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# ACUTE, SUB-ACUTE AND SUB-CHRONIC TOXICITY STUDY OF *Psychotria sarmentosa* LEAVES USED IN TRADITIONAL PORRIDGE IN SRI LANKA

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## Introduction

*Psychotria sarmentosa,* generally known as ":*Gonica*" in Sinhala, is a climber of the Rubiaceae family. Leaves and stems of this plant are used to treat bone fractures, in the *Deshiya Chikista* system of medicine in Sri Lanka. Males in rural areas in Sri Lanka drink an aqueous extract of leaves and stems of this plant, when they are physically assaulted [1]. In addition, immature leaves are widely used by the community make a traditional leafy porridge ("*kola kenda*") and a tempered vegetable salad. Our previous studies have shown that freeze dried aqueous extract of leaves of this plant has significant ($p < 0.05$) acute and chronic anti-inflammatory activity in *in-vivo* models. Further it was found that it has significant ($p < 0.05$) anti-histamine, anti-nociceptive, *in-vivo* and *in-vitro* anti-oxidant, nitric oxide scavenging activities as well as cyclooxygenase-2 and prostaglandin $E_2$ inhibitory activity. Although, it shows different biological actions no scientific data are available regarding its potential adverse effects. The insufficiency of information regarding the toxicity of this plant limits the possible long-term use in chronic disease conditions. Hence, in the present study an attempt has been made to evaluate the acute, sub-acute and sub-chronic toxicity of *P. sarmentosa* leaves.

## Material and Methods

### *Plant material*

Fresh *P. sarmentosa*, stems with leaves were purchased from a local market and authenticated by Dr. D. S. A. Wijesundara, Director General, Royal Botanical Gardens, Peradeniya, Sri Lanka. A voucher specimen (KMR001) was deposited at National Herbarium, Department of National Botanic Garden, Peradeniya, Sri Lanka.

*Ethical clearance*

The protocol for animal experiments was approved by the Ethics Review Committee of the Faculty of Medical Sciences, University of Sri Jayewardenepura, Gangodawila, Nugegoda, Sri Lanka (No. 35/15).

*Animals*

Healthy adult female, Wistar rats weighing 150-200 g were purchased from Medical Research Institute, Colombo 8, Sri Lanka. Rats were housed under standard conditions with a natural light-dark cycle and fed with standard diet and water *ad libitum*. The animals were acclimatized for at least one week to the laboratory conditions prior to the experiment.

*Preparation of aqueous extract of P. sarmentosa leaves*

Fresh leaves of *P. sarmentosa* (100 g) were crushed in a mortar and pestle with 200 mL of water. The extract was filtered. The greenish filtrate was boiled for 5 minutes and cooled and then freeze dried. The extract yielded 2.4 g of green coloured freeze dried powder. The required amounts of freeze dried aqueous extract of *P. sarmentosa* leaves (FAPL) were dissolved in distilled water for oral administration to the rats.

*Toxicity study of P. sarmentosa leaves*

To evaluate the safety of the aqueous extract of *P. sarmentosa* leaves, a limited dose acute oral toxicity study, sub-acute oral toxicity study (28 days) and sub-chronic oral toxicity study (90 days) were carried out in compliance with the Organization for Economic Co-operation and Development (OECD) guidelines. In each assay negative control group was administered with 1.0 mL of distilled water (DW). In the limited dose test, treated group received 5000 mg/kg b. w. of FAPL. The sub-acute toxicity was done for the therapeutic effective dose of FAPL as found in anti-inflammatory assays (100 mg/kg b. w.) and doses which are lesser and higher than this dose, i.e.: 50 mg/kg b. w. and 2000 mg/kg b. w. respectively for 28 days. The sub-chronic toxicity study was done only for the therapeutically effective dose. In sub chronic assay recovery groups were kept for further 28 days without any oral treatments. In each assay, assessment of mortality and the behavior of the animals were carried out by the general observations of each animal twice daily from the stage of dosing to the end of the study. Further, changes in the body weight, water consumption and food consumption were compared with the control group. In addition to this haematological and biochemical parameters were measured to evaluate the safety of the plant extract. Further relative organ index was calculated to assess the safety of FAPL on different organs. Histopathological studies were also done.

**Results and Discussion**

All studies have shown that there were no mortalities during the entire period of the study following administration of FAPL. As mortality is the main criteria in

assessing the acute toxicity of any drug, the absence of mortality by FAPL is an indicator of the safety. Further absence of any changes or abnormalities in the condition of fur, urine color, faeces or signs such as diarrhea, damaged skin, subcutaneous swelling or lumps, wetness or soiling of perineum, salivation and breathing abnormalities in treated group in comparison to the control group indicated that FAPL in acute, sub-acute and sub-chronic dosing.

The body weight is also an important factor to monitor the health of an animal. The OECD guidelines of toxicity testing place considerable emphasis on reporting on changes in weight gain of each animal. Loss in body weight is frequently the first indicator of the onset of an adverse effect. A dose which causes 10 % or more reduction in the body weight, is considered to be a toxic dose [2]. As FAPL treated groups in each assay have not shown the body weight reduction throughout the study periods, it provides the evidence for safety of usage of FAPL. Further, the results showed that, there are no significant ($p > 0.05$) difference in food and water consumption in treated groups as compared to negative control.

When considering the serum clinical profiles, all tested parameters in the rats treated with FAPL comparable with those of the control rats of each study. The results on sub-chronic study are given in Table 3.1. As AST, ALT, ALP and γ- GT are good enzymatic indicators of hepatic diseases, the absence of any significant difference ($p > 0.05$) of those markers in FAPL treated group indicates its safeness on liver. Further, absence of any significant difference ($p > 0.05$) in serum urea and creatinine levels in FAPL treated rat group as compared to the control group indicate its safeness on kidney. The absence of difference in relative weights of vital organs in treated as compared to the control group also provide scientific evidence for the safety of the test extract.

In addition to clinical chemistry profile, haematological parameters are also good indices of physiological and pathological status in humans as well as the animals. Hence, haematological parameters also measured and any abnormal haematological parameters levels were not found in FAPL treated groups. The results on sub-chronic study are given in Table 3.2.

As histological assessment is also important in toxicology studies the collected tissues were subjected for histopathology observations include leukocyte infiltration, necrosis in liver, hemorrhages in kidneys and many others in each toxicity study. The results have shown that there were no any morphological changes in microscopic examination of tissues. Hence, it also provide strong evidence for the non-toxicity of FAPL.

**Table 1. Clinical chemistry data of Wistar rats in the sub-chronic oral toxicity study of *P. sarmentosa* leaves**

| Clinical chemistry parameters | Group 1 | Group 2 | Group 3 | Group 4 |
|---|---|---|---|---|
| Albumin (mg/dL) | 4.4 ± 0.1 | 4.3 ± 0.1 | 4.5 ± 0.1 | 4.4 ± 0.04 |
| ALP (IU/L) | 94.3 ± 16.6 | 113.8 ± 2.0 | 110 ± 2.8 | 108 ± 3.0 |
| ALT (IU/L) | 42.0 ± 2.3 | 43.6 ± 5.0 | 42.6 ± 2.5 | 45.8 ± 2.8 |
| AST (IU/L) | 78.2 ± 2.3 | 73.0 ± 3.1 | 79.1 ± 3.1 | 73.6 ± 2.4 |
| Calcium (mg/dL) | 10.8 ± 0.3 | 11.8 ± 0.3 | 11.1 ± 0.3 | 11.7 ± 0.6 |
| Cholesterol (mg/dL) | 72.3 ± 3.0 | 68.0 ± 1.8 | 73.2 ± 3.4 | 75.0 ± 3.6 |
| Creatinine (mg/dL) | 0.6 ± 0.02 | 0.7 ± 0.04 | 0.6 ± 0.04 | 0.6 ± 0.04 |
| γ – GT (IU/ L) | 22.2 ± 0.9 | 22.4 ± 0.9 | 22.4 ± 1.1 | 24.1 ± 0.9 |
| Glucose (mg/dL) | 83.7 ± 2.0 | 73.8 ± 5.3 | 75.5 ± 1.2 | 83.4 ± 1.3 |
| Phosphorous(mg/dL) | 18.3 ± 1.4 | 20.7 ± 1.4 | 19.0 ± 2.2 | 19.3 ± 1.3 |
| Total bilirubin (mg/dL) | 2.8 ± 0.2 | 3.0 ± 0.3 | 2.7 ± 0.2 | 3.2 ± 0.3 |
| Urea (mg/dL) | 3.8 ± 0.3 | 3.9 ± 0.5 | 3.4 ± 0.3 | 3.4 ± 0.1 |

Values for clinical chemistry parameters are expressed as mean ± SEM, (n=6/group), *p<0.05 compared with healthy control

Group 1: Healthy control group (Distilled water), Group 2: Treated group (100 mg/kg b. w., FAPL in DW), Group 3: Healthy control recovery group (DW), Group 4: FAPL Treated recovery group (100 mg/kg b. w., FAPL in DW)

**Table 2. Haematological parameters in Wistar rats of the sub-chronic oral toxicity study of *P. sarmentosa* leaves**

| parameters | Group 1 | Group 2 | Group 3 | Group 4 |
|---|---|---|---|---|
| Haemoglobin (g/dL) | 14.9 ± 0.4 | 15.0 ± 0.4 | 15.0 ± 0.4 | 14.7 ± 0.2 |
| RBC (x $10^3$ / L) | 7.6 ± 0.8 | 7.7 ± 0.1 | 7.9 ± 0.1 | 8.0 ± 0.1 |
| PCV (%) | 42.5 ± 1.2 | 43.7 ± 0.9 | 43.7 ± 0.8 | 43.1 ± 0.9 |
| Platelet count (x$10^3$/L) | 863 ± 55 | 847 ± 67 | 822 ± 43 | 848 ± 69 |
| MCV (fL) | 111 ± 1.5 | 113.5 ± 0.5 | 113 ± 1.2 | 108.6 ±1.7 |
| MCH (pg) | 39.0 ± 0.6 | 38.9 ± 0.4 | 40.0 ± 0.4 | 37.1 ± 0.5 |
| MCHC (g/ dL) | 70.2 ± 0.3 | 68.8 ± 0.7 | 70.8 ± 0.3 | 68.4 ± 0.4 |
| WBC ( x $10^9$ / L) | 6.7 ± 0.4 | 8.5 ± 0.6 | 6.8 ± 0.4 | 8.2 ± 0.4 |
| Granules ( x $10^9$ / L) | 0.5 ± 0.2 | 0.6 ± 0.1 | 0.5 ± 0.2 | 0.9 ± 0.3 |
| Lymphocytes(x$10^9$/ L) | 5.4 ± 0.2 | 6.9 ± 0.5 | 5.5 ± 0.2 | 6.4 ± 0.6 |
| Monocytes( x $10^9$ / L) | 0.8 ± 0.2 | 0.8 ± 0.2 | 0.7 ± 0.2 | 0.8 ± 0.1 |

Values for haematological parameters are expressed as mean ± SEM, (n=6/group), *p<0.05 compared with healthy control

Group 1: Healthy control group (Distilled water), Group 2: Treated group (100 mg/kg b. w., FAPL in DW), Group 3: Healthy control recovery group (DW), Group 4: FAPL Treated recovery group (100 mg/kg b. w., FAPL in DW)

## Conclusion

As the aqueous extract of *P. sarmentosa* leaves showed absence of acute, sub-acute and sub-chronic toxicity in Wistar rats, it is safe in using of short term as well as long term consumption. As aqueous extract of *P. sarmentosa* leaves has anti-inflammatory properties, it can be recommended as a safe treatment for chronic inflammatory disease conditions such as arthritis.

## References

[1] Ratnasooriya, W. D, Dharmasiri, M. G (1999) A water extract of leaves and stems of *Psychotria sarmentosa* has analgesic and antihyperalgesic activity in rats, *Medical Science Research*, 27: 715 – 718.

[2] Allan, J. J., Bhide, R. M., Agarwal, A. (2012) Safety assessment of Zigbir ®: A polyherbal formulation in Sprague-Dawley rats, *Journal of Toxicology*, volume 2012, Article ID 589520, http://dx.doi.org/10.1155/2012/589520

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