Effect of Subacute Dose of Mitragyna Speciosa Korth Crude Extract in Female Sprague Dawley Rats

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***Abstract*—*Mitragyna speciosa* Korth (*MS*) leaves are widely used as a traditional remedy. The main aim of the present study was to observe the subacute toxicity of *MS* crude extract on the liver, kidney and uterus in female Sprague Dawley rats. Thirty two rats (150-200g) were randomly divided into four groups: control acute (CA); control subacute (CS); experimental acute (EA) and experimental subacute (ES). CA and CS groups were only given 15% Tween-80. Group EA rats were administered single oral dose of 1000mg/kg *MS* extract for 14 days. Group ES rats were administered repeated dose of 500 mg/kg *MS* methanol for 28 days. Liver of ES group showed severe sinusoidal congestion with enlarged hepatocytes and numerous vacuolation compared to EA group. The lining of epithelial cells of uterine tissue in ES group showed more vacuolated cells with increasing in height. No changes were observed in kidney with both doses.**

***Index Terms*— *Mitragyna speciosa*, subacute, toxicity, liver, kidney, uterus**

I. Introduction

Herbal medicines are widely used as an alternative treatment in developing countries including Malaysia, Thailand and Myanmar. It is widely used as self-medication as consumption of herbs is believed to be safe with little or no side effects. [1] *Mitragyna speciosa* (*MS*) Korth is one of the psychostimulant plant, belongs to the family *Rubiaceae*. It is locally known as 'ketum' or 'biak-biak' in Malaysia and 'kratom' in Thailand. [2] Mature leaves of *MS* consist of abundant biologically active alkaloids. Mitragynine is the major constituent which is 66.2% based on the crude base and followed by its analogues paynantheine (8.6%), speciogynine (6.6%) and speciociliatine (0.8%). [3] Mitragynine has opioid like properties which can develop to addiction. [4]

MS leaves has been tradiationally used as an alternative treatment by local people for the treatment of fever, cough, malaria, hypertension, diarrhea, to prolong sexual intercourse and as a substitute for the treatment of opiate addiction like morphine. [5], [6] Previous studies reported that *MS* crude extract or mitragynine possessed anti-depressant [7], anti-inflammatory [8] and antinociceptive properties [9] in experimental animals. However, there were few side effects following consumption of *MS* such as dry mouth, weight loss, diuresis, constipation, jerky movement of limbs and aching of muscles and bones. [5]

Nowadays, the abuse of *MS* leaves has gained a lot of attention in developing countries. Recently, several case studies showed toxicity effects in human following the consumption of *MS* leaves. It is either due to acute overdose or long term consumption of *MS* extract. [10]-[13] Several studies have been conducted based on the effect of *MS* extract with an acute dose. However, it is important to ascertain the information regarding the toxicity of *MS* crude extract with the minimal dose while on prolong consumption. The toxicity study of *MS* crude extract with regard to subacute dose is not well documented, to date. Majority of the studies have been performed on the mitragynine compound and its toxicological as well as therapeutical effects. Therefore, it is essential to investigate the subacute dose of *MS* crude extract since the majority of population prefer to consume fresh *MS* leaves instead of the isolated compound. Thus, the present study was designed to investigate subacute toxicity of *MS* crude extract in female Sprague Dawley rats by observing its physiological behaviour, biochemical and histological changes. Since the toxicity is related to major organs such as liver and kidney, we observed the changes in these major excretory organs. We studied the histological changes occuring in liver and kidney of experimental animals following the subacute dose of *MS*. In addition, we also observed the microstructural changes in the uterus tissue of the animals.

II. Materials and Methods

A. Plant Material

The fresh green leaves of *MS* were collected from Gunung Kriyang, Alor Setar, Kedah. Authentication of the plant was carried out at the Herbarium, Faculty of Science and Technology, Universiti Kebangsaan Malaysia (UKM), Bangi, Malaysia with the specimen voucher number UKMB 30028.

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B. Methanol Extract Preparation

The fresh leaves were dried under sunlight for 4-5 days and blended using universal grinder. The dried blended leaves (1kg) were sent to Forest Research Institute Malaysia (FRIM) for methanol extract preparation. The methanol extract was sealed in bottle and stored at 4 ℃ until being further used. [14]

C. Animals

A total of thirty two (n=32) female Sprague Dawley rats aged 6-8 weeks (weighing 150-200g) were used in this study. These rats were obtained from the Animal Resource Unit, Faculty of Science and Technology, Universiti Kebangsaan Malaysia (UKM), Bangi. The care and handling of animals were conducted based on ethical norms approved by Universiti Kebangsaan Malaysia Animal Ethics Committee (UKMAEC). The experimental rats were kept one rat per cage. All the animals were kept at room temperature of 27 ℃ ± 2 ℃ with standard environmental condition (12-hours light/ dark cycle) at the Anatomy Department Animal House. Food and water were available *ad libitum* throughout the study. All the experimental rats were acclimatized for one week before the commencement of the study.

D. Study Design and Selection of Doses

Thirty two (n=32) female Sprague Dawley rats were equally divided into 2 main groups (n=16): Acute toxicity group (for 14 days) and Subacute toxicity group (for 28 days). Both groups were further subdivided into: Control Acute (CA) group and Experimental Acute (EA) group, Control Subacute (CS) group, and Experimental Subacute (ES) group. Each group contained n=8 in number of rats. The acute (1000 mg/kg *MS*) and subacute (500 mg/kg *MS*) doses were chosed based on previous study. [14]

E. Extract and Drug Administration

The dried methanol extract was dissolved in 15% Tween-80 and prepared for oral administration. CA and CS groups were administered 15% Tween-80. EA group was administered a single dose of 1000mg/kg *MS*. For the ES group, *MS* was administered daily 500 mg/kg for 28 days. The maximum volume of extract administered was not greater than 0.4 ml/100g body weight.

F. Histological Study

At the end of the experiment, the rats were sacrificed and liver, kidney and uterus were harvested. The collected tissues were washed with 0.9% normal saline to remove excess blood and then fixed in 10% formalin for histological analysis. The fixed tissues were dehydrated in ascending series of ethanol, cleared in xylene and embedded in paraffin wax. The 4-5μm tissue sections were stained with Haematoxylin and Eosin (H&E) to analyze the morphological changes and degenerative alterations. [15]

III. Results

The histological assessment of liver, kidney and uterus tissues was performed using H&E staining. The histological changes of the liver were shown in Fig. 1. The CA and CS groups showed normal parenchymal architecture with cords of hepatocytes radiating from the central veins (Fig. 1a & 1b). The EA group showed hypertrophy of hepatocytes with mild cytoplasmic vacuolation and sinusoidal congestion (Fig. 1b). The ES group (Fig. 1d) showed severe hypertrophy of hepatocytes with numerous vacuolation and severe sinusoidal congestions compared to the EA group.

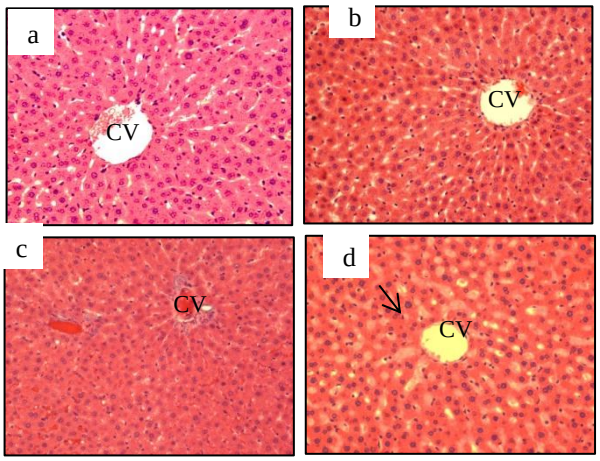

Figure 1. Group CA (a) and CS (b) showing normal liver architecture. Group EA (c) showing sinusoidal congestion and enlarged hepatocytes. Group ES (d) showing numerous vacuolated hepatocytes with severe sinusoidal congestion. Abbreviation: CV, central vein; Arrow indicates vacuolated hepatocytes. (H&E x 10)

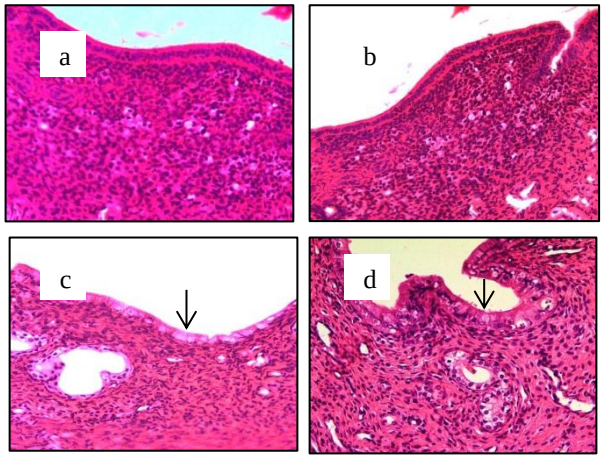

Figure 2. Photomicrographs of uterus. Note the lumen lined by columnar epithelium with loose stroma in control groups, CA (a) and CS (b). The EA (c) and ES (d) groups showed vacuolated epithelium with dense stroma. Abbreviation: Arrow indicates vacuolated epithelial cells. (H&E x 200)

The histological change in the uterine tissue was shown in Fig. 2. In the endometrial layer of CA and CS groups, the uterine lumen was lined by simple columnar epithelial cells with their nuclei situated at the same level and the underlying stroma was loosely arranged (Fig. 3a & Fig. 3b). The EA and ES groups showed the presence of vacuolated epithelial cells lining the lumen with densely packed stroma. However, the severe morphological changes such as tall columnar cells with

prominent vacuolation were found in ES group compared to the EA group (Fig. 3b & Fig. 3d).

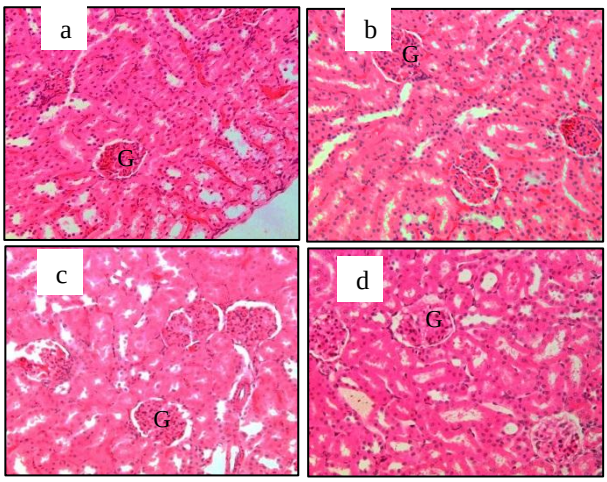

Figure 3. Photomicrographs of kidney. Note that numerous glomeruli and the convoluted tubules present in cortex. Glomerulus was surrounding by Bowman's capsule which lined by simple squamous epithelium in all groups (CA, EA, CS & ES. Abbreviation: G, Glomerulus, a, Group CA; b, Group CS; c, Group EA; d, Group ES. (H&E x200).

The Fig. 3 showed histological analyses of kidney. Normal histology of the glomerulus and tubules were found in the CA and CS groups. There were numerous glomeruli and the convoluted tubules in the cortex and the glomerulus was surrounded by Bowman's capsule which was lined by the simple squamous epithelium (Fig. 3a & Fig. 3b). There was no overt morphological change observed in the kidney tissue of EA & ES groups (Fig. 3c & Fig. 3d) compared to the control groups.

IV. Discussion

Histological observation of the liver showed abnormal morphology characteristics in all *MS* extract groups. The histomorphological studies are the most reliable evidence of hepatotoxicity in routine toxicological testing of pharmaceuticals. [16] In the present study, all the rats administered with *MS* extract revealed vacuolated hepatocytes and sinusoidal congestion. Harizal et al. [17] observed similar findings such as centrilobular necrosis, congestion of sinusoids, fatty change. They also found the haemorrhage hepatocytes, and increased number of Kupffer cells in the liver of rats administered by acute dose of *MS* extract at 1000mg/kg. However, in the present study, the *MS* extract at subacute dose of 500mg/kg revealed more severe morphological changes in liver tissue than that of acute dose 1000mg/kg. The subacute dose in the present study revealed numerous vacuolated hepatocytes with severe sinusoidal congestion. Swelling of hepatocytes with vacuolation of cytoplasm is frequently observed in acute liver injury caused by viruses or hepatotoxic agents. The vacuolation occur due to disturbance of cell membrane integrity, resulting in intracytoplasmic accumulation of fluid or due to accumulation of glycogen. [16] Vacuolated hepatocytes reflect a cellular adaptation beneficial to the host rather than a degenerative damage.

In the present study, there were no obvious morphological changes in the kidney tissue in all the groups. It was in agreement with the previous study regarding acute dose of 1000 mg/kg of *MS* extract which did not reveal any morphological changes in kidney in all experimental groups. [14] However, the study tested with the high dose of mitragynine (100mg/kg) which is the primary alkaloid of *MS* revealed the swollen glomerular capsule and the presence of red blood cells in lumens. It was accompanied with a significant increase in the serum urea level. [15] Mitragynine accounts for two-thirds of the total alkaloid extract of *MS* and possess opiod like properties. [9] Our finding was contradictory to the previous reported results probably because the test agent in the present study was the crude extract of *MS* which might contain other biologically active substances such as speciogynine, speciociliatine, paynantheine and mitraphylline apart from mitragynine. [17]

The rats administered with acute and subacute dose of *MS* extract revealed disorganization of uterine epithelial layer with vacuolated cells, increased height of the cells and dense endometrial stroma. The subacute dose of 500 mg/kg revealed more vacuolated cells with increased height of epithelial cells. The similar results were obtained in female rats treated with nandrolone decanoate [18] which promoted vacuolation and oedema in endometrial stroma of female monkeys and rats. [19] Nandrolone decanoate is a type of steroid used for the treatment of endometriosis. [20] In addition to that, the finding was also similar to an earlier study which used high doses of ethinyl estradiol on uterus and showed hypertrophy and increased luminal epithelial cell with vacuolation. [21] These features may cause the endometrium to be unfavourable for blastocyte implantation thereby leading to infertility [22].

V. Conclusion

In conclusion, long term consumption of minimal dose of *MS* crude extract proved to have disturbance in histological integrity of liver tissue and uterine endothelial cells while compared to single maximum dose. However, both doses showed no effect on the kidney tissue. Further detailed studies on chronic toxicity of crude extract of *MS* are needed to evaluate its toxicity.

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