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Evaluation of hematological and hepatorenal functions of methanolic extract of *Moringa oleifera* Lam. root treated mice

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Methanolic extract of *M .oleifera* root was found to contain some alkaloids (total alkaloid 0.2%). Effects of multiple weekly (35,46,70 mg/kg) and daily therapeutic (3.5,4.6,7.0 mg/kg) ip doses of the crude extract (CE) on liver and kidney functions and hematological parameters in mice were studied. No alteration in hematological and biochemical parameters at low and moderate dose level of daily and low dose level of weekly treatment of the extract was observed. However, the extract at moderate dose level in weekly treatment changed serum aminotransferase and plasma cholesterol levels significantly. High dose in addition to the above parameters changed total bilirubin, non protein nitrogen, blood urea and plasma protein. High dose of daily treatment and moderate and high dose of weekly treatment of CE increased WBC count and decreased clotting time significantly. The results indicate that the weekly moderate and high dose (>46mg/kg body wt.) and daily/ therapeutic high dose (7mg/kg) of CE affects liver and kidney functions and hematological parameters whereas the weekly dose (3.5mg/kg) and low and moderate daily/therapeutic dose (3.5 and 4.6 mg/kg) did not produce adverse effects on liver and kidney functions.

Moringa oleifera (Moringaceae) a perennial plant (sajna in Bengali and soanjna in Hindi) is found and cultivated frequently in West Bengal. Different parts of this plant possess multifarious medicinal properties like antidiarrhoeal, purgative, laxative etc[1].The root of the plant is used by some tribal groups of India as antiepileptic agent[2]. Preliminary analysis suggested that LD_{50} value of crude extract of root (CE) is 2.8 g/kg ip in mice (unpublished data), indicating a less toxic action of CE. The present study has been undertaken to observe chronic toxicity of CE on hepatorenal functions and hematological parameters in mice.

Chemical investigation of CE - From the preliminary analysis, it was found that CE of root contains some alkaloids (total alkaloids 0.2%) [3]. Qualitative tests for alkaloids were also found positive when tested with Dragendorff's, Mayer's and Wagner's reagents.

Three fractions from CE were separated by column chromatographic method. Fraction 1 was eluted with $CHCl_3$ saturated with NH_4OH, showed characteristic IR peaks for ketals (1200 cm^{-1}) and spiroketals of steroids (1360 cm^{-1}) which also gave positive color test for steroids. Fraction 2, eluted with $CHCl_3$: MeOH (2:1) mixture was found to be identical with benzyl amine (identical with moringine)[3],confirmed by superimposable IR (Perkin Elmer IR-297). Fraction 3, eluted with $CHCl_3$: MeOH (1:1) showed characteristic IR peaks for -OH (1050 cm^{-1}, 3350 cm^{-1}, 3600cm^{-1}), 1-alcohol and C=N-OH (oxime) groups (3620 cm^{-1}), suggesting the structural similarity of Fraction 3 with sympathomimetic group of bases [4].

As the yield of separated fractions were very poor, total CE was used for the present investigation.

Effect of CE on hematological and hepatorenal functions - Root pieces of *M.oleifera* were collected during May-June, sundried, grinded and extracted by methanol. The extract was evaporated to dryness and dissolved in propylene glycol. Swiss albino mice of either sex weighing between 20-25 g were fed standard pelleted diet and given tap water *ad libitum*. Preliminary study indicates that the CE is less toxic as the LD_{50} value was found to be 2.8 g/kg when tested on mice computed according to the method of Litchfield and Wilcoxon[5]. For chronic toxicity studies, the mice were divided into 10 groups of 10 each. CE was injected, ip once in a week for 4 weeks at the dose levels of 35(low), 46(moderate) and 70(high) mg/kg to the animals of 3rd,4th and 5th groups respectively. CE at dose levels of 3.5(low), 4.6(moderate) and 7.0(high) mg/kg was injected daily

Table 1—Effect of crude extract of *M.oleifera* (weekly treatment) on hematological and hepatorenal functions

[Values are mean±SE of 10 experiments]

Parameters	Saline (5ml/kg)	Vehicle (5ml/kg)	Crude extract (mg/kg) 35	46	70
Hemoglobin (%)	13.6±0.30	13.4±0.23	13.5±1.21	13.5±1.21	13.7±0.38
WBC (count/mm^3)	4300±0.06	4390±0.31	5240±0.35	6132±0.91*	7900±0.19*
RBC (count x 10^6/mm^3)	7.6±0.12	7.4±0.18	7.4±1.09	7.6±0.01	7.63±0.03
Clotting time (sec)	78.0±2.15	81.7±0.58	83.0±0.32	97.5±0.09*	105.7±1.15*
Serum aspartate-aminotransferase[a]	53.0±0.62	53.2±0.14	62.7±1.03	68.4±0.71*	76.2±1.09*
Serum alanine-aminotransferase[a]	25.1±0.03	25.2±0.02	25.7±0.19	26.6±0.11	28.7±0.32*
Total bilirubin[b]	1.6±0.85	1.6±0.04	1.4±1.13	1.3±0.04*	0.85±0.09*
Plasma cholesterol[c]	72.2±3.14	72.6±0.91	76.2±3.31	82.6±1.91*	85.4±2.48*
Blood urea[c]	12.8±0.39	12.9±0.03	17.5±0.12	19.9±2.18*	22.6±1.72*
Plasma protein[d]	8.3±1.33	8.5±0.51	11.2±2.18	14.4±0.19*	16.7±0.31*
Non protein nitrogen[c]	26.2±0.96	26.7±1.84	28.4±0.09	31.6±1.44*	33.8±3.52*

a - units/ml serum ; b - mg/100ml serum ; c - mg/100ml blood ; d - g/100ml blood

P values for saline control with respect to vehicle control is insignificant. So all *P* values are calculated with reference to saline control.

* $P<0.001$.

Table 2—Effect of crude extract of *M.oleifera* (daily treatment) on hematological and hepatorenal functions

[Values are mean±SE of 10 experiments]

Parameters	Saline (5ml/kg)	Vehicle (5ml/kg)	Crude extract (mg/kg) 3.5	4.6	7.0
Hemoglobin (%)	13.7±0.85	13.4±0.39	13.4±0.72	13.6±0.66	13.6±0.03
WBC (count/mm^3)	4365±0.07	4371±0.09	4620±0.91	4784±0.34*	5080±0.01*
RBC (count x 10^6/mm^3)	7.2±0.09	7.4±1.31	7.4±1.91	7.4±0.35	7.6±0.84
Clotting time (sec)	76.0±1.28	79.2±0.43	81.7±0.74	85.4±1.22*	94.5±0.29*
Serum aspartate-aminotransferase[a]	51.42±0.51	51.74±0.83	59.21±1.72	61.41±0.28*	64.28±1.36*
Serum alanine-aminotransferase[a]	24.30±1.21	24.85±0.14	25.21±0.19	25.74±1.09	28.82±0.21*
Total bilirubin[b]	1.42±0.08	1.41±0.42	1.33±0.72	1.21±0.05*	0.96±0.11*
Plasma cholesterol[c]	73.40±0.54	73.61±0.98	76.52±1.51	78.410.09*	83.26±1.32*
Blood urea[c]	13.42±1.39	13.53±2.54	13.57±0.05	13.60±2.51	13.65±3.12
Plasma protein[d]	8.72±2.41	9.03±0.56	9.23±1.33	9.85±1.87	10.01±0.39*
Non protein nitrogen[c]	25.73±1.44	25.91±2.84	26.05±2.61	26.28±0.52	27.31±1.94*

a - units/ml serum ; b - mg/100 ml serum ; c - mg/100 ml blood ; d - g/100ml blood.

P values for saline control with respect to vehicle control is insignificant. So all *P* values are calculated with reference to saline control.

*$P < 0.001$

for 4 weeks to the mice of 8th,9th and 10th groups respectively. Groups 1 and 2 received normal saline (0.9% NaCl, w/v ; 5ml/kg) and propylene glycol (5ml/kg) respectively, once in a week for 4 weeks. Groups 6 and 7 received normal saline (5ml/kg) daily for 4 weeks. Animals from each group were decapitated after 24 hr of the last dose.

Serum was separated from clotted blood for the estimation of bilirubin [6],serum aspartate amino transferase (SGOT) and serum alanine amino transferase (SGPT)[7] . Heparinised whole blood was taken for estimation of urea [8], plasma protein [9], cholesterol [8,10] and non protein nitrogen [11] . For hematological parameters blood was drawn from tail

and used for RBC count, hemoglobin content, clotting time [12] and differential leucocyte count [13].

The data were statistically analysed by Student's unpaired *t* test [14].

The results are presented in Tables.1 and 2

The weekly moderate dose level of CE changed serum amino transferase, plasma cholesterol, WBC count and clotting time significantly. High dose level of CE changed all the above parameters of weekly treated mice including total bilirubin, non protein nitrogen, blood urea and plasma protein. High dose level of the extract in daily treated mice changed serum amino transferase, plasma cholesterol, total bilirubin, clotting time and WBC count. But no significant alteration was observed at low and moderate dose levels of weekly treatment of CE.

Increase in plasma cholesterol at high dose level of CE may be due to a) decrease in cholesterol catabolism due to liver dysfunction ; b) inhibition of lipoprotein lipase activity which would decrease the removal of lipoprotein from plasma. Another possibility of increase of cholesterol may be due to the intake of CE itself as it was found to be steroid in nature. Moringinine - the alkaloid present in the root of *M.oleifera* belongs to the sympathomimetic group of bases, which inhibits the tone and movements of involuntary muscles of gastrointestinal tract [15]. Both transaminase are elevated in serum of patients with acute hepatic deseases and are good indicators of liver damage [16]. Thus elevated level of serum GOT and GPT in moderate and high dose level of weekly and high dose level of daily treated mice may be due to improper liver function . Decrease in serum bilirubin may be due to liver dysfunction at high doses of CE treatment. Prolongation of clotting time arises out of defficiencies of standard factors or out of an increase of circulatory anticoagulants [17]. Increased urea and NPN content in blood have been observed with impaired renal function or in acute renal failure [8]

CE treated mice when tested for anticonvulsive property revealed increased level of brain serotonin in mice (unpublished data) . Increased serotonin often caused kidney disturbance by slowing the glomerular filtration rate [18]. This may be another cause of high NPN and urea content in blood of CE treated mice in high dose. Increment of total plasma protein is an indication of liver diseases [8].

Thus the present study showed that the weekly moderate and high doses (46 and 70 mg/kg) of CE affect liver and kidney functions and metabolism and also alter hematological parameters.

The daily high dose (7mg/kg) of CE slightly affects liver and kidney functions and metabolism (alteration takes place in case of transaminases, plasma cholesterol and total bilirubin) and hematological parameters (WBC and clotting time). However, low and moderate doses (3.5 and 7.0 mg/kg) did not exhibit any remarkable toxic action.

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