

**TOXICOPATHOLOGICAL EVALUATION OF TRADITIONAL MEDICINE  
PALAKALYANA GHRITHA IN SPRAGUE DAWLEY RATS**

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**ABSTRACT**

Palakalyana Ghrita is a polyherbal traditional medicine commonly used in Sri Lanka to revitalize and enhance reproductive functions of human. However there is no scientific evidence on toxic- pathological effects of this drug if any. Present study evaluated presence of any acute and subacute toxic effects of this drug in Sprague Dawley rats. In acute toxicity study, four groups of rats (n = 5 / group / sex) were orally treated with single dose of 0.25, 0.5, 2.0 and 4.0 g / kg body weight followed by 24 hr observation for signs of toxicity. Subacute toxicity test was performed by oral administration of doses of PKG 0.5, 2.0 and 4.0 g / kg body weight daily for 14 days. Any signs of toxicity, weight, basic haematological parameters, liver function tests, and histopathological evaluation of selected organs were performed on sub-acute treated and control groups. There was no toxic signs observed in the acute toxicity study compared to control group. Biochemical and hematological parameters were unaltered in both test and control rats of the sub-acute group. (P> 0.05). Moreover, two male rats treated with 4.0 g / kg, for 14 days showed renal tubular epithelial necrosis while rats subjected to other dosages did not reveal any organ impairment. As no significant changes of histopathological, biochemical or haematological parameters occurred in rats subjected to the dosage up to 2 g / kg / body weight of both genders, it can be used as the maximum non- toxic dose.

**KEYWORDS:** Acute toxicity, Palakalyana Ghrita, Sub- acute toxicity, Sprague Dawley.

**1. INTRODUCTION**

Herbal formulations are currently popular among the people in developed and developing countries as therapeutic agents in various ailments that impact the quality of life. People rely on herbal drugs considering their affordability, ancient background knowledge on medicinal plants, and a belief that they are harmless<sup>[1]</sup> Long term used of plant medicines people expect to have less toxicity. The recent researches have illustrated that many medicinal plants included in traditional formulas showed adverse effects.<sup>[2, 3]</sup> Therefore the data of the acute and sub -acute toxicity studies on herbal preparations should be obtained in order to enhance the confidence in their safety to humans, particularly for use in the development of pharmaceuticals.

Palakalyana Ghrita (PKG) a poly herbal formulation consists aqueous extract of *Asparagus resemosus* (roots) *Terminalia chebula* (fruits), *Terminalia bellirica* (fruits), *Phyllanthus emblica* (fruits), *Picrorhiza kurroa* (rhizomes), *Curcuma domestica* (rhizomes), *Rubia cordifolia* (roots), *Vitis vinifera* (fruits), *Nymphoides*

*crystata* (Flowers), *Santalum album* (wood), *Saussure lappa* (roots), *Glycyrrhiza glabra* (rhizomes), *Pterocarpus santalinus* (wood), *Sida cordifolia* (roots), *Ipomoea peniculata* (tubers), *Coscinium fenestratum* (stem), *Sacchrum officinarum* (stem), *Vernonia cineria* (whole plant), *Withania somnifera* (roots) and cow's ghee and cow's milk. Herbs included in PKG possess aphrodisiac, enhancing fertility, immunomodulatory, anti- ulcer and anti -anemic properties.<sup>[4, 5, 6, 7]</sup>

Fertility enhancement of PKG based on long term use by humans. Even though the formula is included in the Ayurveda pharmacopoeia of Sri Lanka<sup>[8]</sup> and Bhaishajja Ratnavali Yoniviyapath Chikithsa<sup>[9]</sup>, there is no data pertaining to the pharmacology and toxic effects of it's as for most other commonly used herbal remedies.<sup>[10]</sup> Efforts to elucidate health safety and efficacy of herbal medicaments should be intensified, especially in the current era where herbal medicine is gaining momentum even in non-traditional settings. Therefore present study was designed to investigate the acute and sub-acute

toxicity effects of PKG in Sprague Dawley rats in order to optimize its safe use.

## 2. EXPERIMENT

### 2.1. Preparation of the drug

All plant materials, pasteurized cow's milk and fresh cow's ghee were purchased from the Ayurveda pharmacy "Osudara" near to the Ayurveda teaching hospital, Borella, Sri Lanka. All plant materials were identified and authenticated by Dr.Sajeewa Harapathdeniya at Department of Materia Medica, Institute of Indigenous Medicine, University of Colombo Sri Lanka Concentrated water extract of *Asparagus racemosus* was prepared using 600 g of the chopped roots infused in 20 L water and boiled at 100 C° for 4-6 hours until reduced to 5 L of volume. Other plant materials were washed and shade dried to a constant weight and powdered separately by an electric grinder and passed through a 10 mesh sieve. 5 L of *Asparagus racemosus*, 5 L of pasteurized cow's milk, 1280 g of fresh cow's ghee and 15 g each of the powdered herbal ingredients were mixed together and was boiled until all the water evaporated. The preparation was stored in glass bottles at room temperature. PKG was prepared in the pharmacy affiliated to the Institute of Indigenous Medicine, University of Colombo, Sri Lanka.

### 2.2. Dosage of the drug

The clinical dose of PKG for humans is 10 g / day. The dose for the experimental animals was calculated by extrapolating the human dose to Sprague Dawley rats (900 mg / kg), based on the body surface area ratio of the standard table of Paget and Barnes.<sup>[11]</sup>

### 2.3 Experimental animals

Sprague Dawley (SD) rats of both sexes, aged 6-8 weeks, weighing 180 g  $\pm$  20 g were obtained from the animal house, Faculty of Medicine, University of Colombo, Sri Lanka. The rats were allowed to acclimatize for 7 days before commencement of the experiment. Male and virgin female rats were caged separately in the animal house, in wire cages on steamed wood shaving bedding at 25  $\pm$  2°C, 44 -56% humidity, with light and dark cycles of 12/12 h respectively. The rats had free access to water and food. They were made to fast 16-18 hours prior to administration of the first dose of PKG, though water was allowed. This experimental study was conducted after obtaining approval from the Ethical Research Committee at the Faculty of Medicine, University of Colombo, Sri Lanka (No: EC-11-158).

### 2.4 Acute toxicity

Experiment to determine the oral median lethal dose (LD<sub>50</sub>) of the PKG were carried out using the method described by Miller and Tainter in 1944.<sup>[12]</sup> Animals randomly divided in five groups of male and five groups of female (n=5 /group). The first group (control group) received orally distilled water. Groups 2, 3, 4, and 5 were orally administrated the drug in doses of 0.25, 0.5, 2.0 and 4.0 g / kg / body weight (b.wt), respectively.

Animals were observed continuously for 4 to 24 hours after administration of PKG for skin changes, food and water intake, morbidity and mortality.

### 2.5 Subacute toxicity study

The sub-acute toxicity study was conducted according to the Organization for Economic Co-operation and Development (OECD) guidelines with slight modification.<sup>[13]</sup> Four groups of Sprague Dawley rats (n=5 / group / sex), caged separately and received PKG orally by oral gavage at single doses of 0.5, 2.0 and 4.0 g / kg /b.wt for 14 consecutive days, while control group received distilled water. The toxic manifestations and mortality were monitored once a day throughout the treatment period. The body weight of each rat was recorded before commencement of dosing, and on the every 7<sup>th</sup> day.

### 2.6 Biochemical and hematological evaluation of sub-acute toxicity

After the 14 days, all animals were fasted overnight and were anaesthetized by using diethyl ether vapor in a chamber to collect blood for biochemical and hematological studies. Non-heparinized blood was collected and centrifuged at 3000 rpm for 10 minutes. Serum samples were then aspirated off for measurement of ALT, AST, ALP, GGT, Total Bilirubin and LDH. These biochemical tests were performed by Vital Micro lab 300 semi auto analyzer, using commercially available kits.[ALT (AS 1268), AST (AL 1268), ALP (AP307), GGT (GT2750), Total Bilirubin (BR 411) and LDH (LC2389) manufactured by Randox laboratories Ltd, United Kingdom. Heparinized blood samples were taken for determining RBC, Hb, MCV, MCHC, MCH, HCT, WBC/DC and PLT. Hematological analyses were performed by Sysmex Automated hematology analyzer using commercially available kits manufactured by Human GmbH, 21-65205 Wiesbaden-Germany.

### 2.8 Necropsy study of sub-acute toxicity

Animals were sacrificed using an overdose of ether on day 15<sup>th</sup>. The internal organs were dissected and were compared in the control and treated groups for gross lesions. Representative samples of liver, lungs, heart, kidneys, Adrenal gland, stomach, esophagus, pancreas, small intestines, large intestines, uterus, ovaries / testis, epididymis, and spleen were weighted individually and preserved in 10% neutral buffered formalin solution. After routine processing the paraffin sections of each tissue were cut at a thickness of 5 $\mu$ m, mounted on glass slides and stained with haematoxylin and eosin for histological examination.

### 2.9 Statistical analysis

The values were expressed as mean  $\pm$  SD. Statistical analysis was performed by one way analysis of variance (ANOVA) followed by the Turkey test. A probability level of less than 5% was considered as significant.

### 3. RESULTS AND DISCUSSION

This study was designed to assess the safety evaluation of PKG, a polyherbal traditional medicament in Sprague Dawley rats. No mortality or hazardous signs were observed after administrating PKG at a range of doses including the high dose of 4.0 g / kg / b.wt during the acute toxicity period. Thus, LD<sub>50</sub> of PKG is considered to be over 4.0 g / kg/ b.wt in both genders.

During the subacute toxicity period all animals showed normal body weight gain and general behavior pattern throughout the treatment period as compared to the control (Data not shown). One animal died in the 4.0 g / kg/b.wt on day 1<sup>st</sup> while another one died in same group on day 2<sup>nd</sup> during the experiment. The death of these animals due to gavage accidents as the behavior pattern and food intake of these animals before death did not

show any changes when compared to other animals in the same group.

Liver and kidney are target organs to evaluate toxicity due to drugs or plant extracts. Elevation of AST, ALT and ALP levels in blood are indicated damage in liver cells. There were no significant alterations of liver function tests or tissue damage in the treated male and female rats compared to the control groups shown in **Table 1 and Table 2.** ( $P > 0.05$ ). LDH is an enzyme especially concentrated in the heart, liver, red blood cells, kidneys, muscles, brain, and lungs<sup>[14,15]</sup> LDH level is most often measured to check for damage to heart, red blood cells, kidneys, muscles, brain, and lungs. As the LDH levels also did not alter in the treated rats compared to the control rats.

**Table 1: Serum biochemical findings of male rats treated with PKG in sub-acute toxicity study.**

Parameters	control	0.5 g / kg	2.0 g / kg	4.0 g / kg
AST (IU/L)	151.7 ± 18.4	156.7± 19.6	163.2 ± 21.9	152.3±15.08
ALT (IU/L)	189.6 ± 12.1	189.4±14.2	186.5 ± 7.9	188.7±13.6
GGT(U/L)	27.07 ± 2.2	26.6 ± 3.9	26.5 ± 3.6	26.6 ± 2.8
LDH (U/L)	641.6 ± 46.9	711.6 ± 73.1	628.7± 124.3	661.6 ± 39.4
Total BIL (mmol/l)	1.41 ± 0.34	0.75 ± 0.55	1.36 ± 0.27	11.95 ± 0.70
ALP (IU/L)	694.4 ± 22.07	612.3 ± 37.1	587.3 ± 103.3	580.1± 64.4

**Table 2: Serum biochemical findings of female rats treated with PKG in sub-acute toxicity study.**

Parameters	control	0.5 g / kg	2.0 g / kg	4.0 g / kg
AST (IU/L)	146.3 ± 28.7	124.2 ± 4.3	144.6 ± 5.2	140.4± 4.2
ALT (IU/L)	185.6±13.1	190.3±15.1	188.04±14.3	190.8 ± 6.1
GGT(U/L)	27.4± 4.5	25.5± 4.7	28.8± 5.2	24.7± 3.5
LDH (U/L)	604.7 ± 111.5	617.0± 116.1	617.4± 62.3	632.2 ± 87.1
Total BIL (mmol/l)	1.22± 0.46	0.83± 0.61	1.26± 0.45	1.56± 0.23
ALP (IU/L)	631.8±80.4	614.1± 68.5	600.2±39.6	588.3± 94.9

**Abbreviations:** AST- Aspartate Aminotransferase, ALT- Alanine Aminotransferase, ALP-Alkaline Phosphatase, LDH-lactate dehydrogenase, BIL -Total Bilirubin, GGT- Gamma Glutamic Transferase.

**Table 3 and Table 4** showed that there was significant elevations of the HCT, MCHC and PLT in the all groups of rats ( $P < 0.05$ ) when compared to the control groups while other hematological values were not significantly affected. . However these changes still remained within the reference range.<sup>[16]</sup> Hematological parameters are also valuable diagnostic tools in the assessment of toxic effects in chemicals. This is because the bone marrow which produces most of the blood cells is vulnerable to toxic damage by chemicals. It has been shown that many medicinal plants consisting PKG possess high total antioxidant which are good in promoting positive health. Additionally there was no change in the lymphocyte, eosinophil and neutrophil counts. This may indicate that PKG does not induce an acute inflammatory reaction as neutrophils are usually elevated in acute inflammatory processes. A significant immune reaction and allergic reactions are also unlikely as no significant changes were detected in the lymphocyte and eosinophil counts. As the RBC, Hb and WBC/DC were not significantly modified

and the PLT still remained within the normal range in treated rats, it could also be assumed that PKG does not have any significant effect on the bone marrow production of RBC, WBC, PLT, in their destruction as well as haemoglobinization of RBC. As the biochemical parameters assessed for hepatotoxicity were also unaltered together with histologically unaffected liver tissue seen in the sub-acute toxicity test, this may imply lack of hepatotoxicity and bone marrow toxicity of PKG in the dosages used in the sub-acute setting (dosing for 14 days) in Sprague Dawley rats.

Histopathological assay of organs which are lungs, heart, liver, adrenal gland, stomach, esophagus, pancreas, small intestines, large intestines, uterus, ovaries or testis, epididymis, and spleen lacked light microscopically detectable toxic changes in all rats treated with different doses of PKG in the sub-acute toxicity test. Acute tubule necrosis involves damage to the tubular epithelial cells of the kidney. This could occur due to lack of oxygen to the

kidney tissues (ischemia) or due to poisons or toxins which are filtered from blood by the glomeruli. These filtered poisons or toxins cause damage to lining epithelial cells of the renal tubules. Each proximal tubule reabsorbs 80% of the sodium, water, chloride and 40% of the filtered urea.<sup>[17,18]</sup> Every proximal tubule has three segments, with the last segment the most vulnerable to xenobiotic (toxic foreign substance) exposures.<sup>[19]</sup> In this study early renal tubular epithelial cell damage with dissolved nuclei (karyolysis) was observed in two male rats included in the group treated with PKG 4 g / kg / b.wt daily for 14 days in the sub-acute toxicity test. This observation could have been further supported if biochemical evaluation of renal function tests were performed, which however was not done in this study. Therefore further study is necessary to confirm the renal toxic effects at a PKG dose of 4 g / kg / b.wt daily for 14 days in rats. To the best of our knowledge there is no documentation regarding different plant material

included in PKG as being poisonous to the kidney. *Asparagus recemosus* is the main ingredient in PKG. Several studies have reported the antioxidant activity of *Asparagus recemosus* and other plants included in PKG.<sup>[20]</sup> Many of the drugs used in this formulation are reported to possess cardio protective, hepato protective, and immune modulatory effect; viz., *Terminalia chebula*<sup>[21,22]</sup>, *Terminalia bellirica*<sup>[23]</sup>, *Phyllanthus emblica*<sup>[24]</sup>, *Rubia cordifolia*<sup>[25]</sup>, *Glycyrrhiza glabra*<sup>[26]</sup>, *Curcuma domestica*<sup>[27,28]</sup>, *Picrorhiza kurroa*<sup>[29]</sup>, *Saussure lappa*<sup>[30,31]</sup>, *Pterocarpus santalinus*<sup>[32]</sup>, *Ipomoea peniculata*<sup>[33]</sup>, *Sida cordifolia*<sup>[34]</sup>, *Vernonina cineria*<sup>[35,36]</sup> and *Withania somnifera*.<sup>[37,38]</sup> Animal research has shown that the antibiotic and diuretic effects of the asparagus root may help to flush out the urinary tract during inflammation and infections.<sup>[39, 40]</sup> However further study is necessary to confirm the actual ingredient/s of the PKG toxic to renal tubular epithelial cells.

**Table 3: Hematological findings of male rats treated with PKG in sub-acute toxicity study.**

Parameters	Control	0.5g/kg	2.0 g/kg	4.0 g / kg
RBC (M/ul)	6.9±0.7	7.0±0.4	5.9±0.8	6.3±1.2
Hb(g/dl)	12.8±1.1	12.5±3.5	11.5±1.0	12.7±5.6
MCV (fl)	52.8±3.3	54.9±2.2	53.5±3.1	54.1±2.5
MCH (pg.)	18.2±1.1	15.4±0.6	16.5±1.1	17.4±0.5
WBC (K/ul)	12.51±1.3	12.54±1.5	12.8±0.6	12.6±0.7
HCT%	41.5±2.2	45.8±6.3	44.8± 5.2	47.2±2.7
MCHC(g/dl)	34.5±2.2	38.4±0.3	38.8± 1.8	38.6±1.6
PLT(10 <sup>2</sup> /L)	680.50±1.5	959.3±139.4	938.2±127.3	1001.5±65.0
Neutrophils (%)	27.8±9.2	25.5±7.3	26.3±12.4	26.5±5.8
Lymphocytes (%)	62.0±7.3	70.3±2.3	73.6±11.5	73.2±5.2
Eosinophils (%)	3.2±0.15	3.3±0.22	2.9±0.42	3.3±0.21
Monocytes (%)	1.12±0.02	1.2±0.2	1.3±0.12	1.22±0.02
Basophils (%)	0	0	0	0

#### Abbreviations

WBC -White blood cell, RBC -Red blood cell, HGB –Hemoglobin, MCV- Mean Corpuscular volume and MCH -mean hemoglobin volume.

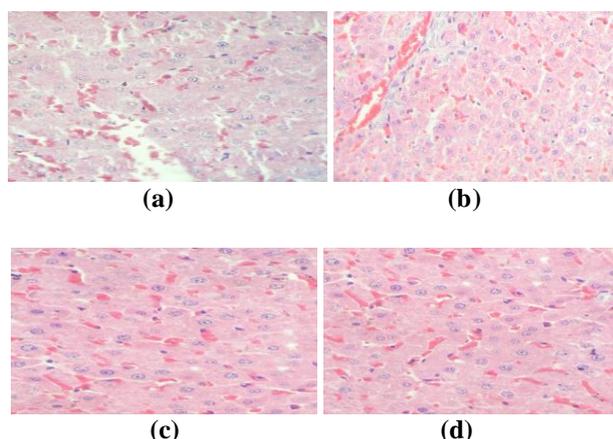
**Table 4: Hematological findings of female rats treated with PKG in sub-acute toxicity.**

Parameter	Control	0.5 g /kg	2.0 g/kg	4.0 g / kg
RBC (M/ul)	5.4±0.5	6.2±0.3	5.6.0±0.2	5.1±0.5
Hb(g/dl)	10.2±1.1	10.5±3.2	11.5±1.2	11.7±4.6
MCV (fl)	51.23±4.2	52.63±1.4	50.14±5.3	53.25±2.3
MCH (pg.)	14.5±2.1	14.2±1.3	13.5±1.1	12.5±0.5
HCT (%)	40.4±3.2	44.3±4.2	45.2±3.2	44.1±2.3
MCHC (g/dl)	34.6±0.2	36.1±0.2	37.4±0.5	37.2±0.7
PLT (10 <sup>3</sup> /L)	681.9±12.1	942.2±128.9	990.6±101.9	984.8±90.9
WBC (K/ul)	12.5±2.1	11.21±1.3	11.65±0.4	12.41±0.3
Neutrophils (%)	26.8±4.1	26.5±5.3	25.3±11.8	26.6±4.7
Lymphocytes (%)	61.0±4.5	62.3±6.3	63.6±9.5	63.2±4.6
Eosinophils (%)	2.5±0.14	3.6±0.14	2.7±0.31	3.2±0.15
Monocytes (%)	1.21±0.02	1.2±0.2	1.3±0.12	1.22±0.02
Basophils (%)	0	0	0	0

#### Abbreviations

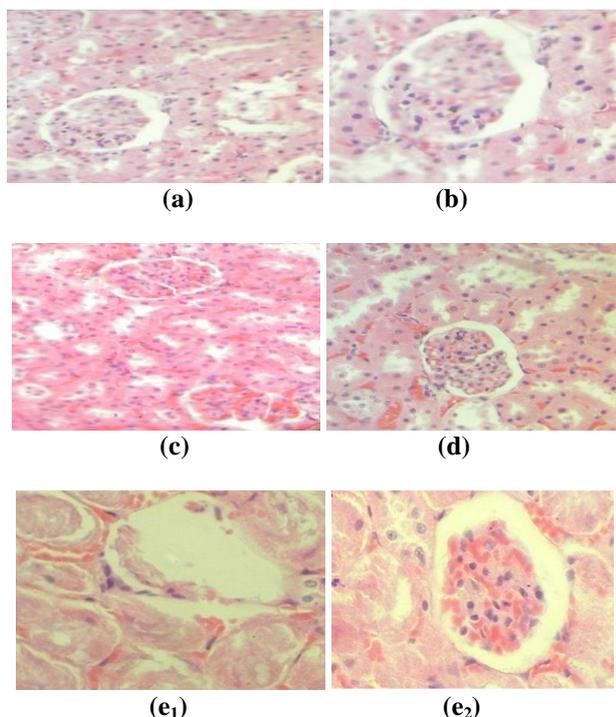
WBC -White blood cell, RBC -Red blood cell, HGB –Hemoglobin, MCV- Mean Corpuscular volume and MCH -mean hemoglobin volume.

Histological examination of organs other than renal tissue, of rats that belonged groups dosed with 0.5, 2 and 4 g / kg / BW of PKG did not show any abnormality attributable to the treatment.



**Figure 1: Histopathological Images of Liver tissues of control and PKG treated Sprague Dawley rats in the sub-acute toxicity.**

Liver section of a rat in the control group (a), Liver section of a rat treated with 0.5g/kg/BW of PKG (b). Liver section of a rat treated with 2.0 g/kg/BW of PKG(c). Liver section of a rat treated with 4.0 g / kg /BW of PKG (d). **Stained by Haematoxylin and eosin sections at 40x magnification.**



**Fig 2: Histopathological images of renal tissues of control and PKG treated Sprague Dawley rats in the sub-acute toxicity.**

Preserved glomeruli and tubules of a rat in the control group (a) Preserved glomeruli and tubules of a rat treated with 0.5 g/kg/BW of PKG (b). Preserved glomeruli and

tubules of a rat treated with 2 g/kg/BW of PKG(c). Preserved glomeruli and tubules of a rat treated with 4.0 g/kg/BW of PKG (d). (e<sub>1</sub>) and (e<sub>2</sub>) are preserved glomeruli and tubules of two rats treated with 4 g /kg/BW of PKG out of the twelve surviving rats. Note tubules lined by swollen epithelial cells with absent nuclei indicating tubular damage. **(Haematoxylin and eosin stained sections at 40x magnification)**

### CONCLUSION

The LD<sub>50</sub> value for hepatotoxic effects of PKG is greater than 4 g / kg / b.wt per 24 hours for Sprague Dawley rats. Furthermore doses of PKG consumed up to 2 g / k g / b.wt daily for 14 days are safe, as it did not cause mortality or morbidity, alter selected biochemical and hematological parameters, or produce any light microscopically detectable toxic effects on selected body organs of both genders under the present experimental conditions. Further study coupled with biochemical renal parameters are necessary to confirm possible renal toxic effects of PKG at higher doses (4g / kg / b.wt/daily) in the rat model studied.

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