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Toxic Effects of *Sapium baccatum* (Ludai) Extract in Rats

(Kesan Toksik Ekstrak *Sapium baccatum* (Ludai) ke atas Tikus)

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ABSTRACT

Sapium baccatum *is usually used as a timber in Malaysia. Its medicinal values are not well known and very little research has been conducted concerning the plant. Consequently, this study was conducted to observe toxicity effects of hexane extract of stem bark of* S. baccatum. *The toxicity effects were assessed through observation of physiological changes of the rats as well as through gross and histological examinations of its livers. The doses for the treated groups were given intraperitoneally for T2, T3 and T4 group which were given 100, 250 and 500 mg/kg, respectively. Meanwhile, control group (T1) was administered with 80% PEG 200 only. The observation period was 14 days. If the rat died, it was dissected and the liver was removed and examined. Some physiological effects observed include ataxia and hind-legs paralysis. The gross observations of the livers, growth of abnormal spots and connective tissues attachment to lobes of the livers were observed. The experiment was followed by histopathological observation, which indicated the presence of abnormal hepatocytes with a distorted shape and undefined cell linings for T2 group. Results also indicated an increase in the distortion of hepatocytes, presence of massive cytoplasm and necrosis of hepatocytes for T3 and T4 groups. The results suggest that non polar extract of the stem bark of* S. baccatum *may promote toxic effects to the animals.*

Keywords: Euphorbiaceae; hexane extract; rats; Sapium baccatum*; toxic effects*

ABSTRAK

Sapium baccatum *merupakan salah satu tumbuhan yang digunakan sebagai kayu balak di Malaysia. Nilai perubatannya masih banyak belum diketahui umum dan kajian terhadapnya juga amat sedikit dilakukan. Justeru, kajian ini dijalankan untuk memerhatikan kesan toksik ekstrak heksan kulit batang* S.baccatum *ke atas tikus. Kesan toksik ekstrak dinilai melalui pemerhatian terhadap perubahan fisiologi, abnormaliti pada morfologi hati secara kasar dan juga melalui slaid histopatologi sel hati tikus kajian. Sembilan ekor tikus telah dibahagikan kepada 3 kumpulan uji kaji (T2, T3 dan T4) dengan setiap kumpulan menerima sebanyak 100, 250 dan 500 mg/kg takaran ekstrak masing-masing melalui suntikan intraperitoneum manakala kumpulan kawalan (T1) pula diberikan 80% PEG 200. Pemerhatian dijalankan selama 14 hari. Tikus yang mati dalam tempoh pemerhatian dibedah dan diambil hatinya untuk pemeriksaan. Hasil kajian menunjukkan berlakunya ataksia dan kelumpuhan bahagian belakang kaki pada kumpulan uji kaji. Pemeriksaan ke atas hati juga menunjukkan kehadiran bintik-bintik tidak normal pada permukaan hati dan pertumbuhan tisu-tisu penyambung antara lobus-lobus hati. Hasil histopatologi menunjukkan kehadiran hepatosit-hepatosit tidak normal dan kehadiran lapisan sel yang tidak dikenal pasti pada kumpulan T2. Kesan ekstrak juga didapati menyebabkan pertumbuhan sel-sel hepatosit yang tidak normal, kehadiran banyak sitoplasma dan nekrosis pada kumpulan T3 dan T4. Oleh sebab itu, hasil kajian ini mencadangkan bahawa ekstrak kasar heksan kulit batang* S. baccatum *mampu memberikan kesan toksik kepada haiwan.*

Kata kunci: Ekstrak heksan; Euphorbiaceae; kesan toksik; Sapium baccatum*; tikus*

INTRODUCTION

Sapium baccatum trees (Ludai) can reach up to 30 m high with its stem reaching up to 60 cm in diameter. Its leaves are pinkish brown when young or withering yellow and the plant can be found easily at Bukit Pelindung reserved forest Kuantan, Malaysia. Ahmed et al. (2010) reported the antimicrobial and cytotoxic effects of the leaves of *S. baccatum*. An alkaloid from the leaves named bukittinggine exhibited antiinflamatory activity in carrageenan-induced hind paw edema and adjuvant-induced arthritis rats (Panthong et al. 1998). The toxicity effect of *S. baccatum* was evaluated in this study. The study was carried out by using *n*-hexane extracts of the stem bark of *S. baccatum* which was administered intraperitoneally into rats. The toxicity effects were assessed by examining the possible toxic symptoms occurring in the experimental animal and through gross as well as histological structure (Jeong et al. 2010) of the livers after administration of *S. baccatum* at different doses. The study provided the

data on toxicity effect of *S. baccatum* in rats after administration of different doses of plant extract. These data were taken into account for the safety of the extracts for the human use.

Experimental Details

General

n-Hexane, ethanol, toluene, xylene, dopexamine (DPX), polyethylene glycol (PEG), neutral buffered formalin, haematoxylin, ethyl eosin were obtained from Merck (Germany).

Plant Material and Extraction

Stembark of *S. baccatum* was collected from Kuantan Pahang, Malaysia in December 2007. The stembark was extracted with *n*-hexane, ethyl acetate and methanol, successively by using soxhlet extractor. The *n*-hexane extract was concentrated *in vacuo* and stored at -4°C until further use.

Animals

The test was performed using healthy (four weeks old) *Sprague Dawley* male rats weighing at 200-250 g. These animals received care compliance with good laboratory animal handling approved by the University Animal Care Committee (Faculty of Medicine, IIUM). The animals were housed in polypropylene cages and fed standard rat pellet diet and water *ad libitum*.

Toxicity Test

The rats were divided into four groups (T1, T2, T3 and T4) containing three rats in each group. T2, T3 and T4 received 100, 250 and 500 mg/kg dose, respectively, while T1 (control group) received 80% PEG 200. The rats that died within 14 days period were dissected and the liver removed. Those rats that did not die were rendered unconscious before dissection. The livers were stored in formalin for fixation.

Histopathological Study

The liver was then sectioned into small blocks of tissues before undergoing routine tissue processing procedures that involved submersion of the liver tissues into various percentage of alcohol solution (50, 70, 80, 95 and 100%) as well as toluene solutions. The tissues were then embedded in paraffin wax to harden the tissues. After processing, slides of the tissues were prepared before being stained using Hematoxicilin Eosin (H&E) staining. The slides were then mounted with DPX and left overnight to dry.

Results

Physiological Observations

The effects were observed within two hours after injection of the extract. Observations that were recorded include staggering gait (ataxia) and hind-legs paralysis. Table 1 shows that when the dose level increased, the ataxia and the concaving of the stomach area were occurred faster. Hind-leg paralysis was also observed in this study.

Gross Observations

Some observations in gross including colour, texture and size changes (Hooser et al. 1989) as well as the formation of white spots or abnormal growth on the surface of the liver (Table 2). Characteristics of the growth include round-shape of spots, white-yellowish color and slightly raised surface area. Overall, the results showed increasing degrees of severity in which the extract may have caused toxic effects towards the livers. The presence of round-shape of spots, white-yellowish color and slightly raised surface area frequency were increase in higher doses (Figures 1(a)-1(d)). Abnormal growths within the lobes of the livers were observable only at the highest dose (Figure 1(e)).

Histopathological Observations

Some of the characteristics that observed for the histopathological observations were the condition of nucleus and the hepatocytes, presence of Kupffer cells and the possible changes in the architectural structure of the

TABLE 1. Summary of the physiological effects observed after intraperitoneal injection

Dose (mg/kg)	Observations
Control	No observable adverse effects
100	Ataxia was observed after 15 min of injection Slight concaving of the stomach area after 20-30 min of injection
250	Ataxia was observed after around 5 min The stomach area concaved inward after 5 min
500	Ataxia occurred immediately Immediate concaving inward of the stomach area Dragging of hind legs were observed after 5 min

TABLE 2. Gross observations of the livers

Dose (mg/kg)	Observations			
	Size	Colour	Surface texture	Changes or abnormal growth
Control	Normal	Dark red	Smooth	No observable abnormal growth or changes
100	Normal	Dark red-brownish	Smooth	Few scatterings of round-shape, white-yellowish spots were observed on several lobes. Connective tissues connected to edge of the largest lobe
250	2 out of 3 were slightly enlarge	Dark red-brownish with slight darkening in a few areas	Smooth	Many clusters and scatterings of round-shaped, white spots in various sizes were found on the lobes. Connective tissues connected to edge and center of largest lobe
500	2 out of 3 were slightly enlarge	Dark red-brownish with darkening of the edges of the lobes	Smooth but slightly rougher surface texture where growth was present	Clusters and scatterings of spots of various sizes were observed throughout the lobes. Large, abnormal growths found in largest lobes. Characteristics of the abnormal growth include: white-yellowish, round-shape, slightly raised surface. Connective tissues firmly connected to several lobes

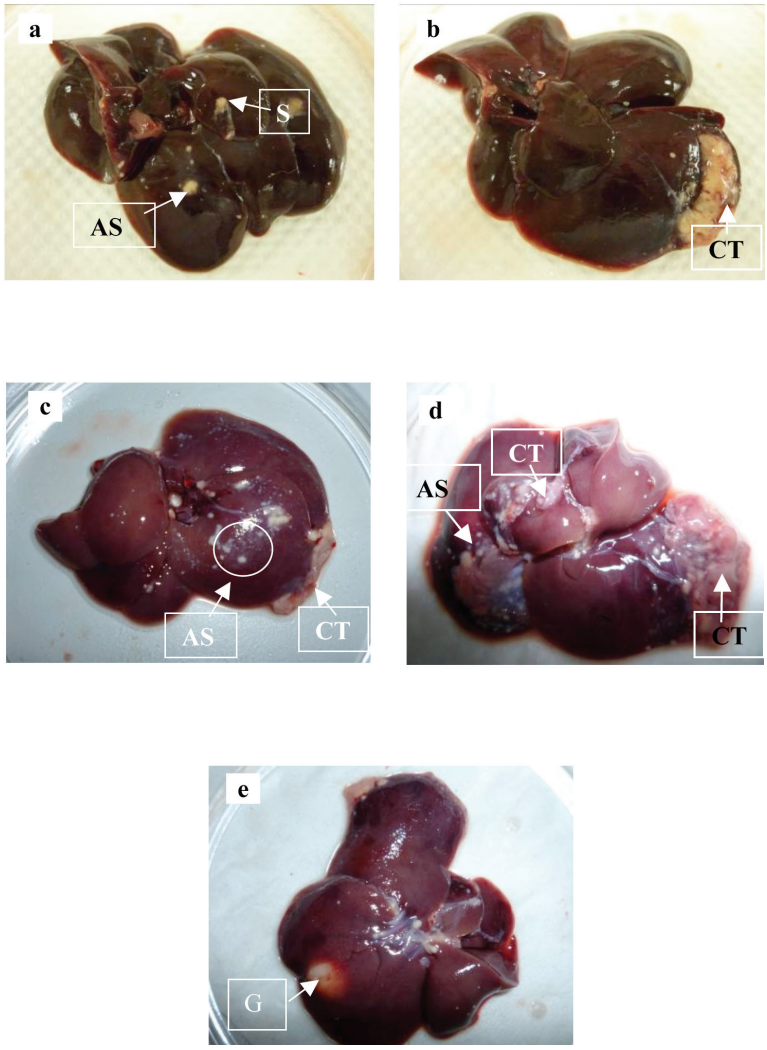

FIGURE 1. Presence of abnormal spots (AS) on the surface of the liver and connective tissues (CT) firmly attached to edge of the largest lobe of the liver for T2 group (100 mg/kg) was observed (a and b). Liver from T3 group (250 mg/kg) where abnormal spots (AS) on the surface of the liver and abnormal connective tissues (CT) were observed (c). Liver from T4 group (500 mg/kg) where abnormal spots (AS) on the surface of the liver and abnormal connective tissues were observed (d). Abnormal growth (G) observed within one of the lobe at 500 mg/kg dose level (e)

liver tissues. Abnormalities of different degrees of severity were also observed in the treated groups.

CONTROL GROUP (80% PEG 200)

The results indicated the existence of polyhedral hepatocytes (H) with normal, round-shaped nuclei (N) and presence of sinusoidals (S) as well as Kupffer cells (K) (Figures 2(a) & 2(b)). The hepatocytes were arranged in plates radiating from the central vein (CV).

LOW DOSE GROUP (100 MG/KG)

The results indicated that although the existence of polyhedral hepatocytes (H) with normal, round-shaped nuclei (N) were still visible, there was also the presence of several damage cells (AC) with distorted shapes (Figures 3(a) & 3(b)).

MEDIAN DOSE GROUP (250 MG/KG)

The results indicated the existence of abnormal cells with distorted shapes and undefined cell linings (Figures 4(a) & 4(b)). There was also hepatocytic vacuolations (HV). The architectural structure of the liver tissues appeared changed as the normal structure had been distorted, possibly by the presence of lipids (L) that was observed.

HIGH DOSE GROUP (500 MG/KG)

The results indicated the existence of abnormal hepatocytes with distorted shapes consisting of an undefined cell lining (CL) (Figures 5(a) & 5(b)). The architectural structure of the liver tissues appeared more prominent and the normal structures more distorted due the presence of a large amount of lipids and abnormal cells as well as due to cytoplasmic (C) changes and increased vacuolation of hepatocytes. Sections of liver tissues that showed abnormal

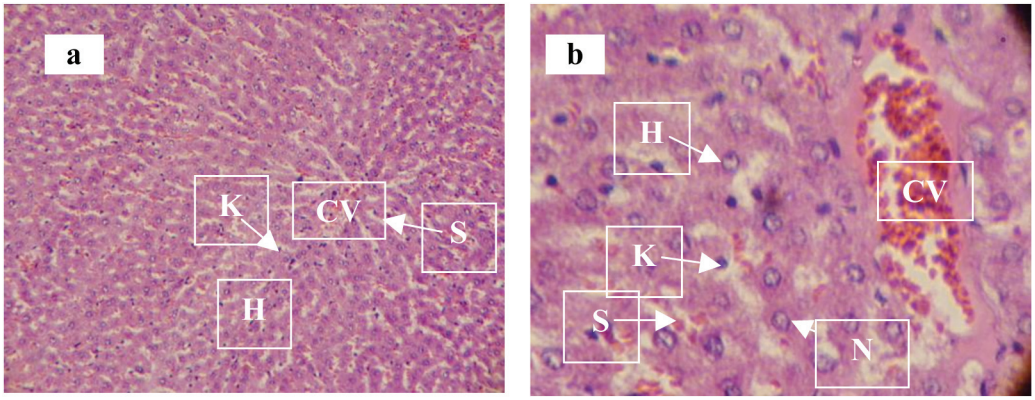

FIGURE 2. Hispathology of liver tissue of T1 (control group). 100 × magnification (a). 400 × magnification (b). T1 received 80% PEG 200 only. CV=central vein, L=lipids, PN=pyknotic nuclei, H=hepatocytes, K=Kupffer cells, N=nuclei, S=sinusoidals

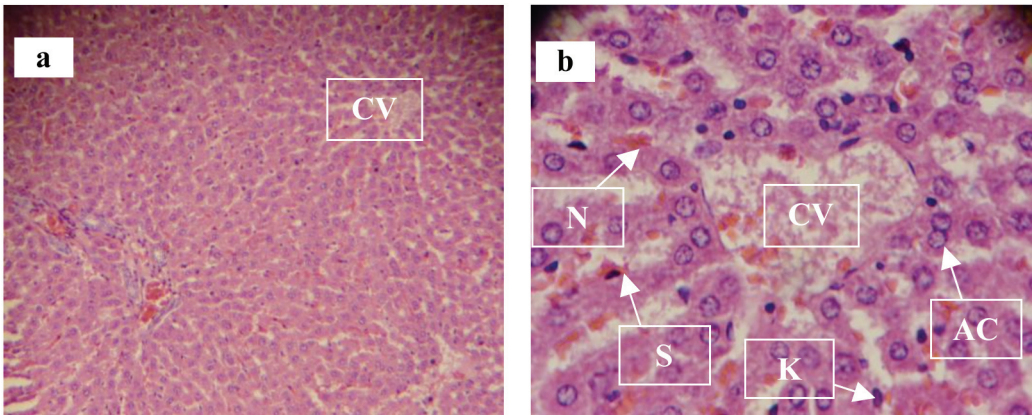

FIGURE 3. Hispathology of liver tissue of T2 group. 100 × magnification (a). 400 × magnification (b). T2 received 100 mg/kg. AC= abnormal cells, CV=central vein, K=Kupffer cells, N=nuclei, S=sinusoidals

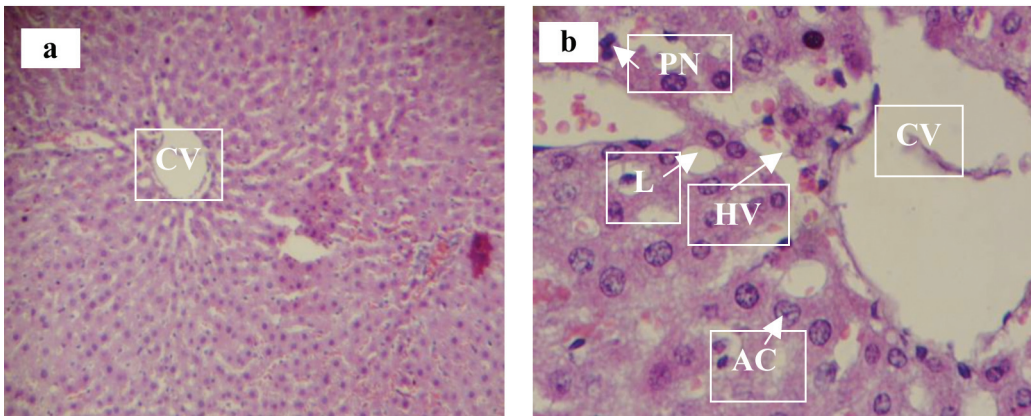

FIGURE 4. Hispathology of liver tissue of T3 group. 100 × magnification (a). 400 × magnification (b). T2 received 250 mg/kg. AC= abnormal cells, CV=central vein, HV=hepatocytic vacuolations, L=lipids, PN=pyknotic nuclei

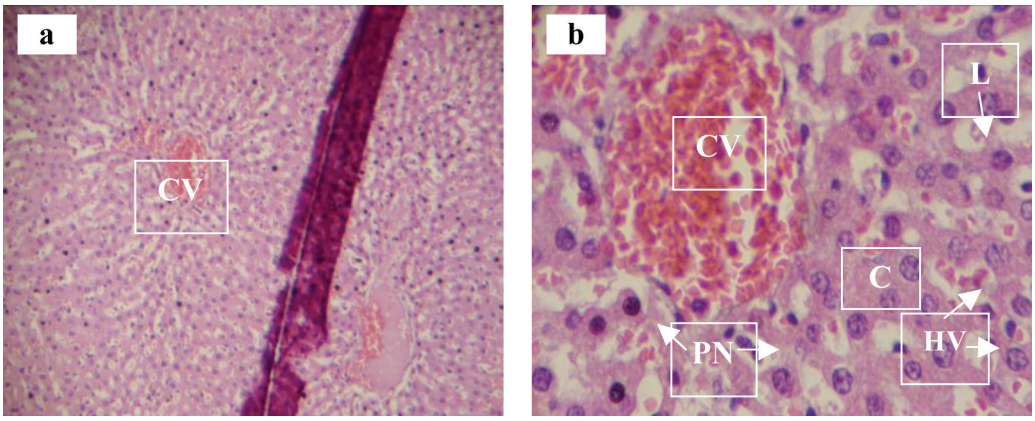

FIGURE 5. Hispathology of liver tissue of T4 group. 100 × magnification (a). 400 × magnification (b). T4 received 500 mg/kg. C= cytoplasmic, CV=central vein, L=lipids, HV=hepatic vacuolations, PN=pyknotic nuclei

growths and spots were also observed (Figure 6). The results showed the presence of unidentified cells (U) and possible presence of lipids among the abnormal cells.

DISCUSSION

PHYSIOLOGICAL OBSERVATIONS

The first physiological changes observed in experimental animal within first two hours after administering the *S. baccatum* extract was ataxia. Ataxia is defined as failure of muscular coordination staggering gait (Hedrich 2004). It was noted that when the dose increased, the ataxia occurred faster. The failure of muscular coordination may have been caused by the presence of alkaloids and other unknown compounds present in *S. baccatum* (Kanjanapothi et al. 1990; Panthong et al. 1998). The Euphorbiaceae family is much diversified and is especially rich in alkaloids and terpenoids (Webster 1986). Additionally, bukittinggine, a type of alkaloid extracted from *S. baccatum*, can cause smooth muscle relaxant activity on various types of smooth muscles (Kanjanapothi et al. 1990; Panthong et al. 1998). Thus, it is postulated that the possible presence of alkaloid in the extract of *S. baccatum* may have caused the relaxing of the muscles of the hind-legs of the rats, which led to slightly uncoordinated movements.

Moreover, the presence of compounds in the extract may also have affected the central nervous system (CNS) of the rat. Ataxia is usually caused by trauma or damage that disrupts the CNS (Tortora & Derrickson 2006). Muscle coordination of the specimen would be affected as their senses could not function normally in order to coordinate the movement of the muscles.

Additionally, the compounds may have influenced the muscles, as well as the CNS of the rats and caused the dragging of the hind-legs of the specimens. This reaction was only observable at the highest dose. This may be due to the presence of a high amount of alkaloid within the extract that caused slight paralysis and reduction of motor reactions of the hind-legs, resulting in slowly movement

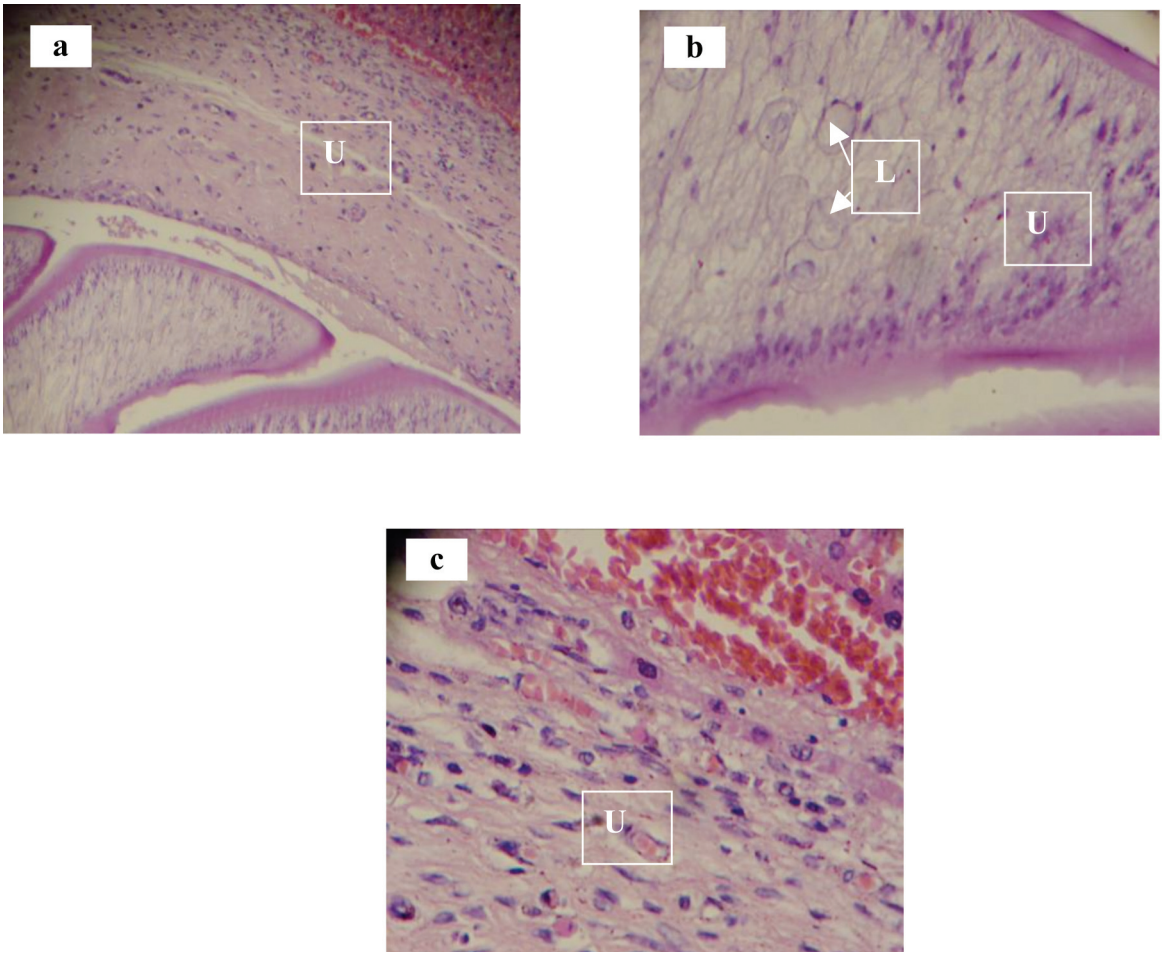

FIGURE 6. Photomicrograph of abnormal liver tissues of T4 group. 100 × magnification (a). 400 × magnification (b) and (c). L=lipids, U=unidentified cells

of those legs. Another effect was the concaving inward of the stomach area of the rats. The smooth muscle relaxant activity may be caused by the various types of phytochemicals presence in the extract.

GROSS OBSERVATIONS

The results showed that the color of treated rat's livers changed noticeably if compared with that of a normal liver of a rat. Polyethylene glycols, in general, are recognized to be bland and low order toxicity (Majeed & Prentice 1978). The level of toxicity of PEG 200 was not high enough to give the adverse effects throughout the experiment.

In addition, the size of the livers was mostly normal except for those from the T3 and T4 dose groups as some of the livers were slightly enlarged. The toxic effects of the compounds of the extract may have promoted the swelling and enlargement of these livers (Hooser 1989). Abnormal growths on and in the livers may have also contributed to the enlargement of the livers.

The presence of white-yellowish spots on the lobes was also observed. It was noted that when the dose was increased, the number and size of the spots located on the surface of the livers also increased. Furthermore, at dose of 500 mg/kg, some of the abnormal growths were found on the surface of the liver. Characteristics of the abnormal growth include white-yellowish colour, round-shape and its size ranging from small to medium.

Another observation was the attachment of connective tissues to several lobes of the livers. The connective tissues were also found to have interconnection to other organs such as the stomach, intestines and the diaphragm. This condition may be related to the abnormal growths observed on the liver, which consequently supports the presumption that extract of *S. baccatum* may have toxic properties. Consequently, it was presumed that these abnormal connective tissues may have undergone metastasis, which is a characteristic of cancer. The traits observed of the connective tissues in this study suggest that the cells of these connective tissues may have metastasized, enabling to spread to other organs of the rat's body system.

HISTOLOGICAL OBSERVATIONS

The liver sections of the treated groups indicated the presence of abnormal hepatocytes which have a distorted

shape and undefined cell linings, as well as massive cytoplasm. Vacuolation of hepatocytes and enlarged nucleus was also observed for T3 and T4 group. The large vacuole in the cell forces the nuclei to the periphery of the hepatocytes which is usually accompanied by nuclear atrophy. These abnormalities not only cause changes in the structure of the hepatocytes, but also to the architectural structure of the whole liver tissues and even some of the sinusoidal spaces were eradicated (Ebaid et al. 2007).

Furthermore, the results pointed towards potential morphologic evidence of necrosis which is shown by the presence of pyknotic nuclei and cytoplasmic changes. When a cell receives a signal to initiate apoptosis, condensation of the nuclear chromatin, also known as pyknosis, occurs. Pyknosis causes the formation of dark-staining masses found against the nuclear membrane (Young et al. 2006). Observations of unidentified, abnormal cells were found directly in areas where abnormal growths were grossly observed. It was presumed that these cells may have undergone abnormal proliferations that lead to the loss in the uniformity of the individual cells and the loss of their architectural orientation (Kumar et al. 2007).

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