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Effect of aqueous extract of *Murraya koenigii* on haematological, hormonal and lipid profile of Albino rats

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## ABSTRACT

**Objective:** To investigate the effect of aqueous extract of *Murraya koenigii* leaves on some haematological, hormonal and serum lipid parameters in rats.

**Methods:** We evaluated whether oral administration of *Murraya koenigii* affected the haematological, hormonal and lipid parameters. After 7 days of oral administration of 250 mg/kg and 500 mg/kg body weight, other profiles were investigated.

**Results:** The results showed that the extract administered significantly increased ( $P < 0.05$ ) packed cell volume, haemoglobin concentration, red blood cell, mean corpuscular hemoglobin, mean corpuscular hemoglobin concentration, mean corpuscular volume and decreased the platelet count ( $P < 0.05$ ) at the dose of 250 mg/kg and 500 mg/kg body weight when compared with control. The extract significantly increased ( $P < 0.05$ ) white blood cell count at all doses administered when compared with control. Results showed that thyronine and thyroxine were increased, while thyroid stimulating hormone was decreased significantly ( $P < 0.05$ ) at high doses compared. The recorded data in the present study showed that the testosterone value was reduced significantly ( $P < 0.05$ ) from control value. Also the extract significantly reduced ( $P < 0.05$ ) the total cholesterol concentration and low density lipoproteins cholesterol concentration in the serum while it had no significant effect on serum high density lipoprotein cholesterol concentration at all doses administered.

**Conclusions:** This study suggests that the extracts may have beneficial effect on serum cholesterol concentration, can act as a stimulant to thyroid functions as well as in anemia and immunity dependent disorders and also as a potent contraceptive.

## 1. Introduction

Plants play an essential role in the health care needs for the treatment of diseases and to improve the immunological response against much pathology[1]. Plant extracts are potentially curative. Some of these extracts can boost the humoral and cell mediated immunity against viruses, bacteria, fungi, protozoa and cancer[2-8].

*Murraya koenigii* Linn. Spreng (*M. koenigii*) belonging to family Rutaceae, commonly known as "Curry patta", is an aromatic plant that has been widely used in India as Ayurvedic herbal medicine. Since antiquity, it is used to treat a wide array of unrelated ailments that include dysentery, diarrhoea, microbial growth and stomach ache. Of the fourteen global species belonging to the genus *Murraya*, only two are available in India, viz. *M. koenigii* and *Murraya paniculata* (Linn.) Jack (syn. *Murraya exotica* Linn).

The former is more popular due to its large spectrum of medicinal properties and also because of the use of its leaves for centuries as a natural flavouring agent in various curries and food items[9]. Triterpenoid alkaloids, cyclomahanimbine, tetrahydroharmine, murrayastine, murrayaline, pyrayafoline carbazole alkaloids and many other chemical compounds have been reported in the leaves of *M. koenigii*[10,11]. Various studies showed its antibacterial properties[12,13] as well as its curative effects in hypothyroidism[14].

Haematological values are widely used to determine systemic relationships and physiological adaptations including the assessment of general health condition of an organism[15,16]. Hormonal profile regulates sexual behavior, growth of the cellular components of tissues and organs. Alteration of blood parameters disrupts normal physiological functions. Likewise, changes in the concentration of hormones can have profound effects on mood, behavior, anatomy and physiology in humans. Inhalation exposure to kerosene, petrol fumes and gasoline has been reported to alter the level of hormone and different components of blood[17,18]. Thyroid dysfunction has a great impact on lipid levels, and a number of other cardiovascular

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risk factors have significant effects on the synthesis, mobilization and metabolism of lipids[19]. The thyroid hormones affect serum cholesterol mainly by altering lipoprotein metabolism[19]. With herbal contraception getting its acceptance, this field needs to be explored more. Various plants and plant products are reported to have antifertility properties[20].

In spite of several studies on the different pharmacological activities of *M. koenigii*, not much has been investigated on hormonal, lipid and hematological profile. Therefore, the present study is aimed to investigate the impact of leaf extract of *M. koenigii* on hormonal, lipid and hematological indices in mammalian animal model.

## 2. Materials and methods

### 2.1. Experimental animals

Adult Albino rats of male sex weighing between 120 and 150 g procured from authorized suppliers were housed under standard environmental conditions in polypropylene cages [(25 ± 1) °C temperature, (55 ± 5)% humidity and 12 h/12 h light/dark cycle]. The animals were allowed free access to drink water and rat feed. The care and handling of rats were in accordance with the protocol approved by Institutional Animal Ethics Committee (process No. 46, page No.137).

### 2.2. Collection of plant materials

The fresh and tender leaves were collected, dried in a shade under (28 ± 2) °C for six to seven days and then crushed into coarse powdery substance by using electric grinder. The coarse powdery substance was dried again and then sieved to get fine powder using the fine plastic sieve, which was then stored in an air tight bottle in the laboratory until required.

### 2.3. Extract preparation

A total of 50 g of the sieved powder was weighed accurately and subjected to extraction in a Soxhlet apparatus at room temperature using 350 mL water. The extract obtained was filtered, concentrated in rotary flash evaporator and maintained at 45 °C. The percentage yield of the extract was calculated and the dried extract was stored in air tight containers at room temperature for further studies[21].

### 2.4. Acute toxicity studies

The acute toxicity studies were performed in accordance with the Organization for Economic Co-operation and Development, guidelines No. 425 (up-and-down procedure). No death was observed till the end of the study. The test samples were found safe up to the dose of 2000 mg/kg and from the results 500 mg/kg was

chosen as the maximum dose for further experimentation[22].

### 2.5. Experimental design

Group 1 was received 1 mL of distilled water orally; Group 2 was received 250 mg/kg body weight of *M. koenigii* orally; Group 3 was received 500 mg/kg body weight of *M. koenigii* orally. The details have been described elsewhere[14].

### 2.6. Estimation of haematological profile

Estimation of total cholesterol, high density lipoprotein (HDL) cholesterol, low density lipoprotein (LDL) cholesterol and triglycerides was done by cholesterol oxidase phenol aminoantipyrine method[23]. The haemoglobin level was measured by the cyanomethemoglobin method. The red blood cell (RBC) and reticulocyte counts were determined by visual method[24]. Packed cell volume (PCV) was measured using microhematocrit method and total white blood cell (WBC) count was estimated by visual method[25]. The RBC indices were calculated from the RBC count, haemoglobin level and PCV estimations[24,25].

### 2.7. Estimation of thyroid hormones

Estimation of serum 3,5,3'-triiodo-L-thyronine (T3), thyroxine (T4) and thyroid stimulating hormone (TSH) was done by chemiluminescence immunoassay method[26].

### 2.8. Estimation of lipid profile

Estimation of total cholesterol, HDL cholesterol, LDL cholesterol and triglycerides was done by cholesterol oxidase phenol aminoantipyrine method[23].

### 2.9. Statistical analysis

Data were expressed as mean ± SEM. For statistical evaluation of the data, Microsoft Office Excel was used (2007 version).

## 3. Results

The data recorded on haematological profile in control and *M. koenigii* fed rats were presented in Table 1. The extract had significant effect on RBC, haemoglobin, mean corpuscular hemoglobin concentration, mean corpuscular hemoglobin, PCV, mean corpuscular volume, neutrophils, basophils, monocytes, lymphocytes and eosinophils. The WBC was significantly elevated ( $P < 0.05$ ) in the group treated with 250 mg/kg body weight as well as in 500 mg/kg body weight. The platelet also significantly increased ( $P < 0.05$ ) in rats treated with both the doses.

The recorded data on thyroid profile showed increase in thyroid

**Table 1**Effect of aqueous leaf extract of *M. koenigii* on haematological parameters in Albino Wistar rats ( $n = 6$ ).

Groups	PCV (%)	MCV (%)	Haemoglobin (g/dL)	RBC ( $\times 10^9/\mu\text{L}$ )	Platelet ( $\times 10^7/\mu\text{L}$ )	WBC ( $\times 10^3/\mu\text{L}$ )	Neutrophil (%)	Lymphocyte (%)	Monocyte (%)	Eosinophil (%)	Basophil (%)	MCH (pg)	MCHC (g/dL)
Control	39.00 $\pm$ 0.02	93.00 $\pm$ 1.02	11.60 $\pm$ 0.76	4.21 $\pm$ 0.48	339.00 $\pm$ 1.81	6.80 $\pm$ 1.62	56.60 $\pm$ 1.40	32.90 $\pm$ 4.50	5.50 $\pm$ 1.51	0.50 $\pm$ 0.07	0.28 $\pm$ 0.09	30.46 $\pm$ 0.35	31.06 $\pm$ 0.28
LD	42.00 $\pm$ 0.01 <sup>a</sup>	94.40 $\pm$ 1.09 <sup>a</sup>	13.50 $\pm$ 0.65	4.73 $\pm$ 0.14 <sup>a</sup>	301.00 $\pm$ 1.09 <sup>a</sup>	6.90 $\pm$ 1.57 <sup>a</sup>	47.50 $\pm$ 0.97 <sup>a</sup>	30.30 $\pm$ 2.05 <sup>a</sup>	8.50 $\pm$ 2.54 <sup>a</sup>	1.20 $\pm$ 0.08 <sup>a</sup>	0.29 $\pm$ 0.04 <sup>a</sup>	29.00 $\pm$ 1.24 <sup>a</sup>	30.60 $\pm$ 1.00 <sup>ns</sup>
HD	44.00 $\pm$ 0.02 <sup>a</sup>	99.40 $\pm$ 0.72 <sup>a</sup>	14.60 $\pm$ 0.27 <sup>a</sup>	4.85 $\pm$ 0.13 <sup>a</sup>	305.00 $\pm$ 1.57 <sup>a</sup>	6.90 $\pm$ 0.59 <sup>a</sup>	46.40 $\pm$ 0.87 <sup>a</sup>	35.90 $\pm$ 2.16 <sup>a</sup>	4.50 $\pm$ 1.94 <sup>a</sup>	4.50 $\pm$ 0.06 <sup>a</sup>	0.30 $\pm$ 0.01 <sup>a</sup>	30.00 $\pm$ 0.71 <sup>a</sup>	30.00 $\pm$ 0.72 <sup>ns</sup>

Values were expressed as mean  $\pm$  SEM from the experiments. <sup>a</sup>:  $P < 0.05$ ; <sup>ns</sup>: Non-significant relative to control; MCV: Mean corpuscular volume; MCH: Mean corpuscular hemoglobin; MCHC: Mean corpuscular hemoglobin concentration; LD: Low destiny (250 mg/kg body weight); HD: High destiny (500 mg/kg body weight).

activity (Table 2). The TSH level was correlated well inversely with T3 and T4 levels. The group which received maximum test dose (500 mg/kg body weight) showed maximum percentage increase in hormone concentration of T3 and T4 whereas a maximum percentage decrease in TSH levels was observed when compared to the other dose levels, which clearly proved that the response was dose effective and the *M. koenigii* leaf extracts could be used in hypothyroidism condition to normalize hormone levels.

**Table 2**T3, T4 and TSH concentration along with their percent increase (+) or decrease (-) in relation to the control values in male rats by *M. koenigii* leaf extract ( $n = 6$ ).

Groups	T3 (ng/dL)	% Increase or decrease	T4 (ng/dL)	% Increase or decrease	TSH (uIU/mL)	% Increase or decrease
Control	30.00 $\pm$ 0.19	-	2.90 $\pm$ 0.86	-	0.81 $\pm$ 0.45	-
LD	39.00 $\pm$ 0.35 <sup>a</sup>	+30	3.50 $\pm$ 0.66 <sup>a</sup>	+20	0.58 $\pm$ 0.85 <sup>a</sup>	-28
HD	69.00 $\pm$ 0.44 <sup>a</sup>	+130	4.70 $\pm$ 0.89 <sup>a</sup>	+62	0.22 $\pm$ 0.87 <sup>a</sup>	-72

Values were expressed as mean  $\pm$  SEM from the experiments. <sup>a</sup>:  $P < 0.05$  relative to control. LD: Low destiny (250 mg/kg body weight); HD: High destiny (500 mg/kg body weight).

The effect of the oral administration of *M. koenigii* leaves aqueous extract on testosterone was presented in Table 3. The present study showed that the testosterone value was reduced significantly ( $P < 0.05$ ) in comparison to the control value [(170.62  $\pm$  1.30) ng/dL, (127.00  $\pm$  0.65) ng/dL and (30.03  $\pm$  0.44) ng/dL for control and treated groups respectively].

**Table 3**Testosterone concentration along with their percent increase (+) or decrease (-) in relation to the control values in male rats by *M. koenigii* leaf extract ( $n = 6$ ).

Groups	Testosterone (ng/dL)	% Increase or decrease
Control	170.62 $\pm$ 1.30	-
Low destiny (250 mg/kg bw)	127.00 $\pm$ 0.65 <sup>a</sup>	-25.29
High destiny (500 mg/kg bw)	30.03 $\pm$ 0.44 <sup>b</sup>	-82.35

Values were expressed as mean  $\pm$  SEM from the experiments. <sup>a</sup>:  $P < 0.05$  relative to control; <sup>b</sup>:  $P < 0.1$  relative to control; bw: Body weight.

The data recorded on lipid profile in control and *M. koenigii* fed rats were presented in Table 4. In case of *M. koenigii* extract, the total cholesterol showed a significant decrease ( $P < 0.05$ ) whereas HDL and LDL showed no significant decrease or increase.

**Table 4**Lipid profile along with their percent increase (+) or decrease (-) in relation to the control values in male rats by *M. koenigii* leaf extract ( $n = 6$ ).

Groups	Total cholesterol	% Increase or decrease	HDL cholesterol	% Increase or decrease	LDL cholesterol	% Increase or decrease
Control	60.00 $\pm$ 0.05	-	23.00 $\pm$ 0.13	-	18.00 $\pm$ 0.54	-
LD (250 mg/kg bw)	55.00 $\pm$ 0.19 <sup>a</sup>	-8.3	23.00 $\pm$ 0.23 <sup>ns</sup>	-	18.00 $\pm$ 0.32 <sup>ns</sup>	-
HD (500 mg/kg bw)	50.50 $\pm$ 0.11 <sup>a</sup>	-17	21.00 $\pm$ 0.03 <sup>a</sup>	-8.6	18.00 $\pm$ 0.42 <sup>ns</sup>	-

Values were expressed as mean  $\pm$  SEM from the experiments. <sup>a</sup>:  $P < 0.05$  relative to control; <sup>ns</sup>: Non significant; LD: Low destiny; HD: High destiny; bw: Body weight.

## 4. Discussion

Assessment of haematological parameters can not only be used to determine the extent of deleterious effect of extracts on the blood of an animal, but it can also be used to explain blood relating functions of a plant extract or its products[27]. The results obtained show significant values of WBC. Therefore, it is clear that an increase in the number of WBC is a normal reaction of rats to foreign substances, which alter their normal physiological processes. Platelets play a major role in the development as well as in the stability of atherosclerotic plaques and as a consequence, anti-platelet agents have been used clinically in patients at risk for myocardial ischemia, unstable angina and acute myocardial infarction[28,29]. Therefore, the doses of *M. koenigii* extract are useful in reducing the platelets which in turn might be useful in reducing the cardiovascular diseases as some studies suggested various mechanisms by which flavonoid exert its antiplatelet property by lowering intracellular  $\text{Ca}^{2+}$  levels, alteration in the metabolism of cyclic adenosine monophosphate, and thromboxane A2[30,31]. The haemoglobin content, RBC and PCV have also significantly increased erythropoietin release in the kidney which is the humoral regulator of RBC production[32,33].

Thyroid hormones play important role in the growth and development of the body and regulate metabolism[34]. So, with an increase in thyroid activity, marked changes in thyroid hormone production, metabolism and action occur. This may result in an increased prevalence of sub-clinical thyroid disease that is associated with thyroid dysfunction[35]. Many factors can influence the concentration of these hormones and therefore disturb the general body metabolism. Thiocyanate from tobacco, smoke, perchlorate and drugs which contain different amounts of iodine can influence the structure and function of thyroid hormones[36]. Various other studies reported that thyroid disorders vary according to age, genders, ethnic and geographical areas[37].

Similar results have been observed in different plant extracts such as Caraway[38], Everyouth and Dreamshape[39]. Also *Ficus carica* leaf extracts showed similar changes in the levels of T3 and T4, where TSH level was not investigated[40].

Among the plant based contraceptives, inhibition of male fertility after administration of natural substances has been related to decreased spermatozoa density[41]. Also, for male contraception, it is not necessary to stop spermatogenesis, rather to eliminate the fertilizing ability of the spermatozoa by causing changes in the morphology or in the function of the sperm[41]. *Saccharum*

*officinarum*, *Momordica dioica* and *Ocimum sanctum* are commonly known plants which possess antifertility activities as reported previously[42]. Raji *et al.* reported that the effects of the ethanol extract of *Azadirachta indica* stem bark on body and organ weights, sperm morphology, counts and viability, serum levels of testosterone, luteinizing hormone and follicle stimulating hormone were studied in Albino rats[43]. *Azadirachta indica* produced dose-dependent reduction in serum testosterone and luteinizing hormone but no change in follicle stimulating hormone levels. In the present study, the testosterone values were reduced significantly, therefore the aqueous extract may have contraceptive properties. However, such deduction needs to be substantiated by other testicular parameters and histopathological studies.

The relationship between serum lipid parameters and thyroid hormones has been found intensively[44,45]. Studies on this subject confirm the presence of an inverse relationship between thyroxin serum levels and cholesterol[46]. Other studies demonstrate the influence of iodothyronine on the catabolism of the very-low-density lipoprotein, showing increase in LDL and very-low-density lipoprotein fractions in untreated hypothyroidism[47].

In some studies, it is shown that total and LDL cholesterol are increased in hypothyroidism[47]. In another study, it is reported that hyperthyroidism induced a decrease in serum cholesterol. However, no alterations were reported in hypothyroidism and euthyroidism[46]. In a population-based study in older women with high TSH, LDL cholesterol is found 13% higher, HDL cholesterol 12% lower and total cholesterol, although not statistically significant, 8% higher than women with normal TSH levels[48].

The presence of hypercholesterolemia in clinical hypothyroidism is well established[19]. It is also reported that total cholesterol and LDL levels are increased in patients with clinical hypothyroidism[49]. This is due to the decreased activity of LDL receptors, resulting in decreased catabolism of LDL[47]. In the present study, *M. koenigii* extract is useful both in hypothyroidism as it can increase the T3 and T4 hormone levels and decrease TSH, and in hypercholesterolemia where it can decrease the total cholesterol.

Therefore, the results of the present study suggest that *M. koenigii* extract studied showed positive haematological activities in rats and can be recommended in the management of anaemia, immunity dependent disorders as well as in regulating the cholesterol and triglyceride levels. Also the extract can be used in hypothyroidism condition to normalize hormone levels and can act as a potent contraceptive too.

### Conflict of interest statement

We declare that we have no conflict of interest.

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