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*Full Length Research Paper*

# Acute and sub-chronic oral toxicity studies of the extracts from herbs in Phikud Navakot

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**This study presents data from oral acute and sub-chronic (90-day) toxicity effect of the extracts from herbs in Phikud Navakot (PN) which is commonly used in Thai traditional medicine for alleviation of the clinical sign of hyperlipidemia, cardiovascular diseases, and cerebrovascular diseases. The single and repeated dose of the extracts were administered to Sprague Dawley rats as described in the OECD code 420 guideline for acute test and OECD code 408 guideline for sub-chronic test respectively. The variables included were body weights; feed consumption, organ weights, hematology and blood clinical chemistry, and histopathology were performed. Acute toxicity test revealed that, the limit dose of 2,000 mg/kg did not cause any mortality or symptoms of toxicity in all rats during the observation period. In the sub-chronic tests, the results did not show any treatment–related effect of toxicity. Therefore, our obtained results suggest that PN is a relatively nontoxic herb for single and repeated oral administration. However, the contraindication of the usage of PN is related to the high levels of uric acid during and after a high dose oral administration.**

**Key words:** Phikud Navakot, oral, acute and sub-chronic toxicity, Sprague Dawley rat.

## INTRODUCTION

Herbal prescriptions and natural products are commonly used for treatment of various diseases in developing countries particularly in Thailand. According to World Health Organization (WHO), it has recommended the use of herbal drugs as an alternative medicine, because almost 80% of the world's population uses medicine from herbal origin for primary health care (Calixto et al., 2000). Like Phikud Navakot (PN) which is composed of nine Thai herbal plant species, *Saussurea lappa*: anti-metastatic agent for prostate cancer (Kim et al., 2012), *Anacyclus pyrethrum*: anticholinesterase effect to ameliorate seizures (Pahuja et al., 2011), *Picrorhiza kurroa*: anti-hyperlipidemia effect (Shetty et al., 2010), *Atractylodes lancea*: increase peristalsis effect to improve the delayed gastric emptying (Nakai et al., 2003), *Artemisia annu* (Efferth et al., 2011), *Terminalia chebula*: inhibitory effect to cancer cells (Kim et al., 2011), *Angelica sinensis*: cardio-protective effect to alleviate cardiovascular diseases (Xin et al., 2007), *Angelica dahurica*: anti-asthmatic effect to alleviate respiratory diseases (Lee et al., 2011), and *Ligusticum sinense*: hypoglycemic effect to alleviate diabetes mellitus (Ignjatovic et al., 2000). National Public Health Ministry of Thailand registered PN in the list of herbal medical product. However, the purified extraction of this herb has not been investigated by toxicity testing yet. To identify the oral toxicity of PN, the acute single dose and sub-chronic repeated dose toxicity of its aqueous extracts was evaluated in Sprague Dawley rat followed by OECD code 420 (OECD, 2001) and 408 (OECD, 1998) guideline respectively.

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**Abbreviation: PN,** Phikud Navakot

## MATERIALS AND METHODS

### Animal husbandry

Animal studies were performed in accordance with the Mahidol

University policy for the care and use of animals for scientific purposes and approved by the institutional animal ethics committee. Healthy young adult eight weeks old Sprague Dawley rats from the National Laboratory Animal Center (NLAC), Mahidol University, Thailand were used. The animal housing environment was controlled by heating, ventilating and air conditioning (HVAC) system to achieve 23±2°C, 55±15% relative humidity, 10 to 15 air change per hour ventilation, 12:12 h of dark and light cycle and provided pasteurization standard diet and 7 to 10 ml chlorinated water *ad libitum*. All rats passed the acclimatization period prior to being used for the test.

#### Test substance

The extracts of PN were kindly provided by the Associate Professor Dr. Uthai Sotanaphun, Department of Pharmacognosy, Faculty of Pharmacy, Silpakorn University, Nakorn Pathom, Thailand. The extraction and preparation were adapted from Farnsworth and Bunyapraphatsara, (1992) briefly as following. Raw materials of nine herbs in PN regimen were mixed and ground into powder. The powder was immersed in ten times volume of 80% ethanol overnight, then boiled for 3 h and filtered to remove the residue. Next, the aqueous extracts were repeatedly boiled for 3 h and filtrated two times. The aqueous extracts were spray dried to remove trace solvent. The percentage yield of the crude extract was roughly 20 to 25% of the raw material.

#### Oral acute and sub-chronic toxicity test

A total number of 121 rats were randomly selected for the studies, six rats for acute test, 110 rats for sub-chronic test, and five rats used as sentinel animal to indicate environmental status in long term study. In the acute test (OECD, 2001), the limit test at dose level of 2,000 mg/kg was administered to three fasted female rats with duplicated group and then observed individually 0.5, 4, 8, 12, and 24 h post-dosing, and at least once daily for 14 days. In the sub-chronic test (OECD, 1998), a group of rats (10 females and 10 males of each dose) were administered daily oral doses of 10, 100, 1,000 mg/kg for 90 days, and 1,000 mg/kg for 90 days with 14 days recovery period (satellite group) while the control group received water at the same volume of the tested extracts with carefully observed clinical sign daily individually. In both studies, individual weights of animals and feed consumption were measured daily.

#### Mortality and clinical sign

During the period of the studies, all rats were observed daily for mortality and clinical sign of toxicity for example, changes in skin, fur, eyes, mucous membranes, occurrence of secretions and excretions, autonomic activity, changes in gait, posture and response to handling, as well as the presence of clonic or tonic movements, stereotypes or bizarre behavior (OECD, 1998; Jaijoy et al., 2010).

#### Hematological and blood clinical chemistry

At the end of the test, 14 days for acute test, surviving rats were euthanized by over dose inhalation of $CO_2$. Whereas 90 days for sub-chronic test, surviving fasted rats were anesthetized by $CO_2$ inhalation. Blood samples were collected by cardiac puncture and rats were sacrificed by exsanguinations. Hematological and blood clinical chemistry were performed by an ABBOTT CELL-DYN® 3500 system (ABBOTT Laboratories, IL, USA) and a Hitachi 902 automated blood analyzer (Hitachi Science Systems Ltd., Ibaraki, Japan), respectively.

#### Relative organ weight and histopathology

All tested rats were subjected to gross necropsy. All gross pathological changes were microscopically examined as described by Jaijoy (2010). In sub-chronic test, heart, kidney, spleen, liver and lung were removed and weighed to identify relative organ weights. Then, all organs were fixed in 10% neutral buffer formalin (NBF). Fixed specimens were processed routinely and embedded in paraffin wax. Sections (4 µm) were mounted on the normal glass slides for staining by haematoxylin and eosin (H&E).

#### Statistical analysis

Quantitative results were expressed as mean ± standard deviation. Data were statistically analyzed with IBM® SPSS® Statistic software version 20, using a one way analysis of variance (ANOVA) followed by Levene's test. To differentiate the difference between groups, the multiple comparison Bonferroni and Dunnett tests were performed for equal and non-equal variance assumption respectively.

## RESULTS

### Survival and clinical sign

There were no treatment-related mortality and clinical signs of toxicity in both acute and sub-chronic studies. Treated and concurrent control group were similar in clinical manifestation.

### Body weights and feed consumption

Table 1 presents the group of mean absolute body weights and feed consumption versus time in acute test. During the period of the study, the rat body weights were normally increased depending on age. At the end of the studies, the percentage of weight gain was 37.9 in male rats and 24.9 in female rats. Tables 2 and 3 present the group of mean absolute body weights and feed consumption versus time in the sub-chronic test in both male and female. For mean absolute body weights, both group of male and female rats administered 10, 100, and 1,000 mg/kg were similar to concurrent control except for few weeks at 100 mg/kg dosing in male that significantly tended to be lower than the concurrent control. However, there was no observed dose responsive to body weight changes in both sexes. Feed consumption data were variable (Tables 4 and 5), especially in male administered 100 and 1,000 mg/kg that significantly tended to be lower than the concurrent control, while females administered 1,000 mg/kg significantly tended to be higher than the concurrent control.

### Organ weights

Table 6 presents a group of mean relative organ weights

**Table 1.** Sprague Dawley mean absolute body weights (g) and feed consumption (g) in acute oral toxicity test.

| Day | Body weight ( $\bar{x}$ ±SD) | | Feed consumption ( $\bar{x}$ ±SD) | |
|---|---|---|---|---|
| | Male | Female | Male | Female |
| 0 | 255.6±5.2 | 193.0±11.4 | - | - |
| 1 | 258.4±8.4 | 201.4±8.5 | 23.8±1.1 | 18.8±2.2 |
| 2 | 246.2±7.8 | 194.0±8.7 | 23.8±4.6 | 16.4±0.9 |
| 3 | 273.8±8.5 | 210.4±7.5 | 21.6±3.6 | 15.6±1.7 |
| 10 | 330.0±14.8 | 229.6±5.8 | 23.2±1.9 | 16.0±1.0 |
| 14 | 352.6±16.2 | 241.2±6.3 | - | - |

**Table 2**. Male Sprague Dawley mean absolute body weights (g) in sub-chronic oral toxicity test.

| Week | Group ( $\bar{x}$ ±SD) | | | | |
|---|---|---|---|---|---|
| | Control | 10 mg/kg | 100 mg/kg | 1000 mg/kg | Satellite |
| 0 | 224.40±5.40 | 224.60±4.55 | 224.30±6.53 | 224.40±5.25 | 224.40±5.68 |
| 1 | 277.60±7.43 | 282.40±12.90 | 282.00±12.75 | 286.10±9.16 | 280.90±16.57 |
| 2 | 338.90±17.03 | 345.40±15.03 | 329.60±14.95 | 341.70±17.85 | 334.10±23.17 |
| 3 | 377.70±24.70 | 388.60±18.36 | 359.50±15.95 | 377.40±23.62 | 367.10±24.36 |
| 4 | 415.20±31.48 | 423.60±22.94 | 383.50±18.06 | 406.30±27.61 | 393.50±29.98 |
| 5 | 445.10±34.35 | 446.80±24.38 | 404.70±18.81[a] | 426.40±31.13 | 409.80±32.00 |
| 6 | 467.70±37.75 | 468.10±25.98 | 419.50±17.84[a] | 442.20±32.01 | 429.30±35.35[a] |
| 7 | 478.20±41.25 | 482.30±27.67 | 429.80±19.96[a] | 452.40±31.75 | 436.00±38.64[a] |
| 8 | 483.60±42.17 | 489.10±25.05 | 445.40±21.10 | 466.10±33.52 | 453.10±40.23 |
| 9 | 504.80±43.19 | 511.20±27.88 | 460.50±22.02[a] | 482.40±33.76 | 466.30±40.35 |
| 10 | 520.70±43.21 | 526.40±30.83 | 472.10±24.66[a] | 495.20±35.57 | 479.00±42.09 |
| 11 | 526.30±45.03 | 536.90±34.28 | 480.40±26.27 | 500.00±36.57 | 488.33±41.67 |
| 12 | 537.60±47.62 | 550.10±34.53 | 491.90±26.10 | 509.90±37.58 | 502.11±44.59 |
| 13 | 543.40±47.19 | 553.30±36.30 | 494.70±25.57 | 511.89±38.86 | 507.33±48.49 |
| 14 | - | - | - | - | 512.22±47.85 |
| 15 | - | - | - | - | 520.11±48.72 |

[a]Significantly different to control ($p<0.01$).

for male and female rats. Both concurrent control and treated rats did not show any significant difference in mean relative organ weights of lung, heart, spleen, liver and kidney.

### Histopathology

No treatment-related gross pathological changes were found in the heart, kidney, spleen, liver and lung of the rats at the dose levels tested. The incidence for histopathological finding was similar in both concurrent control and treated rats as shown in Figure 1.

### Hematology and clinical chemistry

In both sexes of sub-chronic studies, group of mean hematological parameters and blood clinical chemistry parameters are shown in Tables 7 and 8. All of the hematological parameters in both concurrent control and treated rats were similar. However, few clinical chemistry parameters in treated rat were significantly higher than concurrent control. Blood urea nitrogen (only in male treated rats), uric acid, total protein and globulin at high dose significantly tended to be higher than low dose and concurrent control. Triglyceride, aspartase tranferase (AST) and alanine tranferase (ALP) were variable. After recovery from dosing, in each satellite group was found that blood glucose and uric acid significantly tended to be higher than the concurrent control.

## DISCUSSION

PN is the combination of nine Thai herbal plant species

**Table 3.** Female Sprague Dawley mean absolute body weights (g) in sub-chronic oral toxicity test.

| Week | Group ( $\bar{x}$ ±SD) | | | | |
|---|---|---|---|---|---|
| | Control | 10 mg/kg | 100 mg/kg | 1000 mg/kg | Satellite |
| 0 | 186.80±8.18 | 185.40±8.17 | 186.50±6.19 | 186.40±7.86 | 186.10±6.17 |
| 1 | 214.60±12.86 | 212.00±15.64 | 211.60±10.86 | 213.80±8.90 | 211.10±10.26 |
| 2 | 233.30±8.27 | 227.56±11.30 | 232.80±8.40 | 235.40±14.42 | 229.60±9.50 |
| 3 | 250.40±9.01 | 238.67±15.13 | 244.90±11.17 | 252.20±14.60 | 248.10±12.66 |
| 4 | 259.90±8.66 | 251.33±12.11 | 256.50±12.88 | 264.30±17.67 | 257.10±9.61 |
| 5 | 269.10±9.62 | 260.11±12.04 | 263.00±13.87 | 272.80±16.75 | 263.90±9.21 |
| 6 | 276.40±9.62 | 269.00±11.54 | 273.10±13.44 | 279.50±18.92 | 271.60±11.96 |
| 7 | 284.20±12.82 | 266.33±12.67 | 275.50±14.68 | 284.50±22.67 | 276.70±9.86 |
| 8 | 284.20±9.27 | 270.67±11.49 | 276.50±15.67 | 285.60±18.89 | 282.20±16.16 |
| 9 | 287.70±8.91 | 276.44±12.97 | 282.50±14.90 | 294.00±18.78 | 285.50±12.04 |
| 10 | 292.80±10.34 | 279.89±12.25 | 286.40±13.61 | 297.67±18.81 | 289.00±13.63 |
| 11 | 291.70±8.78 | 281.11±12.54 | 287.40±14.27 | 297.22±22.07 | 288.80±11.55 |
| 12 | 299.00±7.12 | 287.22±13.90 | 296.20±13.69 | 305.89±21.76 | 295.40±14.03 |
| 13 | 298.70±10.86 | 294.33±16.44 | 297.90±13.74 | 311.33±27.21 | 296.90±14.96 |
| 14 | - | - | - | - | 300.20±13.77 |
| 15 | - | - | - | - | 304.00±16.38 |

**Table 4**. Male Sprague Dawley mean feed consumption (g) in sub-chronic oral toxicity test.

| Week | Group ( $\bar{x}$ ±SD) | | | | |
|---|---|---|---|---|---|
| | Control | 10 mg/kg | 100 mg/kg | 1000 mg/kg | Satellite |
| 0 | 23.2±0.7 | 23.6±0.8 | 23.2±2.3 | 22.9±1.2 | 21.1±0.9[a] |
| 1 | 28.7±1.1 | 25.4±2.7 | 20.3±1.8[a] | 23.3±2.7[a] | 21.2±1.6[a] |
| 2 | 29.1±3.0 | 26.7±3.8 | 21.5±3.6[a] | 23.5±1.4[a] | 22.5±2.0[a] |
| 3 | 28.3±3.5 | 24.0±2.8 | 18.2±1.4[a] | 20.0±1.4[a] | 18.8±1.9[a] |
| 4 | 23.6±4.0 | 21.9±2.2 | 20.1±1.7[a] | 18.5±2.1[a] | 18.5±3.3[a] |
| 5 | 20.0±1.7 | 21.2±1.7 | 19.6±1.7 | 19.9±1.2 | 19.6±2.4 |
| 6 | 20.5±1.7 | 22.0±1.4 | 18.3±1.0[a] | 18.6±1.8 | 19.4±2.4 |
| 7 | 19.6±0.9 | 21.8±1.6 | 20.8±1.1 | 20.0±0.9 | 19.6±3.3 |
| 8 | 27.3±3.4 | 23.7±2.2 | 20.9±2.3 [a] | 21.2±2.4[a] | 21.4±1.8[a] |
| 9 | 22.1±2.5 | 24.4±4.8 | 20.2±0.8 | 21.8±1.4 | 20.7±2.3 |
| 10 | 21.2±1.5 | 24.6±3.5[a] | 22.2±1.0 | 21.7±2.0 | 21.5±2.5 |
| 11 | 21.6±2.1 | 23.6±2.0 | 22.3±1.6 | 21.5±2.6 | 22.9±2.1 |
| 12 | 21.3±1.4 | 22.2±1.4 | 21.2±1.4 | 21.8±1.7 | 21.9±3.0 |
| 13 | 20.0±0.9 | 19.5±2.8 | 20.4±1.2 | 19.4±2.1 | 21.0±1.9 |
| 14 | - | - | - | - | 19.4±5.5 |
| 15 | - | - | - | - | 18.0±1.4 |

[a]Significantly different to control ($p<0.01$).

which are normally prescribed as Thai traditional medicine to relieve several kinds of symptoms in many diseases, especially cardiovascular and respiratory diseases (Ignjatovic et al., 2000; Nakai et al., 2003; Xin et al., 2007; Shetty et al., 2010; Efferth et al., 2011; Kim et al., 2011; Lee et al., 2011; Pahuja et al., 2011; Kim et al., 2012). However, there is no report related to PN in terms of toxicological studies and its combination effect. The present work evaluates the acute and sub-chronic toxicity of the extracts from nine herbs in PN. In acute toxicity test, no adverse effect was observed. All animals treated survived beyond 14 days observation period. Therefore, it can be suggested that the dose 2,000 to ∞ mg/kg is safe for acute oral toxicity test.

**Table 5.** Female Sprague Dawley mean feed consumption (g) in sub-chronic oral toxicity test.

| Week | Group ( $\bar{x}$ ±SD) | | | | |
|---|---|---|---|---|---|
| | Control | 10 mg/kg | 100 mg/kg | 1000 mg/kg | Satellite |
| 0 | 16.5±0.8 | 15.1±1.5 | 16.0±1.5 | 17.9±1.2 | 16.5±1.9 |
| 1 | 15.7±2.3 | 14.8±1.9 | 14.2±2.7 | 14.9±1.2 | 14.6±0.6 |
| 2 | 15.7±2.8 | 15.3±0.8 | 15.2±1.5 | 15.7±1.5 | 16.2±1.0 |
| 3 | 13.2±0.4 | 13.1±1.9 | 13.3±1.5 | 14.7±1.5 | 13.2±0.6 |
| 4 | 15.2±1.1 | 14.9±1.8 | 14.7±1.6 | 16.1±2.4 | 14.2±1.1 |
| 5 | 14.9±1.0 | 14.6±1.6 | 13.9±1.2 | 14.4±1.0 | 13.7±1.0 |
| 6 | 15.1±0.7 | 14.2±1.5 | 14.0±1.8 | 14.3±1.8 | 13.4±0.9[a] |
| 7 | 15.1±2.5 | 14.8±1.7 | 15.2±1.0 | 15.2±1.0 | 15.6±0.5 |
| 8 | 13.8±1.5 | 14.7±1.8 | 14.4±1.2 | 15.4±0.8[a] | 14.1±1.3 |
| 9 | 15.3±2.0 | 14.9±2.1 | 14.5±2.4 | 16.9±1.1 | 15.4±0.5 |
| 10 | 16.6±1.5 | 16.1±1.3 | 14.2±2.3 | 14.7±3.4 | 16.2±0.9 |
| 11 | 15.9±1.3 | 16.1±1.6 | 17.6±1.7 | 19.6±2.5[a] | 16.9±1.1 |
| 12 | 17.0±1.2 | 16.1±1.9 | 17.1±2.0 | 15.3±1.9 | 15.1±1.2 |
| 13 | 14.6±2.0 | 15.1±1.9 | 15.3±1.8 | 17.1±1.7[a] | 14.8±0.8 |
| 14 | - | - | - | - | 15.7±1.5 |
| 15 | - | - | - | - | 12.8±0.9 |

[a]Significantly different to control ($p<0.01$).

**Table 6.** Sprague Dawley mean relative organ weights (g) in sub-chronic oral toxicity test.

| Group | Heart ( $\bar{x}$ ± SD) | Lung ( $\bar{x}$ ± SD) | Liver ( $\bar{x}$ ± SD) | Kidney R ( $\bar{x}$ ± SD) | Kidney L ( $\bar{x}$ ± SD) | Spleen ( $\bar{x}$ ± SD) |
|---|---|---|---|---|---|---|
| **Male** | | | | | | |
| Control | 0.32±0.33 | 0.30±0.33 | 2.34±1.62 | 0.26±0.18 | 0.24±0.17 | 0.20±0.02 |
| 10 mg/kg | 0.32±0.01 | 0.29±0.01 | 2.36±1.64 | 0.26±0.18 | 0.25±0.18 | 0.19±0.01 |
| 100 mg/kg | 0.32±0.01 | 0.32±0.05 | 2.29±1.59 | 0.26±0.19 | 0.26±.018 | 0.19±0.01 |
| 1,000 mg/kg | 0.29±0.10 | 0.29±0.12 | 2.11±1.82 | 0.24±0.21 | 0.23±0.20 | 0.17±0.06 |
| Satellite | 0.29±0.10 | 0.28±0.11 | 2.18±1.88 | 0.24±0.21 | 0.22±0.19 | 0.16±0.06 |
| **Female** | | | | | | |
| Control | 0.32±0.12 | 0.40±0.04 | 1.83±1.58 | 0.25±0.02 | 0.22±0.18 | 0.25±0.17 |
| 10 mg/kg | 0.42±0.22 | 0.49±0.26 | 2.67±2.95 | 0.28±0.15 | 0.26±0.32 | 0.26±0.31 |
| 100 mg/kg | 0.36±0.04 | 0.45±0.08 | 2.07±1.43 | 0.23±0.02 | 0.28±0.15 | 0.27±0.14 |
| 1,000 mg/kg | 0.34±0.02 | 0.43±0.06 | 2.06±1.56 | 0.22±0.02 | 0.23±0.18 | 0.23±0.17 |
| Satellite | 0.35±0.01 | 0.45±0.05 | 1.97±1.36 | 0.22±0.02 | 0.24±0.17 | 0.24±0.17 |

[a]Significantly different to control ($p<0.01$).

Sub-chronic studies showed that 90 days administration of 10, 100, 1,000 mg/kg together with satellite group did not observe any death and clinical signs of toxicity. 13 weeks mean body weights of treated animals were similar to concurrent control, unless few weeks in which male rats had a bit variation. It should be related to gavage studies during which drinking and feeding activity may be randomly altered (OECD, 1998). There were no significant changes in the relative organ weights between concurrent control and treated animals. It is in accordance with no gross lesions associated to toxic effects of the extracts in both concurrent control and treated rats, however both of them were found non treatment-related lesions in association with gavage related reflux (Damsch et al., 2011) for example, perivascular edema and ventricular degeneration related to pulmonary hypertension and edema, and degenerative change related to age and sex for example, renal tubular hyaline cast, frequently found in senile and mature male rats as spontaneous occurrence, renal tubular calcification

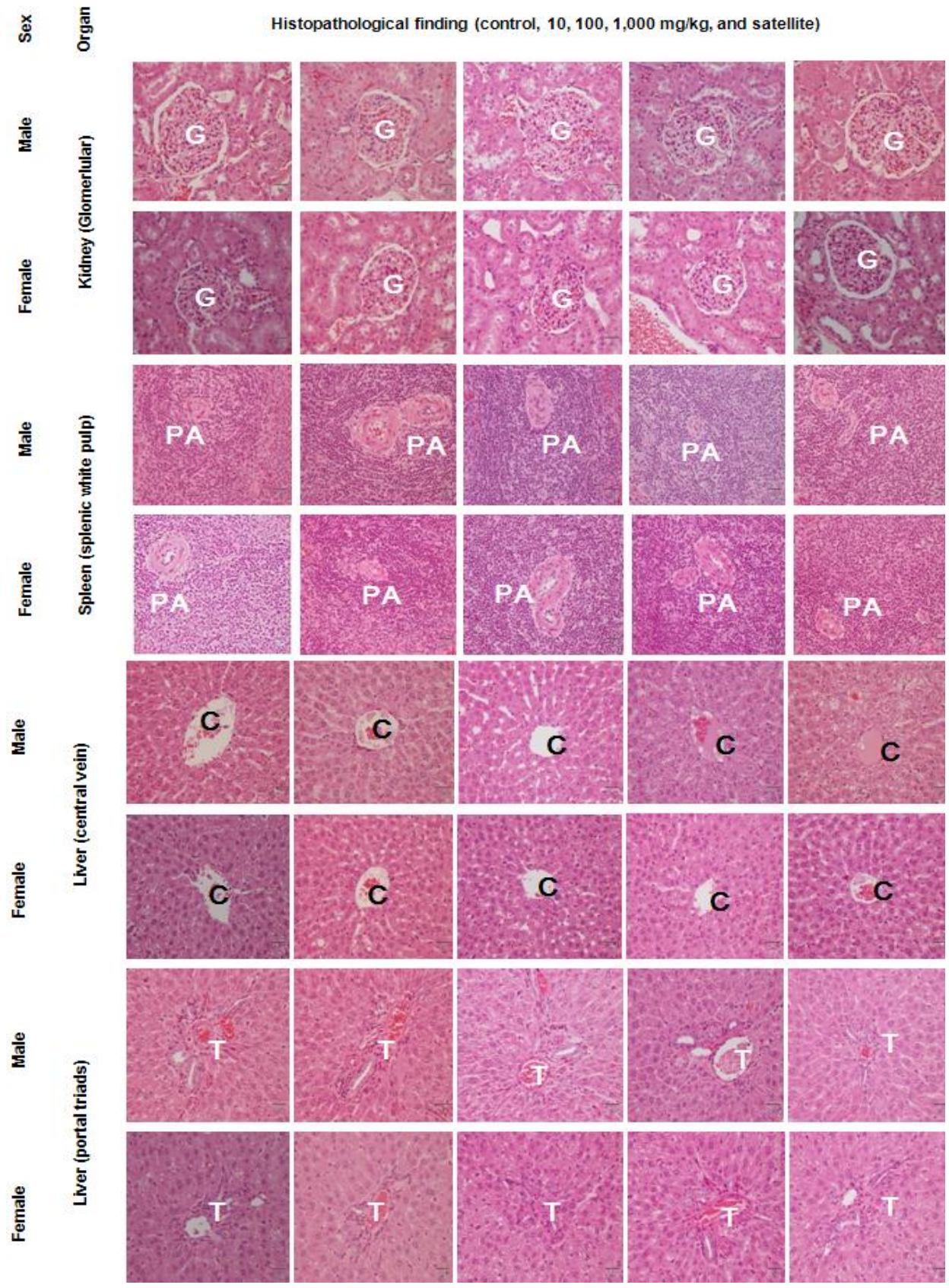


**Figure 1.** Histopathological finding of the kidney, spleen and liver in sub-chronic study. G, glomerulus; PA, periarterial lymphatic sheath; C, central vein; T, portal triad. H&E, ×100. H&E, Haematoxylin and eosin.

**Table 7**. Sprague Dawley mean hematological value in sub-chronic oral toxicity test.

| Group | | WBC ($10^3$ µl) | RBC ($10^6$ µl) | HGB (g/dl) | HCT (%) | MCV (fl) | MCH (pg) | MCHC (g/dl) | PLT ($10^5$ µl) | RDW (%) | PDW (fl) | MPV (fl) | PCT (%) | Differential count (%) | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | | | | | | | | | | Nuet | Lymph | Eosi | Mono |
| **Male** | | | | | | | | | | | | | | | | | |
| Control | Mean | 6.92 | 8.27 | 15.87 | 43.53 | 52.63 | 19.23 | 36.54 | 843.30 | 17.39 | 17.59 | 7.67 | 0.65 | 12.87 | 76.06 | 1.48 | 9.58 |
| | SD | 1.95 | 0.50 | 0.65 | 2.68 | 0.62 | 0.95 | 1.90 | 52.29 | 0.99 | 0.42 | 0.42 | 0.05 | 7.72 | 10.85 | 0.50 | 3.98 |
| 10 mg/kg | Mean | 6.86 | 8.57 | 16.22 | 44.77 | 52.28 | 18.96 | 36.27 | 907.50 | 17.56 | 17.73 | 7.53 | 0.68 | 14.18 | 75.77 | 2.06 | 8.75 |
| | SD | 1.50 | 0.30 | 0.45 | 1.67 | 0.73 | 0.67 | 1.29 | 87.59 | 0.81 | 0.53 | 0.31 | 0.09 | 6.21 | 6.92 | 2.27 | 5.06 |
| 100 mg/kg | Mean | 7.72 | 8.52 | 16.65 | 45.30 | 53.17 | 19.54 | 36.79 | 841.60 | 17.86 | 17.89 | 7.45 | 0.63 | 12.86 | 77.08 | 1.18 | 8.85 |
| | SD | 1.72 | 0.35 | 0.77 | 1.52 | 1.19 | 0.71 | 1.26 | 60.27 | 0.54 | 0.74 | 0.27 | 0.05 | 4.67 | 6.04 | 0.44 | 3.17 |
| 1,000 mg/kg | Mean | 7.44 | 8.72 | 16.48 | 45.20 | 51.83 | 18.89 | 36.48 | 882.75 | 17.52 | 17.96 | 7.68 | 0.68 | 10.84 | 79.89 | 1.26 | 8.00 |
| | SD | 2.38 | 0.27 | 0.64 | 1.25 | 1.03 | 0.62 | 1.20 | 57.68 | 0.85 | 0.63 | 0.23 | 0.04 | 5.08 | 8.47 | 0.35 | 4.15 |
| Satellite | Mean | 6.94 | 8.64 | 14.88 | 46.44 | 53.76 | 17.21 | 32.02 | 866.67 | 18.99 | 18.36 | 7.62 | 1.30 | 12.53 | 75.35 | 1.17 | 10.95 |
| | SD | 2.39 | 0.22 | 0.38 | 1.28 | 0.79 | 0.26 | 0.24 | 64.47 | 0.65 | 1.25 | 0.37 | 1.92 | 2.23 | 4.59 | 0.19 | 3.94 |
| **Female** | | | | | | | | | | | | | | | | | |
| Control | Mean | 4.98 | 7.75 | 15.44 | 42.80 | 55.49 | 20.04 | 36.13 | 724.09 | 16.30 | 18.68 | 7.71 | 0.54 | 7.32 | 72.40 | 1.97 | 10.27 |
| | SD | 1.13 | 0.70 | 0.81 | 2.02 | 3.15 | 1.27 | 0.75 | 248.94 | 0.64 | 1.99 | 0.70 | 0.19 | 2.03 | 26.11 | 1.24 | 4.45 |
| 10 mg/kg | Mean | 5.94 | 7.99 | 15.42 | 43.30 | 54.22 | 19.32 | 35.61 | 695.17 | 16.68 | 17.59 | 7.60 | 0.59 | 9.19 | 79.97 | 1.84 | 9.00 |
| | SD | 2.09 | 0.20 | 0.57 | 0.84 | 1.20 | 0.72 | 1.02 | 249.05 | 0.67 | 0.44 | 0.58 | 0.05 | 3.36 | 5.14 | 0.67 | 3.50 |
| 100 mg/kg | Mean | 7.04 | 8.03 | 15.70 | 43.49 | 54.14 | 19.55 | 36.13 | 717.50 | 16.76 | 17.54 | 7.72 | 0.61 | 8.44 | 81.00 | 2.10 | 8.46 |
| | SD | 1.79 | 0.22 | 0.64 | 1.09 | 0.56 | 0.49 | 1.03 | 252.96 | 0.65 | 0.46 | 0.28 | 0.06 | 1.86 | 5.12 | 0.63 | 4.49 |
| 1,000 mg/kg | Mean | 6.83 | 8.03 | 15.33 | 43.44 | 54.11 | 19.10 | 35.33 | 845.67 | 16.60 | 17.87 | 7.78 | 0.66 | 8.28 | 75.65 | 1.65 | 7.32 |
| | SD | 1.84 | 0.29 | 0.64 | 1.74 | 1.44 | 0.57 | 0.67 | 79.27 | 0.73 | 0.58 | 0.47 | 0.06 | 3.38 | 21.09 | 0.59 | 5.61 |
| Satellite | Mean | 6.76 | 8.05 | 14.24 | 43.79 | 54.37 | 17.68 | 32.51 | 758.70 | 16.63 | 17.94 | 7.84 | 0.58 | 8.24 | 74.19 | 1.15 | 9.02 |
| | SD | 2.78 | 0.23 | 0.37 | 0.91 | 0.81 | 0.21 | 0.46 | 130.36 | 0.86 | 1.25 | 1.08 | 0.05 | 3.16 | 22.01 | 0.025 | 2.60 |

WBC, White blood cell; RBC, red blood cell; HBG, hemoglobin; HCT, hematocrit; MCV, mean corpuscular volume; MCH, mean corpuscular hemoglobin; MCHC, mean corpuscular hemoglobin concentration; PLT, platelet; RDW, red cell distribution width; PDW, platelet distribution width; MPV, mean platelet volume; PCT, plateletcrit; Neut, neutrophil; Lymph, lymphocyte; Eosi, eosinophil; Mono, monocyte.

frequently found in female (Hard et al, 1999; Haschek et al., 2010).

Analysis of blood parameters is relevant to risk evaluation as the predictive value for human toxicity (Olsen et al., 2000). All hematological profiles of treated rats showed no significant difference with concurrent control. Blood clinical chemistry profiles of treated rats showed some significant difference with concurrent control. Blood urea nitrogen (only male treated rats) and uric acid indicated dose related effect exhibited by an increasing higher dose. Although blood urea nitrogen (BUN) was increased, creatinine in treated rats was not affected, since the increased serum creatinine has been a good indicator of

**Table 8.** Sprague Dawley mean blood clinical chemistry value in sub-chronic oral toxicity test.

| Group | | GLU (mg/dl) | BUN (mg/dl) | CREA (mg/dl) | CHOL (mg/dl) | TG (mg/dl) | URIC (mg/dl) | TP (g/dl) | ALB (g/dl) | GLOB (g/dl) | Bili-T (U/L) | AST (U/L) | ALT (U/L) | ALP (U/L) |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **Male** | | | | | | | | | | | | | | |
| Control | Mean | 165.38 | 24.62 | 0.41 | 103.40 | 96.30 | 1.54 | 6.86 | 4.64 | 2.24 | 0.10 | 75.42 | 59.02 | 89.90 |
| | SD | 28.74 | 2.32 | 0.03 | 12.32 | 16.72 | 0.68 | 0.32 | 0.16 | 0.30 | 0.01 | 9.87 | 6.70 | 11.03 |
| 10 mg/kg | Mean | 176.59 | 25.84 | 0.44 | 112.10 | 98.10 | 1.49 | 7.53 | 4.82 | 2.72 | 0.12 | 85.05 | 67.97 | 91.20 |
| | SD | 44.58 | 2.97 | 0.04 | 14.69 | 16.39 | 0.66 | 0.41 | 0.18 | 0.30 | 0.02 | 16.19 | 17.93 | 13.90 |
| 100 mg/kg | Mean | 171.25 | 27.46[a] | 0.44 | 115.20 | 100.70 | 1.90 | 7.43 | 4.86 | 2.60 | 0.11 | 85.71 | 62.36 | 91.30 |
| | SD | 46.09 | 2.49 | 0.05 | 11.05 | 29.15 | 0.91 | 0.38 | 0.21 | 0.32 | 0.02 | 11.34 | 8.13 | 17.02 |
| 1,000 mg/kg | Mean | 199.29 | 30.02[a] | 0.50 | 129.33 | 134.89[a] | 2.14[a] | 8.77[a] | 5.41 | 3.36[a] | 0.13 | 103.79[a] | 78.99 | 101.44 |
| | SD | 57.58 | 4.79 | 0.11 | 34.27 | 36.45 | 0.82 | 1.75 | 0.70 | 1.08 | 0.03 | 24.99 | 25.96 | 14.27 |
| Satellite | Mean | 309.06[a] | 27.36[a] | 0.55 | 112.44 | 140.44[a] | 4.61[a] | 7.52 | 4.77 | 2.76 | 0.08 | 82.16 | 73.12 | 95.11 |
| | SD | 119.61 | 2.86 | 0.10 | 17.91 | 34.85 | 1.73 | 1.08 | 0.56 | 0.55 | 0.01 | 28.25 | 11.76 | 23.11 |
| **Female** | | | | | | | | | | | | | | |
| Control | Mean | 189.46 | 30.95 | 0.59 | 127.40 | 140.44 | 1.51 | 8.55 | 5.87 | 2.67 | 0.11 | 101.47 | 73.18 | 97.50 |
| | SD | 28.74 | 3.30 | 0.05 | 13.40 | 34.85 | 0.31 | 0.51 | 0.34 | 0.23 | 0.02 | 13.78 | 6.70 | 14.95 |
| 10 mg/kg | Mean | 215.17 | 28.60 | 0.53 | 133.11 | 151.80 | 1.96 | 8.12 | 5.69 | 2.44 | 0.12 | 101.36 | 66.54 | 108.33 |
| | SD | 50.12 | 3.22 | 0.06 | 17.38 | 15.72 | 0.79 | 0.40 | 0.26 | 0.21 | 0.02 | 21.46 | 6.70 | 29.22 |
| 100 mg/kg | Mean | 219.83 | 26.55 | 0.53 | 129.60 | 111.33[a] | 2.37 | 8.03 | 5.55 | 2.48 | 0.12 | 100.20 | 56.53 | 85.50 |
| | SD | 32.66 | 2.71 | 0.04 | 20.26 | 27.27 | 0.68 | 0.32 | 0.24 | 0.25 | 0.02 | 17.08 | 6.70 | 9.51 |
| 1,000 mg/kg | Mean | 226.47 | 30.22 | 0.62 | 146.67 | 99.60[a] | 2.79[a] | 9.17[a] | 6.12 | 3.04[a] | 0.13 | 99.62 | 58.57 | 87.78 |
| | SD | 52.80 | 2.91 | 0.07 | 18.12 | 39.52 | 1.37 | 0.68 | 0.36 | 0.34 | 0.02 | 11.44 | 6.70 | 16.35 |
| Satellite | Mean | 253.54[a] | 27.98 | 0.61 | 129.80 | 99.50[a] | 2.95[a] | 8.38 | 5.60 | 2.78 | 0.12 | 129.64[a] | 56.81 | 59.80[a] |
| | SD | 66.85 | 4.46 | 0.09 | 19.74 | 28.67 | 0.98 | 0.76 | 0.46 | 0.33 | 0.02 | 58.29 | 6.70 | 9.22 |

[a]Significantly different to control ($p<0.01$); GLU, glucose; BUN, blood urea nitrogen; CREA, creatinine; CHOL, cholesterol; TG, triglyceride; TP, Total protein; ALB, albumin; GLOB, globulin; Bili-T, total bilirubin; AST, aspartate aminotransferase; ALT, alanine transaminase; ALP, alkaline phosphatase.

of negative impact on kidney functions (Rhiouani et al., 2008). In association with the commonly quoted paradigm that damage to 75% of nephrons is required prior to an increase in BUN, is not accurate in well-controlled toxicity studies, particularly in rat studies where the group size and narrow range of BUN values of concurrent control rats facilitates detection of relatively small but relevant increases in group mean BUN values, secondary to renal insult (Haschek et al., 2010). These results may suggest that the kidney functions are not altered. Serum total protein (TP) concentration includes the total specific proteins in plasma. Albumin accounts for35 to 50% of total serum protein, while globulin is the rest. The most

frequent causes of hyperglobulinemia are dehydration or a polyclonal gammopathy secondary to antigenic stimulation (Haschek et al., 2010). Within our treated rats, it seems to be probably related to dehydration rather than disease stage since the range of albumin:globulin ratio had 1.7 to 2.0.

Uric acid is the final product of purine metabolism. The blood levels of uric acid are a function of the balance between the breakdown of purines and the rate of uric acid excretion, which is approximately 70% by the kidneys. Hyperuricemia may occur because of decreased excretion (underexcretors), increased production (overproducers), or a combination of these two mechanisms (Maesaka and Fishbane, 1998). In our treated rat cases, it might be related to overproduction because the kidney function was preserved. There were some effects from the treatment probably, (1) directly from PN purine content or (2) its enhancement effect to increase purine nucleotide breakdown. It is noteworthy that, the increase of serum uric acid in the high dose treated rats might remind us of the hyperuricemia effect associated to PN.

Animals in the satellite group were scheduled for follow-up observations and kept for 14 days without treatment; it was found that there was significant increase in serum glucose levels in the satellite recovering rats which may indicate some hypoglycemic effects of PN. It may be useful as alternative medicine for the control of the blood glucose level of patients who have diabetes mellitus. However, there exhibited prolong effect of high level of uric acid. Hence, caution and close monitoring of uric acid levels might be necessary when patients are placed on this herbal remedy (Aniagu et al., 2005). The animal husbandry quality control was shown in the sentinel animals; all rats were healthy during the period of the study. This guarantees that the test condition was appropriate for long term toxicological study.

In conclusion, the present investigation demonstrates that the extracts from herbs in PN may be considered as relatively safe of toxicity, as it did not cause any lethality nor produced any remarkable physiological, behavioral, haematological and anatomical adverse effects both in acute and sub-chronic toxicity studies in rats. However, the contraindication of the usage for high dose oral administration should be considered.

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