectively. The mean values of creatinine showed decrement by 1.75% at 500mg/kg and by 5.26% at 1000mg/kg, while 0.57 ± 0.02 mg/dl with the controls. The mean values of total protein decreased from 6.28 ± 0.15 mg/dl to 6.24 ± 0.17 mg/dl as the dose increased from 500mg/kg to 1000mg/kg (Table-2).

The mean amounts of total protein showed decrement by 0.47% at 500mg/kg and by 1.11% at 1000mg/kg of the fraction, while 6.31 mg/dl with the controls. The mean amount of ALT and AST were 106.00 ± 4.67 IU/L and 214.17 ± 10.89 IU/L at 500mg/kg and the mean values at 1000mg/kg were 95.17 ± 8.8 IU/L and 229 ± 14.07 IU/L respectively. The mean values of ALT decreased by 0.94% at 500mg/kg and by 11.93% at 1000mg/kg, while 107.00 ± 11.41 IU/L with the controls. The AST mean values also showed decrement by 11.06% at 500mg/kg and by 5.83% at 1000mg/kg of the fraction, while 243.17 ± 25.71 IU/L with the controls. The mean amounts of ALP were 115.67 ± 10.27 IU/L at 500mg/kg and 121.17 ± 18.54 IU/L at 1000mg/kg. The mean values of ALP showed increment by 3.74% at 500mg/kg and by 8.67% at 1000mg/kg of the fraction, while 111.5 ± 8.11 IU/L with the controls. As summarized in table 2, all the above changes (except glucose, ($p < 0.05$)), were not statistically different ($p > 0.05$) as compared with the control group.

Table: 2. Comparison of the effect of butanol fraction on biochemical parameters.

Biochemical parameters	Control	500 mg/kg	% of mean difference	1000 mg/kg	% of mean difference
Glucose(mg/dl)	166.00 ± 14.92	$127.5 \pm 4.14^*$	- 23.19	$129.83 \pm 3.24^*$	- 21.78
Urea(mg/dl)	41.00 ± 0.97	44.74 ± 1.97	9.12	41.83 ± 0.79	2.02
Creatinine(mg/dl)	0.57 ± 0.02	0.56 ± 0.013	- 1.75	0.54 ± 0.014	- 5.26
Protein(mg/dl)	6.31 ± 0.19	6.28 ± 0.15	- 0.47	6.24 ± 0.17	- 1.11
ALT(IU/L)	107.00 ± 11.41	106.00 ± 4.67	- 0.94	95.17 ± 8.80	- 11.93
AST(IU/L)	243.17 ± 25.71	214.17 ± 10.89	- 11.06	229 ± 14.07	5.83
ALP(IU/L)	111.5 ± 8.11	115.67 ± 10.27	3.74	121.17 ± 18.54	8.67

Values are expressed as Mean \pm SEM, n= 6/group, $p < 0.05$ statistically significant.

V. DISCUSSION

The present study was aimed to evaluate the effects of butanol fraction of the leaves of *M. stenopetala* on some biochemical and hematology parameters of the blood in experimental rats. During the experimental period no death or apparent behavioral changes were observed as compared with the control group. This might suggest the non-toxic nature of the fraction.

Assessment of hematological parameters can be used to determine the extent of harmful effect of foreign compounds including plant materials on blood (Yakubu et al., 2008). In the present sub-chronic toxicity study, the hematological parameters (RBC, WBC, PLT, HGB, HCT, MCH, and MCHC) of the treated groups (500mg/kg and 1000mg/kg) were within the reference range for rats (Table-1). The reference values of RBCs, HGB, HCT, MCH, and MCHC for rats are $7 - 10 \times 10^6/\mu\text{l}$, 11-19.2g/dL, 35-64%, 14.3 -19.5 pg and 26.2 – 40g/dL respectively (Pass and Freeth, 1993; Delaney, 2008). In the current study, slight decrements in the mean values of RBCs, HGB, HCT, MCH, and MCHC were observed with the fraction at the doses of 500mg/kg and 1000mg/kg (Table 1). However, the changes were within the reference range for rats, and were not statistically significant ($p > 0.05$) as compared with the controls. The absence of significant effect of the fraction may indicate that the fraction may not possess toxic substances that can cause anemia or other abnormalities. This is in agreement with the work of other researchers, including *Bougainvillea spectabilis* leaves administration in rats (Adebayo et al., 2005); *Acacia karroo* stem bark administration in rats and mice (Adebayo et al., 2008); *M. stenopetala* leaves administration in mice (Ghebreselassie et al. (2011); and *Ginidiastenophylla* root extracts administration in mice (Getachew, 2012).

The blood of normal adult rat possesses about 6 - 18 x10³ WBCs per μ L of blood (Pass and Freeth, 1993). In this study, slight decrement in the mean WBCs counts were observed with the fraction at the doses of 500 and 1000mg/kg (Table 1), though the changes were within the reference range for rats and were not significant ($p>0.05$). From this study, it may be inferred that the fraction of *M. stenopetala* has no toxic effect on the WBCs count. Similar results were reported on the non-toxic effects of plants on WBCs as in the *Acacia karroo* stem bark (Adebayo et al., 2008), and the leaves of *M. stenopetala* (Ghebreselasse et al. 2011).

Usually, the normal value of platelets in adult rat is 500-1,300 x 10³/ μ L (Delaney, 2008). In this study, though slight decrement in platelet count was observed with the fraction (500mg/kg and 1000mg/kg), the changes were within the reference range for rats and were not statistically significant ($p>0.05$). This may imply that the fraction did not pose toxic effect on the platelet amount or did not alter blood clotting. The result was also in agreement with the work of Ghebreselassie et al., (2011) and Getachew, (2012) who reported the insignificant effect of *M. stenopetala* and *G. stenophylla* on the number of platelets.

In toxicological evaluation, biochemical parameters have significant roles because of their response to clinical signs and symptoms produced by toxicants. In the present study, the biochemical parameters did not show significant changes except for the mean values of glucose (Table 2).

In this study, the mean amounts of glucose levels decreased by 23.19% (127.5 ± 4.14 mg/dl) at 500mg/kg and by 21.78% (129.83 ± 3.24 mg/dl) at 1000mg/kg, while 166.00 ± 14.92 mg/dl with the controls. There was significant ($p<0.05$) decrease/change in the mean serum glucose levels with the fraction. The decrease in serum glucose level with the fraction observed in this study may be due to presence of insulin like substance in the butanol fraction, either promote glucose uptake and metabolism by the liver or inhibit hepatic gluconeogenesis, supporting the traditional use of the leaves for treatment of diabetes. The result was in agreement with the works of Sileshi, (2010); Nardos et al., (2011), and Toma et al., (2012), who reported that treatment with the ethanol extract and butanol fraction of the leaves of *M. stenopetala* decreased blood glucose level in normal and diabetic mice. Similarly, findings by Mussa et al., (2008) and Ghebreselassie et al., (2011), showed that aqueous extract and isolated fraction of the leaves of *M. stenopetala* decreased blood glucose level in normal and diabetic mice.

Measurement of plasma urea has been used for many years as an indicator of kidney function (Tietz, 2000; Feres et al., 2006). In this study, the mean values of urea showed a slight increment though insignificant ($p>0.05$) and the changes were within the reference range. Similar results were reported by Khileifat et al., (2002), who found that the level of urea and enzymes increased following treatment of experimental animals with several medicinal plants.

Creatinine is produced endogenously and released into body fluids at a constant rate; and its plasma concentration and renal clearance have been used as markers of the glomerular filtration rate (Tietz, 2000). In the current study the mean amounts of creatinine showed a slight decrement but not significant ($p>0.05$). The reference value of creatinine in adult rat is about 0.2- 0.8 mg/dL (CRL, 1998 and Delaney, 2008). In this study, the changes were within the reference range for rat and were not supported by the histopathological examination of the kidneys. The result was in agreement with the finding of Dirikolu et al., (2011).

In the current study, the amounts of total protein showed a slight decrement but not significant ($p>0.05$). The reference value of total protein in the serum of adult rat is in the range of 5.6-7.6mg/dL (Pass and Freeth, 1993). The mean values of total protein were within the reference range for rats, which was also supported by the absence of histopathological changes in the kidneys of treated rats. The result is also in agreement with the findings of Ghebreselassie et al., (2011), who asserted that the aqueous extract of *M. stenopetala* leaves did not affect the level of creatinine and total protein.

The abnormal elevation of the liver enzymes (ALT, AST and ALP) may indicate liver damage or alteration in bile flow (Tietz, 2000). In this study, the mean values of ALT and AST showed decrement but not significant ($p>0.05$) as compared with the controls. This was supported by the absence of histopathological changes in the liver of treated rats. The result was in line with the report of several related studies on various plant extracts (Ghebreselassie et al., 2011; Saha et al., 2011 and Getachew, 2012). In the present study, the mean values of ALP showed increment but not significant ($p>0.05$) as compared with the controls. This hypothesis was also supported by the absence of histopathological changes in the liver of treated rats. The result was in line with the findings of Feres et al., (2006) who asserted that treatment of rats and mice with *Dimorphandramollis* increased serum levels of liver enzymes.

VI. CONCLUSION

In the current toxicity study, results showed that the fraction did not produce adverse effects on hematological and biochemical parameters the blood. These findings indicate that sub-chronic exposure to the butanol fraction of leaves of *M. stenopetala* does not lead to blood toxicity. Further a brief study should be carried out in other species of animals (rabbits, and dogs) and on other additional vital organs (such as thyroid gland, pancreas, stomach, intestine etc).

VII. REFERENCES

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