Hormonal profile and haematological parameters of Wistar albino rats treated with methanolic and aqueous leaf extracts of Schleichera oleosa

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Research Article

Keywords: Schleichera oleosa, haematological parameters, hormonal parameters, testosterone

DOI: https://doi.org/10.21203/rs.3.rs-199227/v1

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Abstract

**Background**: The present study aims to study the Hormonal profile and haematological parameters of Wistar albino rats treated with methanolic and aqueous extracts of *Schleichera oleosa*.

**Result**: The amount of RBC was found to be $9.6 \pm 0.05710^6/\text{mm}^3$, $9.7 \pm 0.0125, 10^6/9.5 \pm 0.003, 10^6/\text{mm}^3$, $9.5 \pm 0.012 10^6/\text{mm}^3$ and $9.5 \pm 0.023 10^6/\text{mm}^3$. The amount of WBC was found to be $13.5 \pm 0.058 10^3/\text{mm}^3, 13.6 \pm 0.0113 10^3/\text{mm}^3, 13.7 \pm 0.021 10^3/\text{mm}^3, 9.5 \pm 0.012 10^3/\text{mm}^3$ and $9.5 \pm 0.023 10^3/\text{mm}^3$. Platelets, $396 \pm 0.577 10^3/\text{mm}^3$, $395 \pm 0.03 10^3/\text{mm}^3$, $397 \pm 0.04210^3/\text{mm}^3$, $398 \pm 0.020 10^3/\text{mm}^3$, and $397 \pm 0.011 10^3/\text{mm}^3$ for controlled group, low dose of methanolic extract, high dose of methanolic extract, low dose of aqueous extract and high dose of aqueous extract respectively. The packed cell volume, haemoglobin, mean corpuscular volume, mean corpuscular haemoglobin, corpuscular haemoglobin concentration and amount of neutrophils, Eosinophils, Lymphocytes, Basophils and Monocytes were measured and no significant change was noticed. The amount of testosterone showed significant increase from $0.75 \pm 0.024 \text{pg/ mL (control)}$, $2.96 \pm 0.088 \text{pg/ mL}$, $3.9 \pm 0.14 \text{pg/ mL}$, $1.85 \pm 0.076 \text{pg/ mL}$ and $2.85 \pm 0.074 \text{pg/ mL}$.

**Conclusions**: The study suggests that the prolonged treatment of Wistar rats with both low dose and high dose of methanolic as well as the aqueous extract of *Schleichera oleosa* is not toxic and they increased the amount of testosterone in the blood which is indicative of their testosterone enhancement.

**Background**

From the earliest time it has been well known that India has affluent oral biodiversity and it has abounded the herbal medicine practitioners with a very helpful collection of ‘natural pharmacy’ which has become a pool and reservoir for selection of plants as ingredients in the extraction and preparation of herbal medicines for the effective treatment, control and management of a great many number of human diseases [1]. The World Health Organization (WHO) has defined medicinal plants as- plant which in one or more of its organs contains substances that can be used for curative purposes, or which are predecessors of useful drugs is called as medicinal plants [2]. There is a grave problem with medicinal claims about many plants as sufficient scientific data is not available concerning the real effects of the concerned medicinal plants [3]. Therefore it is necessary to establish scientific bases for their ethno medical uses and toxicological profiles.

For many decades it is well accepted that Haematological values and parameters are widely used to find out common relationships and physiological conditions including the evaluation and study of general health condition of an organism [4,5]. Hormones- as is well known from the previous researches - in the body of an organism regulate various bodily functions especially they regulate sexual activity, development of cellular structures of tissues and organs. Changes in the values of blood parameters interferes with the natural physiological functions and in the same way alteration in the hormone concentrations in the body brings great impacts on human anatomy, physiology, mood, behaviour,
response to the environment etc. [6,7]. Thus study of hormonal and haematological parameters are very relevant for the humanity today.

As is known Schleichera is genus of plant that belongs to the family Sapindaceae. Schleichera oleosa is a simple ordinary tree found in the forests and villages of India and Southeast Asia and they are called as ‘Kusum’ in ‘Hindi’. They are home to a large number of insects specially the well known lac insect and are therefore used for lac insect culture. All parts of the tree namely its leaves, twigs and seed-cake are used as fodder to feed cattle. The wood is strong, very good for making furniture and as firewood and forms excellent charcoal. ‘Kusum oil’ extracted from its seed is used in food for cooking and for illuminating houses in villages of India where electrification has not taken place. They are used by the village healers to cure of itching, acne, burns, other skin troubles, rheumatism (external massage), hair dressing and for promoting hair growth [8]. M. Lakshmi Santha et al has reported the anti-inflammatory and anti arthritis activity of Schleichera oleosa [9]. Sophy Jose et al., have reported the phytochemistry and antibacterial efficacy of Schleichera oleosa [10]. The anti hypertensive activity of aqueous and methanolic leaf extracts of Schleichera oleosa in described by Jose S et al., [11]. Soundararajan Muthukrishnan et al have reported the antidiabetic activity of Schleichera oleosa [12]. Till this date no report of the haematological and hormonal impacts of Schleichera oleosa are available in the literature. The present study was undertaken to explore the hormonal profile and haematological parameters of male Wistar albino rats treated with methanolic and aqueous extracts of Schleichera oleosa.

Methods

Experimental animals

As experimental material, 30 male Wistar albino rats (Rattus norvegicus) weighing 120 – 200g were taken. They were kept in metallic cages (40 x 15 x 16 cm) under laboratory conditions for one week of acclimatization and were divided into five groups. One control group, second group treated with high dose of aqueous leaf extract of Schleichera oleosa, the third group treated with low dose of aqueous leaf extract of Schleichera oleosa, the fourth group was fed with high dose of methanolic leaf extract and the fifth group was treated with low dose of aqueous leaf extract. The rats were fed with normal rat chow (guinea feeds product) and tap water. They were kept in well ventilated room at ambient temp. of 30 ± 5ºC under 12 hr light / dark cycle. The animals in both control and treated groups were maintained in normal diet while animals in the treated group were administered orally 20 mg / 100 g body weight of methanolic and aqueous leaf extract of Schleichera oleosa by using an oral feeding pipe [13] and acute oral toxicity and dose were determined as per OECD-423 guidelines [14].

Collection of plant Material

Fresh mature leaves of Schleichera oleosa were collected from trees that are found in the campus of St. Xaviers's college, Ranchi district, Jharkhand state, with prior permission from the principal of the college. This is a plant used for culturing lac insect and has been identified and referred in various studies earlier. A deposition number is not available.
Preparation of extract

Collected leaves were washed and dried for 14 days. The dried plant of *Schleichera oleosa* was powdered and was subjected to soxhlet extraction with methanol and water for 24 hrs. The blackish green extracts thus obtained were evaporated to dryness in a flask evaporator at room temperature and the residue designated as methanolic extract and aqueous leaf extracts of *Schleichera oleosa* were used as toxicant for further studies.

The mammalian model will be Wister albino rats (*Rattus norvegicus*) was procured from Chakraborty Rats (Kolkata, India) and was maintained on standard food (pallet diet and water). Rats were acclimatize to the conditions of our laboratory in zoology department for one week before any experimental work was undertaken. Rats were separated into groups and the aqueous and methanolic plant extract was fed with oral feeding pipe and acute oral toxicity and dose was determined as per OECD-423 guidelines. They were given a normal laboratory pellet diet and water. The experiments were accurately designed and conducted according to ethical norms approved by Ministry of Social Justices and Empowerment, Government of India and the Institutional Animal Ethics Committee Guidelines (IAEC No. 01/034/04).

The mammalian model Wister albino rats

The mammalian model for the present study was Wister albino rats (*Rattus norvegicus*). The rats were acquired from Chakraborty Rats (Kolkata, India). They were maintained on standard food (pallet diet and water). Rats were made to become accustomed to the conditions of our laboratory in zoology department Ranchi University for one week before the experimental work was undertaken. Rats were separated into groups and the aqueous and methanolic plant extract was fed as per OECD-423 guidelines. They were given a normal laboratory pellet diet and water. The experiments were accurately designed and conducted according to ethical norms approved by Ministry of Social Justices and Empowerment, Government of India and the Institutional Animal Ethics Committee Guidelines (IAEC No. 01/034/04).

Haematological analysis

After 14 days of oral treatment, blood sample was collected by cardiac puncture in sterilized vials separately for control and treated groups of rats. Samples were analysed for total RBC count and haemoglobin amount by fully automated bi – directional 5 part differential analysers technology [15].

Hormonal analysis

The steroid hormone testosterone for *Schleichera oleosa* and *Scoparia dulcis* leaf extracts was measured by radioimmunoassay as described by Banu *et al.* [16] in ng / mL. Numerical data were expressed as mean ± SD and independent-samples and the results were statistically analyzed by Microsoft Office-Excel (2007 version).

Results
Table 1. Effect of prolonged administration of methanolic and aqueous leaf extracts of *Schleichera oleosa* on haematological parameters (mean SD) of Wistar rats

| TEST          | S. oleosa Met L.D | S. oleosa Met H.D | S. oleosa Aqu. L.D | S. oleosa Aqu. H.D | Control |
|---------------|-------------------|-------------------|-------------------|-------------------|---------|
| RBC (10^6/mm³)  | 9.6±0.057#       | 9.7±0.0125#      | 9.5±0.003#       | 9.5±0.012#       | 9.5±0.023 |
| WBC (10³/mm³)   | 13.5±0.058#      | 13.6±0.0113#     | 13.7±0.021#      | 13.7±0.021#      | 13.8±0.002 |
| Hb (g/dl)       | 11.4±0.011#      | 11.8±0.0342#     | 11.5±0.004#      | 11.6±0.001#      | 11.5±0.023# |
| PCV (%)         | 43.6±0.023#      | 44.6±0.0234#     | 44.7±0.013#      | 45.5±0.115#      | 44.5±0.042# |
| Platelets (10³/mm³) | 396±0.577#    | 395±0.03#        | 397±0.042#       | 398±0.020#       | 397±0.011# |
| MCV (fl)        | 48.3±0.022#      | 48.5±0.022#      | 48.6±0.022#      | 48.8±0.012#      | 48.7±0.0123# |
| MCH (Pg)        | 16.5±0.046#      | 16.3±0.013#      | 16.4±0.033#      | 16.6±0.032#      | 16.5±0.034# |
| MCHC (g/dl)     | 35.0±0.173#      | 35.1±0.0577#     | 35.2±0.014#      | 35.2±0.003#      | 35.0±0.014# |

( Significantly higher; Significantly lower; # no significant difference; mean SD, n=3)

Table 2. Effect of prolonged administration of methanolic and aqueous leaf extracts of *Schleichera oleosa* on haematological Indices (mean ) on Wistar rats
Table 3. Mean free serum testosterone level (pg/mL) among groups of albino rats treated with aqueous and methanolic leaf extracts of *Schleichera oleosa*.

| TEST               | Control   | *S. oleosa* Met L.D | *S. oleosa* Met H.D | *S. oleosa* Aqu. L.D | *S. oleosa* Aqu. H.D |
|--------------------|-----------|---------------------|---------------------|----------------------|----------------------|
| **Testosterone** (pg/mL) | 0.75± 0.024 | 2.96± 0.088         | 3.9± 0.14           | 1.85± 0.076          | 2.85± 0.074          |
| **p Value**        | < 0.05    | < 0.05              | < 0.05              | < 0.05               | < 0.05               |

(Significantly higher; Significantly lower; # no significant difference; mean SD, n=3)

**Discussion**

The effects of oral administration of Methanolic and aqueous leaf extracts of *Schleichera oleosa* on haematological parameter are shown in table 1 and 2 and hormonal profile of Wistar albino rats treated with methanolic and aqueous extracts of *Schleichera oleosa* are shown in Table 3 and figure 1.

The amount of RBC for the controlled group was found to be 9.6±0.05710⁶/mm³. The group treated with low dose of methanolic extract of *Schleichera oleosa* expressed 9.7±0.0125 10⁶/mm³ of RBC the group
treated with high dose of methanolic extract showed 9.5±0.003 $10^6$/mm$^3$ of RBC The group treated with low dose of aqueous extract of *Schleichera oleosa* was found to have 9.5±0.012 $10^6$/mm$^3$ of RBC, The group treated with high dose of aqueous extract of *Schleichera oleosa* possessed 9.5±0.023 $10^6$/mm$^3$ of RBC. The amount of WBC for the controlled group was found to be 13.5±0.058 $10^3$/mm$^3$. The group treated with low dose of methanolic extract of *Schleichera oleosa* expressed 13.6±0.011 $10^3$/mm$^3$ of WBC the group treated with high dose of methanolic extract showed 13.7±0.021 $10^3$/mm$^3$ of WBC, The group treated with high dose of aqueous extract of *Schleichera oleosa* possessed 9.5±0.023 $10^3$/mm$^3$ of WBC.

The group treated with low dose of methanolic extract of *Schleichera oleosa* expressed 395±0.03 $10^3$/mm$^3$ of Platelets the group treated with high dose of methanolic extract showed 397±0.042 $10^3$/mm$^3$ of Platelets The group treated with low dose of aqueous extract of *Schleichera oleosa* was found to have 398±0.020 $10^3$/mm$^3$ of Platelets, The group treated with high dose of aqueous extract of *Schleichera oleosa* possessed 397±0.011 $10^3$/mm$^3$ of Platelets. The amount of Packed cell volume for the controlled group was found to be 43.6±0 %. The group treated with low dose of methanolic extract of *Schleichera oleosa* expressed 44.6±0.023 $10^3$/mm$^3$ of Packed cell volume the group treated with high dose of methanolic extract showed 44.7±0.013 $10^3$/mm$^3$ of Packed cell volume. The group treated with low dose of aqueous extract of *Schleichera oleosa* was found to have 45.5±0.115 $10^3$/mm$^3$ of Packed cell volume, The group treated with high dose of aqueous extract of *Schleichera oleosa* possessed 44.5±0.042 $10^3$/mm$^3$ of Packed cell volume. The amount of haemoglobin for the controlled group was found to be 11.4±0.011 (g/dl). The group treated with low dose of methanolic extract of *Schleichera oleosa* expressed 11.8±0.034 (g/dl) of haemoglobin the group treated with high dose of methanolic extract showed 11.5±0.004 (g/dl) of haemoglobin The group treated with low dose of aqueous extract of *Schleichera oleosa* was found to have 11.6±0.001 (g/dl) of haemoglobin, The group treated with high dose of aqueous extract of *Schleichera oleosa* possessed 11.5±0.023 (g/dl) of haemoglobin. The amount of mean corpuscular volume for the controlled group was found to be 48.3±0.022 fl. The group treated with low dose of methanolic extract of *Schleichera oleosa* expressed 48.5±0.022 fl. of mean corpuscular volume the group treated with high dose of methanolic extract showed 48.6±0.022 fl of mean corpuscular volume The group treated with low dose of aqueous extract of *Schleichera oleosa* was found to have 48.8±0.012 fl of mean corpuscular volume, The group treated with high dose of aqueous extract of *Schleichera oleosa* possessed 48.7±0.0123 fl of mean corpuscular volume. The amount of mean corpuscular haemoglobin for the controlled group was found to be 16.5±0.046 Pg. The group treated with low dose of methanolic extract of *Schleichera oleosa* expressed 16.3±0.013 Pg. of mean corpuscular haemoglobin the group treated with high dose of methanolic extract showed 16.4±0.033 Pg of mean corpuscular haemoglobin The group treated with low dose of aqueous extract of *Schleichera oleosa* was found to have 16.6±0.032 Pg of mean corpuscular haemoglobin, The group treated with high dose of aqueous extract of *Schleichera oleosa* possessed 16.5±0.034 Pg of mean corpuscular haemoglobin. The amount of mean corpuscular haemoglobin for the controlled group was...
found to be 16.5±0.046 pg. The group treated with low dose of methanolic extract of *Schleichera oleosa* expressed 16.3±0.013 pg of mean corpuscular haemoglobin. The group treated with high dose of methanolic extract showed 16.4±0.033 pg of mean corpuscular haemoglobin. The group treated with low dose of aqueous extract of *Schleichera oleosa* was found to have 16.6±0.032 pg of mean corpuscular haemoglobin. The group treated with high dose of aqueous extract of *Schleichera oleosa* possessed 16.5±0.034 pg of mean corpuscular haemoglobin.

The amount of mean corpuscular haemoglobin concentration for the controlled group was found to be 35.0±0.173 g/dl. The group treated with low dose of methanolic extract of *Schleichera oleosa* expressed 35.1±0.0577 g/dl. of mean corpuscular haemoglobin concentration. The group treated with high dose of methanolic extract showed 35.2±0.014 g/dl of mean corpuscular haemoglobin concentration. The group treated with low dose of aqueous extract of *Schleichera oleosa* was found to have 35.2±0.014 g/dl of mean corpuscular haemoglobin concentration. The group treated with high dose of aqueous extract of *Schleichera oleosa* possessed 35.0±0.014 g/dl of mean corpuscular haemoglobin concentration.

The amount of neutrophils for the controlled group was found to be 39.2±0.021 10^3/mm^3. The group treated with low dose of methanolic extract of *Schleichera oleosa* expressed 39.3±0.002 10^3/mm^3 of neutrophils. The group treated with high dose of methanolic extract showed 39.4±0.021 10^3/mm^3 of neutrophils. The group treated with low dose of aqueous extract of *Schleichera oleosa* was found to have 39.6±0.002 10^3/mm^3 of neutrophils. The group treated with high dose of aqueous extract of *Schleichera oleosa* possessed 39.6±0.002 10^3/mm^3 of neutrophils. The amount of eosinophils for the controlled group was found to be 2.0±0.02 10^3/mm^3. The group treated with low dose of methanolic extract of *Schleichera oleosa* expressed 2.2±0.021 10^3/mm^3 of eosinophils. The group treated with high dose of methanolic extract showed 2.4±0.023 10^3/mm^3 of eosinophils. The group treated with low dose of aqueous extract of *Schleichera oleosa* was found to have 2.6±0.021 10^3/mm^3 of eosinophils. The group treated with high dose of aqueous extract of *Schleichera oleosa* possessed 2.5±0.02310^3/mm^3 of eosinophils.

The amount of lymphocytes for the controlled group was found to be 78±0.033 10^3/mm^3. The group treated with low dose of methanolic extract of *Schleichera oleosa* expressed 79±0.0310^3/mm^3 of lymphocytes. The group treated with high dose of methanolic extract showed 77±0.0510^3/mm^3 of lymphocytes. The group treated with low dose of aqueous extract of *Schleichera oleosa* was found to have 76±0.03410^3/mm^3 of lymphocytes. The group treated with high dose of aqueous extract of *Schleichera oleosa* possessed 78±0.033 10^3/mm^3 of lymphocytes.

The amount of basophils for the controlled group was found to be 1.0±0.0110^3/mm^3. The group treated with low dose of methanolic extract of *Schleichera oleosa* expressed 1.0±0.01 10^3/mm^3 of basophils. The group treated with high dose of methanolic extract showed 1.0±0.0110^3/mm^3 of basophils. The group treated with low dose of aqueous extract of *Schleichera oleosa* was found to have 1.0±0.01 10^3/mm^3 of basophils. The group treated with high dose of aqueous extract of *Schleichera oleosa* possessed 1.0±0.01 10^3/mm^3 of basophils. The amount of monocytes for the controlled group was found to be 2.4±0.001 10^3/mm^3. The group treated with low dose of methanolic extract of *Schleichera oleosa* expressed 2.5±0.002 10^3/mm^3 of monocytes. The group treated with high dose of methanolic extract showed 2.4±0.001 10^3/mm^3 of monocytes.
Schleichera oleosa was found to have 2.5±0.00210³/mm³ of Monocytes, The group treated with high dose of aqueous extract of Schleichera oleosa possessed 2.4±0.001 10³/mm³ of Monocytes

It was observed that oral administration of aqueous and methanolic leaf extracts of Schleichera oleosa did not have any major impact on the haematological parameters.

It has been reported that persistent treatment with Vanadate, Ammonium metavanadate for 7 to 8 days caused a dose dependent significant decrease of RBC from 11.29 ± 1.2 to 5.67 ± 0.9 and 11.350 ± 1.4 to 4.245 ± 1.02 g / dL respectively (Ahmad, F.,1995). Ashour et al., [17] also observed oral administration of 1000 or 2000 ppm lead acetate significantly decreased red blood cell count, hemoglobin level and hematocrit value at 20, 40 and 60 days compared with control groups of male wistar albino rats. According to Terayama[18], lead could affect the rat erythrocyte membrane and decrease their mobility, it may also induce oxidative stress in RBCs [19]. Our results showed that aqueous and methanolic leaf extracts of Schleichera oleosa did not cause any major changes in the haematological parameters. From the result it could be said that methanolic and aqueous leaf extracts of Schleichera oleosa have no negative impact in the haematological parameters and do not cause toxicity on albino rats. Therefore they can be considered safe when they are used as medicine.

The amount of testosterone for the controlled group was found to be 0.75± 0.024 pg/ mL. The group treated with low dose of methanolic extract of Schleichera oleosa expressed 2.96± 0.088 pg/ mL of testosterone the group treated with high dose of methanolic extract showed 3.9± 0.14pg/ mL of testosterone The group treated with low dose of aqueous extract of Schleichera oleosa was found to have 3.9± 0.14 pg/ mL of Testosterone, The group treated with high dose of aqueous extract of Schleichera oleosa possessed 2.85± 0.074 pg/ mL of Testosterone.

It showed significant increase from 0.75± 0.024 pg/ mL (control) 2.96± 0.088 pg/ mL (treated with low dose of methanolic extract) and 3.9± 0.14pg / mL (treated with high dose of methanolic extract) to 1.85± 0.076 pg/ mL (treated with low dose of aqueous extract) and 2.85± 0.074 pg/ mL (treated with high dose of aqueous extract). Mean serum testosterone level of rats treated with of the Schleichera leaf extract for 14 days significantly increased (p < 0.05) compared with the controls.

Male sex hormone testosterone is responsible for the growth, development and proper functioning of male urinogenital system and the accessory sex organs. A study conducted by Nagendra Singh Chauhan et al has stated that crude extracts of plants that are used for treating sexual disorders have the potential to improve sexual behaviour, spermatogenesis and as a whole reproduction. [20]. M. Carpentier, et al said that plants have the capacity to rectify erectile dysfunction [21]. W. Low et al proved the some Asian traditional medicine can be used for erectile dysfunction [22,23]. Improvement in the testosterone will improve the sexual functions and reproductive functions of the body [24,25]. Our study also proved the same as it is shown in table 3 the prolonged treatment of Wistar rats with both low dose and high dose of methanolic as well as the aqueous extract of Schleichera oleosa increased the amount of testosterone in
the blood which is indicative of the testosterone enhancement function of leaf extract of *Schleichera oleosa*.

**Conclusion**

Thus the present concludes that the prolonged administration of aqueous and methanolic leaf extracts of *Schleichera oleosa* is not toxic and have no altering haematological impacts on Wistar rats. Thus it may be safe to administer them for various therapeutic purposes. The leaf extracts have testosterone enhancement quality therefore it can be used in the male reproduction enhancement treatments.

**Abbreviations**

| Abbreviation | Description                  |
|--------------|------------------------------|
| LD           | low dose                     |
| HD           | High dose                    |
| WHO          | World Health Organization    |
| OECD         | Economic Co-operation and Development |
| SD           | Standard deviation           |

**Declarations**

**Ethical approval and consent to participate**: Ethics approval and consent to participate was given by Zoology Department, Ranchi University. The experiments were accurately designed and conducted according to ethical norms approved by Ministry of Social Justices and Empowerment, Government of India and the Institutional Animal Ethics Committee Guidelines (IAEC No. 01/034/04).

**Consent for Publication**: ‘Not Applicable’

**Availability of data and material**: Availability of data was by the experiments conducted in the laboratory of Department of Zoology Ranchi University and plant material for the experiment that is leaf of *Schleichera oleosa* was obtained from the campus of St. Xavier’s College, Ranchi.

**Plant authentication**: Branch of ‘National Medicinal Plants Board, Ministry of Ayush, Government of India’ in Ranchi collaborating closely with Department of Botany and Zoology, Ranchi University authenticated the plant leaf of *Schleichera oleosa*.

**Competing interests**: There are no competing interests.

**Authors Contribution**: Collection of materials and data by SJ and substantively revised it by M.P.S. Both authors read and approved the final manuscript.
Acknowledgements- The authors acknowledge the facilities provided by Department of Zoology, Ranchi University, Ranchi, India for carrying out the work.

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