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Subacute Toxic Effects of *Melastoma malabathricum* Linn. Aqueous Leaf Extract on Liver and Kidney Histopathology of Rats

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Abstract: Many plant based medicines used since time immemorial have never undergone vigorous scientific testing, despite countless reports on cases of health hazards arising from their unbridled usage. In light of this, since recent research has unearthed a novel anticoagulant property present in the *Melastoma malabathricum* leaf extract, this study evaluated its subacute safety profile. Sprague-Dawley rats of both sexes were orally administered with deionized water (vehicle control), 50, 75 and 100 mg kg^{-1} aqueous leaf extract daily for 28 days. At day 29, all animals were scarified. Blood samples were collected and analyzed blood biochemical enzymes; while, organs were harvested, weighed and subjected to tissues histopathological examination. There were no aberrant physical and behavioral changes in the treated rats. However, blood chemistry analysis revealed significant elevation of aspartate aminotransferase (AST), alanine aminotransferase (ALT) and alkaline phosphatase (ALP) levels. Correspondingly, histopathology of liver and kidney tissues showed extensive toxic manifestations. All hepatic venous structures appeared dilated and congested. Tissue hemorrhage and tubular damage were seen in the kidneys. These results collectively highlight the predisposition of *Melastoma* extract to be hepato- and nephrotoxic if taken repeatedly via oral route for a long period. This is the first exploratory study on the nature of *Melastoma* leaf's aqueous extract subacute oral toxicity.

Key words: Subacute toxicity, *Melastoma malabathricum*, histopathology, liver, kidney

INTRODUCTION

The indiscriminate use of phytomedicines has never stopped gaining popularity as opposed to the paradoxically scarce scientific evidences on their safety profiles. The notion that natural products are generally harmless and thus the safest forms of medication further ignite the usage of plant-based medicines (Ashafa *et al*., 2010; Catania *et al*., 2010). In view of this, a local shrubby plant belonging to the family Melastomataceae, commonly known as 'senduduk' among the indigenous folks in Malaysia, was recently shown to possess potent anticoagulant activity (Manicam *et al*., 2010). Traditionally, numerous medicinal properties have been attributed to various parts of this versatile plant (Susanti *et al*., 2007; Whitmore, 1972). However, this plant may have a low safety margin despite its various therapeutic properties. Therefore, it was imperative to thoroughly investigate and substantiate the toxicology and safety profiles of oral administration of *Melastoma* leaf extract in an experimental animal model. Although, there are no specific diagnostic criteria for plant-induced toxic injury, long-term toxicity study is a valuable tool to identify the target organ(s) affected as well as to establish threshold dosages that cause the damage (Fan *et al*., 2010; Yang *et al*., 2010). A 28 day repeated dosing, sub-acute toxicity assessment of plant extracts is widely recognized as adequate to envisage possibilities of human hazards using suitable animal models (Singh *et al*., 2009). Considering the potential medicinal value of *Melastoma malabathricum* leaf extract as an anticoagulant agent, coupled with the lack of a complete toxicological profile aqueous extraction of this plant, studies presented herein tested the effects of the extract administration on Sprague-Dawley rats for subacute toxicity. The present investigation is a sequel of the previous acute toxicity study of this extract, which warranted the *Melastoma* leaf extract safe for oral ingestion at a single dose administration (data unpublished). Test animals subjected to daily dosing of

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three different concentrations of the extract for 28 days were compared to those in the control group gavaged with deionized water (vehicle). Post-mortem investigation of the rats encompassed of analyses of various blood chemistry parameters and histopathology of liver and kidney tissue samples.

MATERIALS AND METHODS

Preparation of plant extract: The aqueous extract of *Melastoma malabathricum* leaves was prepared according to the hot water extraction method previously documented (Manicam *et al*., 2010). Briefly, 500 g of pulverized fresh leaves was extracted with 1 L of hot water under reflux for 5 h. The infusion was filtered, lyophilized and stored at -20°C until use.

Animals: Healthy Sprague-Dawley rats of both sexes, aged 6 weeks old and weighing between 150-175 g were procured from the Animal Experimental Unit of Faculty of Medicine and Health Sciences, Universiti Putra Malaysia (UPM) Serdang, Selangor, Malaysia. All animals were separated by gender and housed in individual cages, with access to commercial laboratory rat chow and drinking water *ad libitum*. Rats were acclimatized to the standard laboratory conditions, with 12 h light-dark cycle for a week prior to the initiation of treatment. The experimental protocols and principles of this study obtained the approval of the Animal Care and Use Committee (ACUC) of the Faculty of Medicine and Health Sciences, UPM Serdang, Selangor, Malaysia. All procedures were conducted in strict compliance to the institutional ethical guidelines for use of small animals in experiments in UPM and National Institute of Health "Guide for the Care and Use of Laboratory Animals" (NRC, 1999).

Subacute oral toxicity: The rats were randomly segregated into four groups after the acclimatization period. The first group, which was the normal control group, consisted of animals gavaged with deionized water (vehicle) at 10 mL kg^{-1} (n = 10 sex^{-1}). Rats in the other three treatment groups received an equal volume of the *Melastoma* leaf extract dissolved in deionized water at 50, 75 and 100 mg kg^{-1}, respectively (n = 10 sex^{-1} $dose^{-1}$). Oral treatment was carried out on a daily basis for 28 consecutive days. Extract samples were prepared fresh at the start of each day of experiment. The study design was based on the Organization for Economic Co-operation and Development (OECD) guideline for repeated dose 28 day oral toxicity study in rodents (OECD, 1995).

Clinical observation: Cage side observations were carried out daily for clinical signs and symptoms of toxic manifestations throughout the course of the experiment at the same time of the day, before and after dosing. These observations comprised of changes in physical appearance (eyes, skin, fur, mucous membranes, excretions or secretions from the eyes, nose and/or mouth), changes in autonomic activities (piloerection, lacrimation, unusual breathing pattern, defecation, urination and pupil size), uncharacteristic behavioral changes (aggression, self-mutilation, backward walking and/or repetitive circling movements and excessive grooming) as well as abnormal responses to handling. Any changes in both eating and drinking patterns that deviate from the norm were also carefully noted.

Body weights determination: Body weight of animals was recorded before the start of treatment (day 0) and everyday thereafter throughout the treatment period (day 1 to day 28). Rats were also weighed immediately before sacrifice on day 29.

Blood biochemical examination: At the conclusion of the 28 days treatment, all animals were fasted overnight and sacrificed on day 29 via cardiac puncture under diethyl ether anesthesia. Blood samples were immediately collected and transferred into tubes without anticoagulants to obtain serum for blood biochemistry analysis. Analysis was performed in an automated chemistry analyzer (Hitachi 902, Roche Diagnostics GmbH, Germany) for alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (ALP), gamma glutamyl transferase (GGT) and blood urea nitrogen (BUN).

Necropsy and histopathology: Sacrificed animals were subjected to thorough necropsy. The cranial, thoracic and abdominal cavities were carefully examined for presence of gross lesions. The following organs were harvested, rinsed in 0.9% saline and blotted free of blood on clean tissue papers prior to weighing: Lungs, heart, liver, spleen, stomach, cecum, left and right kidneys (weighed separately), uterus and left and right testes (weighed separately). Organ-to-body-weight ratio (%) was calculated as follows: g organ weight g^{-1} b.wt.×100. Liver and kidney tissue sections were fixed in 10% phosphate buffered formaldehyde solution, routinely processed, embedded in paraffin wax, sectioned at 5 μm and stained with hematoxylin and eosin (H and E) for examination under light microscope (Nikon YS100, Instech Co. Ltd., Japan). A pathologist unaware of the treatment regimen subjected the tissue histology to blind evaluation. Microscopic examination of each organ was done at multiple sections and magnifications. Images representative of the typical histological profile for the particular treatment group were further scrutinized under an imaging camera attached to the microscope

(Microlambda, Germany). Photomicrographs with the best magnification, with particular emphasis on the presence of lesions, were chosen for imaging.

Statistical analysis: Data were presented as Means±Standard Error mean (SEM) for body weight gains and as Means±Standard Deviation (SD) for other parameters tested. Significant difference between control and treated groups was established by a one-way analysis of variance (ANOVA), followed by a post-hoc Dunnett's multiple comparison tests to compare between groups. Significance level was reported at p-value less than 0.05 ($p<0.05$).

RESULTS

Clinical observation, body and organ weight determination: Oral administration of *Melastoma* leaf extract for 28 days did not produce any demonstrable clinical signs and symptoms of toxicological significance in the rats. There was no treatment-related mortality or morbidity throughout the study period. All animals were consistently healthy and survived until the day of scheduled euthanasia. Rats in the treated groups showed comparable patterns of mean body weight gains to those in the normal control group over the course of investigation, regardless of gender (Fig. 1). This result corroborated well with normal intake of food and water (results not shown). Likewise, the organ-to-body-weight ratios of several organs harvested did not show deviations from the control for both male and female animals (Table 1, 2).

Table 1: Relative organ weights of male rats in subacute oral toxicity study of *Melastoma* leaf extract

	Relative weight (%)*			
Organ	Control	50 mg kg^{-1}	75 mg kg^{-1}	100 mg kg^{-1}
Cecum	0.66±0.23	0.68±0.20	0.67±0.12	0.62±0.07
Heart	0.34±0.06	0.34±0.04	0.35±0.05	0.35±0.09
Liver	3.58±0.47	3.59±0.44	3.63±0.41	3.63±0.45
Lungs	0.59±0.10	0.59±0.07	0.64±0.10	0.61±0.17
Left kidney	0.39±0.07	0.40±0.03	0.43±0.05	0.38±0.04
Right kidney	0.39±0.07	0.40±0.04	0.42±0.04	0.38±0.03
Spleen	0.21±0.06	0.21±0.04	0.21±0.06	0.21±0.05
Stomach	0.73±0.22	0.73±0.14	0.75±0.15	0.75±0.05
Left testicle	0.53±0.11	0.52±0.06	0.54±0.06	0.52±0.02
Right testicle	0.54±0.12	0.53±0.04	0.54±0.09	0.53±0.03

*Relative organ weight was calculated as g organ weight/g body weight×100 and expressed as Means±SD (n = 10 animals/dose group)

Blood biochemistry analysis: The clinical blood chemistry profiles of dosed and control rats of both sexes are summarized in Table 3. Male rats in all treated groups showed significant ($p<0.001$) increased levels of aspartate aminotransferase (AST), alanine aminotransferase (ALT) and alkaline phosphatase (ALP) enzymes. However,

Table 2: Relative organ weights of female rats in subacute oral toxicity study of *Melastoma* leaf extract

	Relative weight (%)*			
Organ	Control	50 mg kg^{-1}	75 mg kg^{-1}	100 mg kg^{-1}
Cecum	0.49±0.15	0.49±0.12	0.43±0.09	0.49±0.12
Heart	0.40±0.04	0.40±0.06	0.42±0.07	0.40±0.07
Liver	3.96±0.51	3.98±0.57	3.84±0.42	3.91±0.47
Lungs	0.73±0.05	0.75±0.11	0.73±0.11	0.71±0.13
Left kidney	0.38±0.07	0.42±0.04	0.39±0.03	0.38±0.06
Right kidney	0.39±0.04	0.41±0.08	0.40±0.04	0.38±0.07
Spleen	0.28±0.05	0.29±0.13	0.25±0.06	0.28±0.04
Stomach	0.61±0.07	0.64±0.06	0.62±0.07	0.60±0.05
Uterus	0.40±0.16	0.42±0.13	0.39±0.12	0.38±0.04

*Relative organ weight was calculated as g organ weight/g body weight×100 and expressed as Means±SD (n = 10 animals/dose group)

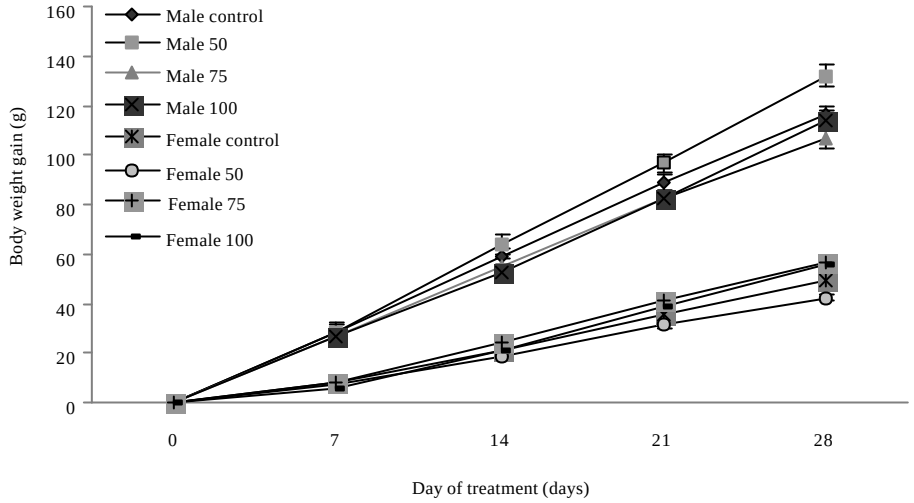

Fig. 1: Effect of *Melastoma* leaf extract on body weight gain of both male and female rats. Rats were orally administered with 10 mL kg^{-1} deionized water (control groups), 50, 75 and 100 g kg^{-1} *Melastoma* leaf extract on a weekly basis for 28 days. Data are expressed as Mean±SEM

Table 3: Effects of *Melastoma* leaf extract on the serum biochemistry of male and female rats

	Groups			
Parameters	Control	50 mg kg^{-1}	75 mg kg^{-1}	100 mg kg^{-1}
Male				
AST (IU L^{-1})	125.24±0.21	140.10±0.14[a]	140.40±0.20[a]	150.20±0.42[a]
ALT (IU L^{-1})	31.10±0.57	32.80±0.85[b]	33.33±0.59[a]	41.30±0.42[a]
ALP (IU L^{-1})	227.35±14.92	308.40±10.83[a]	288.20±17.84[a]	279.20±29.56[a]
GGT (IU L^{-1})	5.78±1.80	5.33±0.93	4.49±0.35	4.70±2.05
BUN (mg dL^{-1})	8.46±0.36	7.76±1.77	8.42±0.74	8.50±0.34
Female				
AST (IU L^{-1})	120.01±0.37	123.33±0.12[b]	126.67±0.31[a]	129.65±0.14[a]
ALT (IU L^{-1})	31.14±0.17	38.33±0.56[a]	44.51±0.21[a]	45.56±0.48[a]
ALP (IU L^{-1})	186.85±2.33	192.65±0.64	191.00±0.76	200.85±29.06
GGT (IU L^{-1})	6.32±1.01	7.20±3.51	7.30±2.11	7.20±1.70
BUN (mg dL^{-1})	6.44±0.40	6.42±0.46	6.39±0.10	6.47±0.68

Values are expressed as Mean±S.D (n = 10 animals /dose group). Animals in the control group received 10 mL kg^{-1} of deionized water. [a]$p<0.001$ in relation to the control group by Dunnett's test, [b]$p<0.05$ in relation to the control group by Dunnett's test

these treatment-related changes occurred in a concentration-dependent fashion for AST and ALT only. Level of AST was increased from 125.24±0.21 IU L^{-1} (control) to 140.10±0.14 IU L^{-1} (50 mg kg^{-1}), 140.40±0.20 IU L^{-1} (75 mg kg^{-1}) and 150.20±0.42 IU L^{-1} (100 mg kg^{-1}). In addition, at 100 mg kg^{-1} of dose treatment of *Melastoma* leaf extract, the level of ALT was the highest which was 41.30±0.42 IU L^{-1}. Then, levels of ALT were decreased orderly at 75 mg kg^{-1} was 33.33±0.59 IU L^{-1}, while the ALT level at 50 mg kg^{-1} was 32.80±0.85. There were no changes attributed to the treatment on the other parameters tested i.e,. Gamma Glutamyl Transferase (GGT) and Blood Urea Nitrogen (BUN). A similar trend of significant ($p<0.001$) elevation of AST and ALT levels was also noted in the blood samples of treated female animals compared to their control counterparts. The measurement of AST levels were showed 123.33±0.12 IU L^{-1} at 50 mg kg^{-1}, 126.67±0.31 at 75 mg kg^{-1} and 129±0.14 at 100 mg kg^{-1} when compared to control at 120.01±0.37 IU L^{-1}. Besides, ALT levels of treated female rats were 38.33±0.56, 44.51±0.21 IU L^{-1} and 45.56±0.48 IU L^{-1} at 50 mg kg^{-1}, 75 kg mg^{-1} and 100 mg kg^{-1}, respectively. Both results showed that levels of AST and ALT for female rats were increased in a dose-dependent manner. Minimal but non-significant increases in ALP and GGT enzymes were measured for female rats in all dose groups. Blood urea nitrogen levels remained similarly unaffected by the treatment of *Melastoma* leaf extract. In general, AST and ALT were significantly higher in both treated male and female groups in a dose-dependent manner in comparison to their respective normal control counterparts administered with deionized water orally.

Histopathology

Histopathology of liver tissues: Evaluations of the liver tissue samples of treated male rats revealed potential treatment-related histomorphological changes. There were severe venous dilatation and congestion in all dosed animals' liver specimens (Fig. 2b-d). Hepatic tissues of control rats administered with deionized water (Fig. 2a) showed normal lobular and venous histology. The pathological severity is apparent in all treated tissues regardless of the dosage of leaf extract administered. It is noteworthy that the pronounced histopathological findings are similar in female tissues as well (results not shown).

Histopathology of kidney tissues: Likewise, there were remarkable histopathological changes attributable to the treatment seen in the kidney tissues of dosed rats, which are worth highlighting. A concentration-dependent, multiple foci of tissue hemorrhage was observed in specimens harvested from both treated male (Fig. 3a-d) and female animals (results not shown) at all doses. However, there were no treatment-related pathologic changes noticed in the cellular architecture of rats dosed at 50 mg kg^{-1} of extract. The renal corpuscles with glomeruli and Bowman's capsule appeared structurally intact and well defined. Similarly, renal tubules appeared normal and distinct. On the other hand, cortex regions of both male and female rats at 75 and 100 mg kg^{-1} demonstrated notable changes in the cellular and structural integrity of tubular organizations. Although, the renal corpuscles retained their normal histoarchitecture, the shapes of tubules appear 'wiped-out' and indistinct due to the marked loss of epithelial arrangement. Nuclei of tubular cells also look pale in comparison to the nuclei of normal control samples, which appeared as prominent bluish spherical structures. These treatment-related adverse changes were more pronounced in the highest concentration of treatment, 100 mg kg^{-1}. In brief, histopathologic investigation of renal tissues of treated animals of both sexes revealed extensive tubular cells

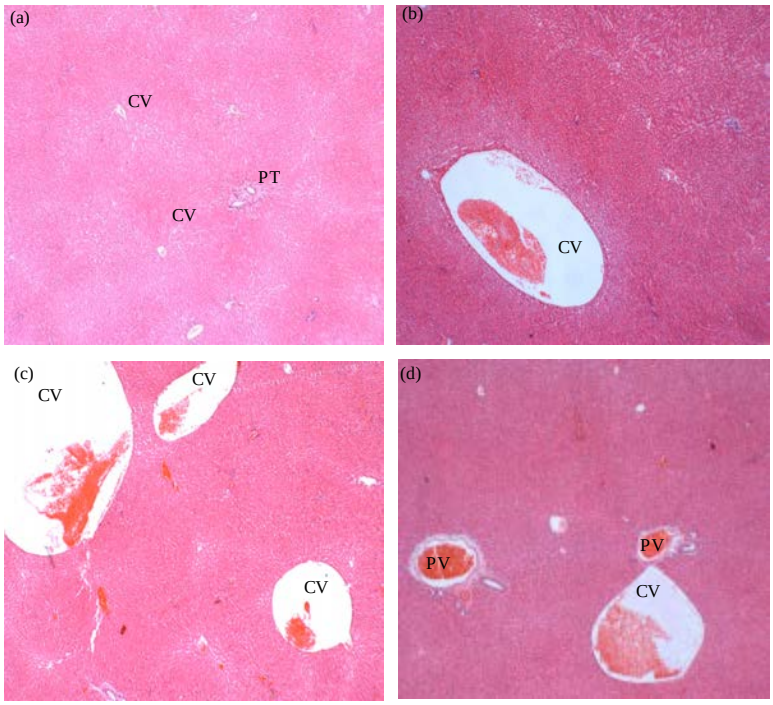

Fig. 2(a-d): Photomicrographs of liver of male rats orally treated with (a) 10 mL kg^{-1} deionized water as normal control (b) 50 g kg^{-1} *Melastoma* leaf extract (c) 75 g kg^{-1} extract and (d) 100 g kg^{-1} extract daily for 28 days. PT indicates portal triad, CV indicates central vein and PV indicates portal vein. H&E stain, magnification: 40×

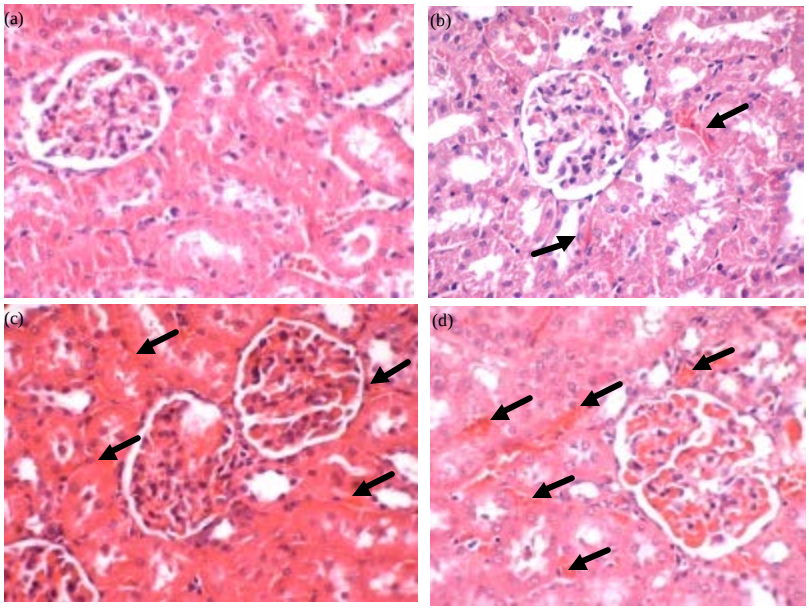

Fig. 3(a-d): Representative photomicrographs of kidney of male rats orally treated with (a) 10 mL kg^{-1} deionized water as normal control (b) 50 g kg^{-1} *Melastoma* leaf extract (c) 75 g kg^{-1} extract and (d) 100 g kg^{-1} extract daily for 28 days. Arrow (→) indicates area of tissue hemorrhage. H&E stain, magnification: 400×

degenerative alterations, with multiple foci of intra-tubular tissue hemorrhage varying in severity, in the absence of glomerular pathology.

DISCUSSION

The pharmacologically active (anti-coagulant) leaf aqueous extract of *Melastoma* was well tolerated by the test animals, as evidenced by zero incidence of mortality and no uncharacteristic changes in the physical appearance and behavior, food and water intake, as well as body weight patterns that do not deviate from the norm throughout the course of investigation. Apart from the use of body weight to assess toxicity potential of test articles on experimental animals, the weights of visceral organs is another sensitive index and a principal requirement in complete toxicity studies (Bailey *et al*., 2004). Weights of certain organs are often useful predictors of potential toxicity due to assault of various toxicants. Liver weight, for instance, has always been used as a sensitive indicator of physiological and metabolic disturbances as a result of toxic insult to an organism. Moreover, the aforementioned parameter scarcely displays inter-species variations and hence, is often a highly reliable and accurate index of disease diagnosis (Michael *et al*., 2007). The present study on various organ weights of dosed animals gavaged with *Melastoma* leaf aqueous extract also showed no deviations from control rats.

The blood samples of the rats subjected to blood chemistry analysis of biomarker enzymes of liver and kidney injuries, however, showed several changes worth highlighting and warranted further examination. Aspartate transaminase (AST) and alanine transaminase (ALT) were elevated significantly in a dose-dependent fashion in the rats. These findings are similar in both male and female animals. Both transaminases are vital indicators of hepatocellular damage, although ALT is primarily specific as a biomarker for liver damage (Mounnissamy *et al*., 2010).

In general, any alteration in ALT levels is often ensued by a parallel pattern of change in AST levels in cases of hepatic cell injury (Kuete *et al*., 2010). Likewise, the results in this study are in agreement with the latter, where both transaminases increased in a dose-dependent trend. Results with respect to the liver function tests obtained suggest that the increase in AST and ALT is largely due to liver damage and the resulting leakage of these enzymes from damaged hepatocytes into the circulation arose from altered plasma membrane permeability (Ksheerasagar and Kaliwal, 2006).

Fluctuations in alkaline phosphatase (ALP) levels were recorded for dosed animals of both genders in comparison to the controls. Although the elevation of this enzyme is slight and non-dose dependent in this study, the changes measured may be indicative of pre-necrotic changes of hepatocytes and suggestive of early onset of cellular disintegration, which will lead to compromise in plasma membrane integrity (Ashafa *et al*., 2010; Eissa and Zidan, 2009). Nonetheless, ALP is predominantly associated with cholestasis or biliary tract inflammation, which could ultimately induce this membrane bound hepatic enzyme to be released into the extracellular fluid following a toxic insult to the liver tissue (Gill *et al*., 2010; Kuete *et al*., 2010). The unfavorable increase in ALP enzyme has been commonly associated with renowned commercial anticoagulant drugs, namely warfarin, dabigatran etexilate and ximelagatran (Arora and Goldhaber, 2006). Results from this investigation on the non-defined trends of ALP levels, although statistically insignificant, warrant caution and require further verification on the probable nature on injury afflicted on the liver tissues of treated rats. This is in light of the potent nature of *Melastoma* leaf aqueous extract as an anticoagulant agent.

Considering the importance of histology for a complete toxicological dossier of *Melastoma* leaf aqueous extract in this study, major organs that are commonly affected i.e., the liver and kidney, were subjected to scrutiny at microscopic level. Organ-specific toxicities especially hepatotoxicity and nephrotoxicity, are often delineated to ingestion of herbal-based products due to their inherent toxic properties (Deng, 2002). Liver and kidney tissues were predominantly chosen for histopathology in this investigation because both organs play central roles in the detoxification and metabolic clearance of harmful substances from the body; and as such, these organs are usually the prime targets of toxic compounds (Elufioye *et al*., 2009; Stickel *et al*., 2005). Nevertheless, histology of kidney and liver samples of dosed animals revealed significant deviant changes attributable to the extract administration.

The kidney tissues of all treated animals, irrespective of gender, displayed moderate to severe progression of hemorrhage in the cortex and medulla regions, dose-dependently. Moreover, medullary tubular cell deterioration was noted apart from the bleeding episodes in the dosed animals. These findings are in accord with the renal histopathological manifestations in rats exposed to the organophosphate pesticide, fenitrothion (Afshar *et al*., 2008). Of major interest in this report is that the administered doses of fenitrothion, which caused the

aforementioned renal pathology, is similar to the dosages of *Melastoma* leaf aqueous extract used in the current work for the same period of 28-day toxicity study.

Tubular damage is often expressed as a sequela of tissue injury and impairment of kidney function, largely due to the highly sensitive tubular epithelial cells to toxicants (Ozer *et al*., 2010). Taken together, although the exact mechanism of renal injury remains unspecified in the present work, the histological pictures of the treated animals point to the administration of *Melastoma* leaf aqueous extract as the etiologic agent of these insidious toxic changes in the kidney. The predisposition of *Melastoma* extract with anticoagulant potency to cause bleeding in the kidney tissues is also of much concern, albeit the unequivocal fact that anticoagulant-related tissue hemorrhage is a common occurrence (Jaffer, 2008). Besides, the progression of tissue deterioration is remarkable and severe in magnitude in the male animals compared to their counterpart. This result, although a minor observation in this investigation, reflects the susceptibility of the males to succumb to the toxic plant metabolites that specifically affect the renal tubules.

Conversely, histology of the liver specimens constituted an altogether different trend of adverse changes in respect to the oral administration of *Melastoma* leaf aqueous extract. All hepatic venous structures of dosed rats appeared profoundly dilated and congested. Interestingly, these histologic findings are reminiscent of that documented earlier in the liver ischemia-reperfusion and resection injury model. Rats subjected to warm ischemia and reperfusion after partial hepatectomy displayed the same pattern of portal and central venous dilatation and congestion (Hossain *et al*., 1999). However, the causative clue in the latter cannot be extrapolated as the etiology to the injurious changes observed in the current study. In retrospect, a toxicity study on paracetamol also revealed similar manifestations of hepatic venous histoarchitecture, which encompassed of severe dilatation and congestion, in addition to significant elevation of the AST, ALT and ALP levels (Arafa, 2009). These pathological discrepancies are consistent with that found in the present toxicity challenge of *Melastoma* leaf aqueous extract, in which similar biochemical alterations confirmed the histologic findings. However, findings of this study were opposed to Alnajar *et al*. (2012) whereas ethanol *Melastoma* leaf ethanol extract did not showed toxic effects in rats for 14 days of acute toxicity study. Meanwhile, methanolic extract of *Melastoma* leaf was also displayed hepatoprotective activity (Mamat *et al*., 2013). The discrepancies of results were due to different solvents used in extraction of *Melastoma* leaf and resulted different composition of biological active components (Tiwari *et al*., 2011). Thus, the existing results of severe liver injury shown after the administration of *Melastoma* leaf aqueous extract can be traced to direct hepatotoxic mechanism of the toxic component(s) of the extract; although the exact underlying mechanism responsible for the observed changes are yet to be unearthed.

CONCLUSION

This is the first exploratory study of the subacute toxicity of *Melastoma* leaf aqueous extract. Collectively, the results of this study revealed toxic changes in both liver and kidney tissues, with alterations in important serum liver enzymes. It is also worth highlighting that many important anticoagulant agents in current clinical use have their fair share of toxic manifestations, especially in hepatic and renal tissues. Nevertheless, they are still being used extensively while concomitant monitoring of the various indices found adversely altered in laboratory evaluations are being carried out. Therefore, since conventional drugs with pharmacologic activities also have side effects, it should not be surprising to find potential adverse reactions in natural, phyto-medicines that are pharmacologically active. The nature of toxic insults inflicted upon the animals subjected to oral administration of different doses of *Melastoma* leaf extract provided valuable reference tool for future clinical studies. Further *in vitro* cytotoxicity evaluations and the isolation of bioactive compound(s) from this extract responsible for the observed toxic manifestations are currently underway.

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