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Evaluation of Sub-Acute Toxicity of *Ficuscarica* Spray-Dried Leaf Extract in Sprague Dawley Mice

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Abstract: ***Toxicity and various hematological and biochemical effects of Ficuscaricaspray-dried leaf extracts were investigated in Sprague Dawleymice. Two doses (1 g/kgand 3 g/kg body weight) of the extract were administered orally to mice that were subsequently sacrificedafter 28 days. The hematological parameters examined were total and differential leukocyte count (WBC), erythrocyte count (RBC), hemoglobin concentration (HGB) and platelet count (PLT).The biochemical parameters studied were serum total protein, alkaline phosphatase (ALP), aspartate aminotransferase (AST), alanine aminotransferase (ALT), urea, creatinine and total bilirubin. The results showed no evidence of sub-acute toxicity in the treated mice,indicating that the extract posed no significant toxicity risk. Hence, there is good likelihood that F. carica extracts are similarly safe with respect to sub-acute toxicity in humans.***

Keywords: *Ficuscarica* ,spray-dried leaf extract, sub-acute toxicity

1. Introduction

Ficuscarica L. is a member of the genus *Ficus*, commonly referred to as "figs" (Dueñas, *et al*, 2008). Many figs produce fruits that are of economic and nutritional value, and they also constitute an important part of the biodiversity in the rainforest ecosystem (Ronsted*et al.*, 2007).*F. carica*has a long history of cultivation for their edible fruit and medicinal properties in other plant parts. The species is believed tohave originatedfrom Western Asia, subsequently spreading to the Mediterranean by human agencies (California, 1996). Turkey,Egypt, Morocco, Italy, Brazil, Spain, Greece, California andother countries that experience mild winters and hot dry summers are major producers of edible figs (Tous and Ferguson, 1996).

Fig leaves are believed to be an important source of phytochemicals with potential therapeutic properties. They have a place in many traditional medicinal practices that are based on the use of plants and plant extracts for primary healthcare. Oliveira (2009) notes that numerousbioactive substances such as phenolic compounds,organic acids, phytosterols, anthocyanins,coumarins, triterpenoids and volatile constituents such as hydrocarbons, aliphatic alcohols, and several otherclasses of secondary metabolites are present in figs. Water extracts of the leaves of *F.carica* have been demonstrated to contain phenolic acids such as 3-*O*- and 5-*O*-caffeoylquinic acids, ferulic acid, quercetin-3-O-glucoside, quercetin-3-*O*rutinoside,psoralen, bergapten, and organic acids (oxalic, citric, malic, quinic, shikimic, and fumaric acids) (Oliveira *et al*., 2009).

Volatile compounds from the leaves of *F. carica*include the aldehydes methylbutanal, 2-methylbutanal, (*E*)-2-pentanal, hexanal, and (*E*)-2-hexanal, the alcohols 1-penten-3-ol, 3-methyl-1-butanol, 2-methylbutanol, heptanol, benzyl alcohol, phenylethyl alcohol, (*E*)-2-nonen-1-ol,the ketones 3-pentanone, the esters methyl hexanoate, methyl butanoate, hexyl acetate, ethyl benzoate, and methyl salicylate, the monoterpenes limonene and menthol, the sesquiterpenes α-cubenene, α- guaiene, α-ylangene, copaene, β-bourbonene, β-elemene, α-gurjunene, β-caryophyllene, β-cubebene, aromadendrene,τ-muurolene, τ-cadinene, α-muurolene, germacrene D, and (+)-ledene, the norisoprenoidβ-cyclocitral, and miscellaneous compounds such as psoralen (Oliveira *et al.*,2010). For centuries, figs have been used in medicine, as recorded in classical Middle Eastern and European medical writings. Figs have been used for the treatment of tumors and other abnormal swellings due to infection or cancer (Ben-Noun, 2003). The bioactive compounds present in figs include coumarins, flavonoids, sterols, triterpenoids, anthocyanins that are found in all part of figs. The pharmacological actions of figs include their antibacterial, anti-inflammatory, antioxidant, gastroprotective, vulnerary, antidiarrheal, antitumor, antispasmodic, anticancer, immunobalancing/ immunoharmonizing, antidiabetic, antihelmintic, antiplatelet, hepatoprotective, anti-inflammatory and antipyretic activities (Anshul *et al.*, 2012). Because of the numerous beneficial compounds in fig leaves, they are potentially valuable when consumed for health or used in alternative treatments. Nevertheless, despite the increasing number of reports on the medicinalbenefits of the *F.carica* leaves, potential deleterious effects such as sub-acute toxicity need to be investigated. Such a study would serve as an importantbaseline for developing this plant as anherbal medicine.

2. Materials and Methods

Extract sample preparation

Fresh leaves of *Ficuscarica* collected in Chuping Valley in Perlis, Malaysia were washed, air dried and ground to small fragments of about 5mm. Maltodextrin (10%) was added to the dry leaf grindings to prepare a spray-dried extract. The extract was stored in an air tight container at room temperature for further experiments on toxicity.

Test animals and extract doses

FortySprague Dawley mice(7 weeks old, 20males and 20females) were used for evaluation, with 5 males and 5 females in each group. The extract in solution was administered by oral gavage daily for 28 days. Food and water intake were observed. The experimental treatments were as follows:

Group 1: Control (received water only)
Group 2: Low dose (received 1g/kg of extract)
Group 3: High dose (received with 3 g/kg of extract)

The main observations in this study were concerned with toxicology evaluations of the fig leaf extracts following the OECD/OCDE Test Guideline 407 over 28 days on the vital organs (liver, kidney) and the biochemical parameters.The body weight and physical appearance of the mice were monitored throughout the experiment for signs of toxicity, with recordings taken once a week.

Haematological and biochemical analysis

At termination, the mice were fasted overnight by removing all food from the cages but were allowed access to water *ad libitum* before blood was collected. The liver and kidneys were harvested for histopathology analysis. Mice were anaesthetized with light ether and blood samples were collected via direct heart puncture and put into two types of tubes, one with anticoagulant (EDTA) and the other without any additive. The EDTA blood samples were analyzed immediately for hematology parameters using the Hematology Analyzer (Medonic CA 620 VET, Stockholm, Sweden). These parameters included total and differential leukocyte count (WBC), erythrocyte count (RBC), hemoglobin concentration (HGB) and platelet count (PLT). The blood samples without additives were used in biochemical tests that included assays for serum total protein (Gornall *et al*., 1949), alkaline phosphatase (ALP) (GSCC, 1970), aspartate aminotransferase (AST), alanine aminotransferase (ALT) urea (Reitman and Frankel, 1957), creatinine (Blass *et al*., 1974) and total bilirubin (Evelyn and Malloy, 1938). The tests were performed using the biochemistry analyzer (Vitalab Selecta, E-series, Netherlands).

Histopathology observations

Small blocks of tissues were sampled from the kidney and the liver of the mice and processed using an automated tissue processor (Leica TP1020). The tissues were then sectioned to a thickness of 5 µM using a rotary microtome and the sections dried overnight in an oven at 37 ◦C. The sections were stained with hematoxylin and eosin (H&E) and examined under the microscope for signs of toxicity.

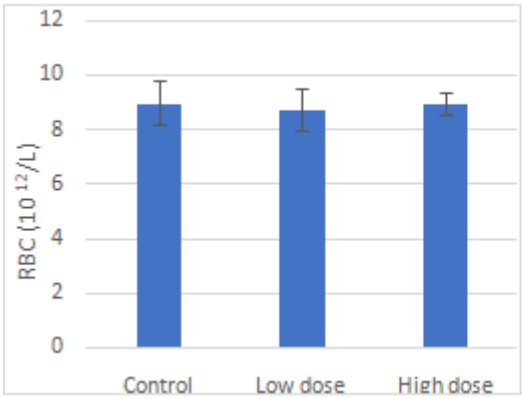
Statistical analyses

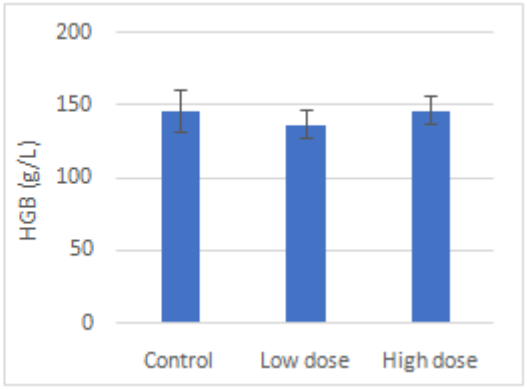
A completely randomised design was used for the experiment. The results were expressed as means ± standard deviations. The t-test was used to compare the serological, histological and biochemical results from the different treatments, where a p value of less than 0.05 was indicative of a significant difference. The Statistical Package for the Social Sciences (SPSS) was used in the statistical analyses.

3. Results

Haematological parameters

Haematology results in the control and treated mice are shown in Figure 1. The mice that were treated with spray-dried extract of figs leaves did not show significantly altered levels of RBC, HGB, PLT and WBC when compared with the controls.





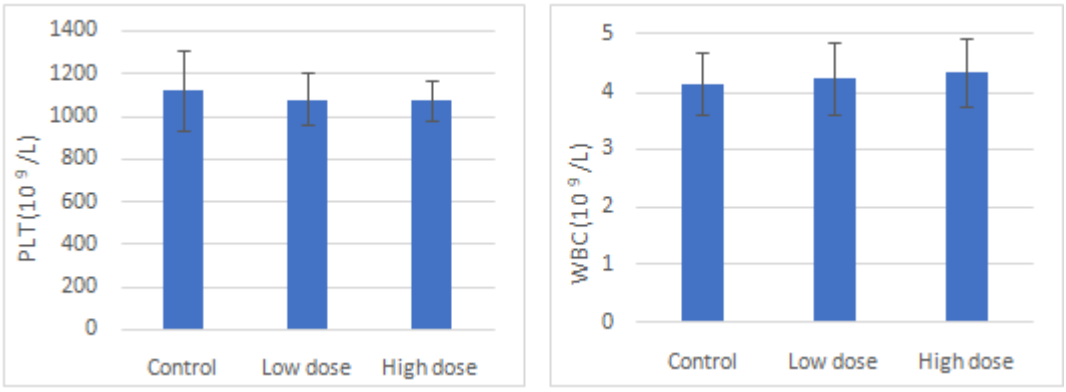


Figure 1: Haematological indices of mice administrated with spray-dried leaf extract of *Ficuscarica.* Means and standard deviations after 28 days are shown

Serum biochemical parameters

The biochemical indicators and activities of the blood serum enzymes are shown in Figure 2. There were no significant differences in the various biochemical test outcomes between the controls and the mice treated with fig leaf extracts, either in low or high doses.

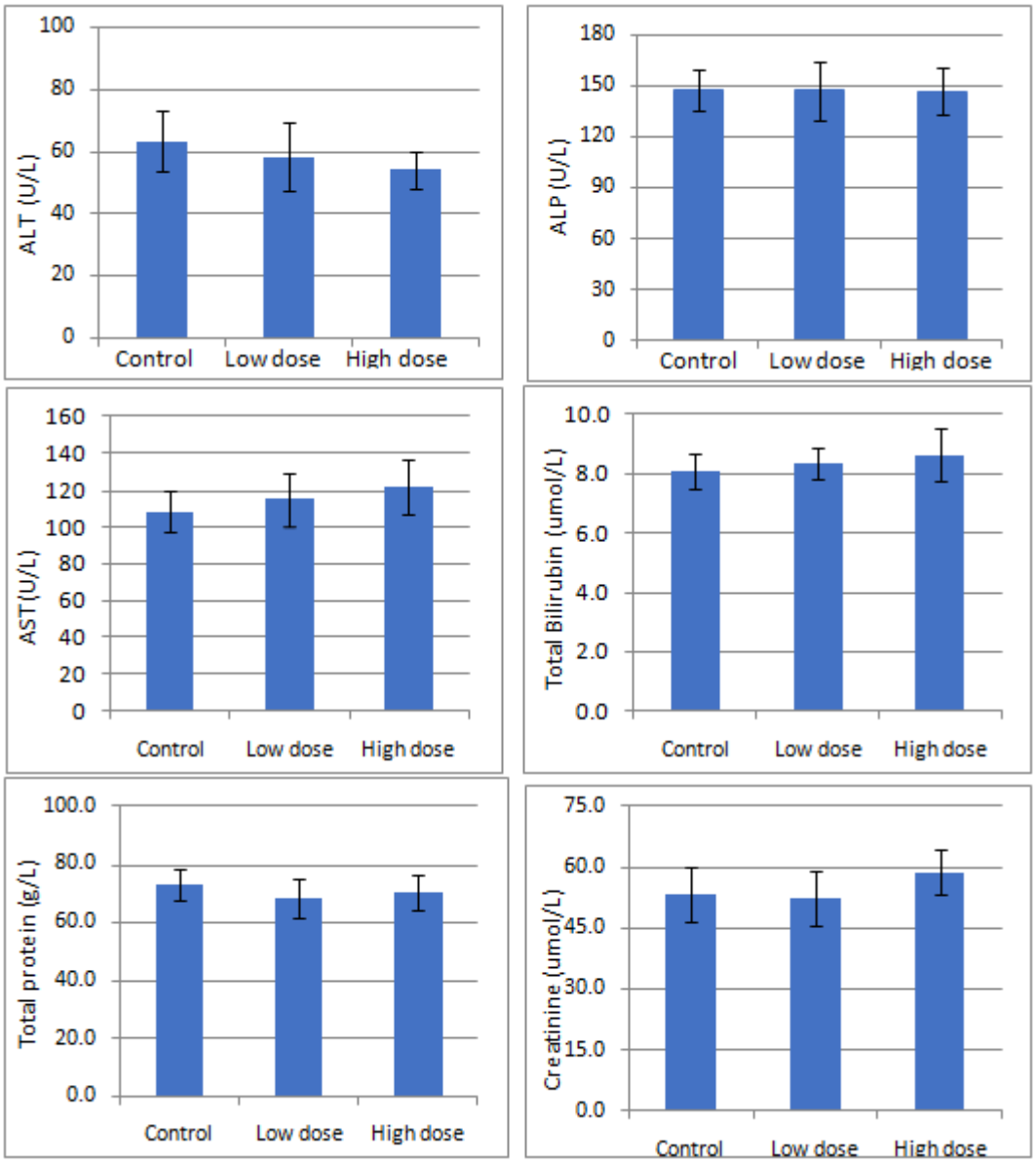


Figure 2: Biochemical tests performed on blood serum of miceadministrated with spray-dried leaf extract of *Ficuscarica.* Means and standard deviations after 28 days are shown

Histopathology observations

Histopathology examination of the vital organs, i.e. the kidney and liver, of the mice treated with fig extract did not reveal any difference as compared with the control (untreated) mice.Treatment with either 1g/kgor 3g/kgdid not produce any toxic symptom, and no change inthe kidney tubules or kidney glomeruliwasvisible under the microscope (Figure 3).

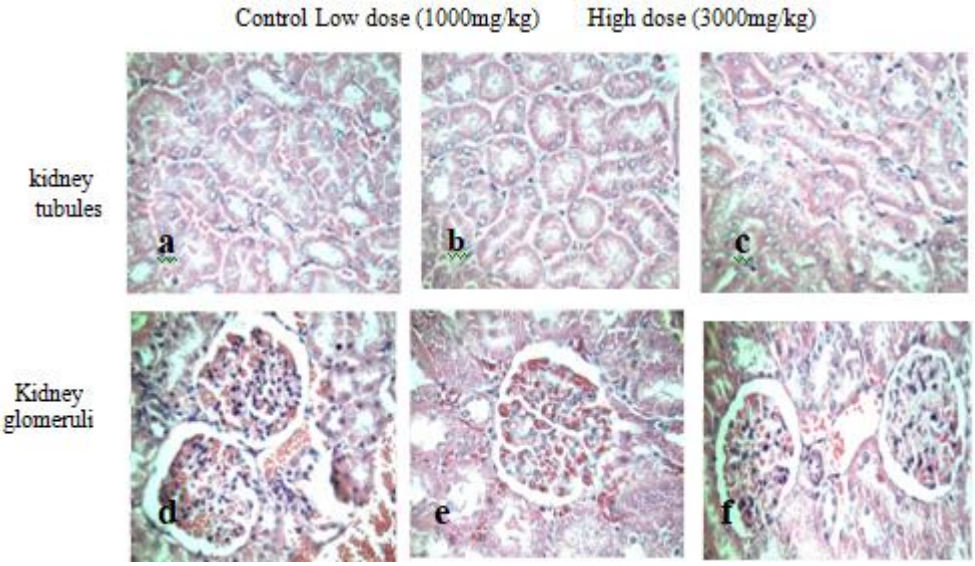


Figure 3: Histological sections of mice kidney tubules in the control (a), low dose treatment (1g/kg) (b) and high dose treatment (3g/kg) (c), andkidney glomeruli in the control (d), low dose treatment (1g/kg) (e) and high dose treatment (3g/kg) (f). (mag 20x)

The results of histopathology changes in the liver tissues are shown in Figure 4. Histological examination of samples from the liver of mice treated with 1 g/kg or 3g/kg *F.carica* extract revealed no abnormalities characteristics that might be indicative of sub-acute toxicity study in bile capillaries, liver cells orblood capillaries in the liver cells. There was no leakage arising from the treatments in bile capillaries of the liver.

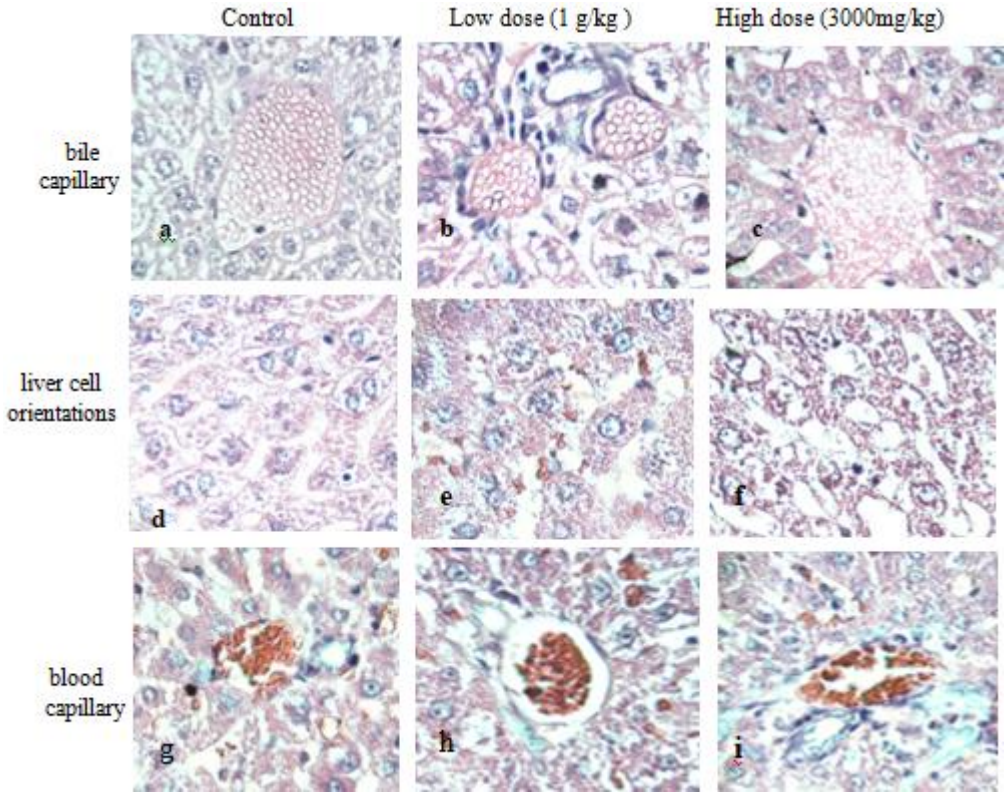


Figure 4: Histological sections of liver tissues: liver bile capillaryin the control (a), low dose treatment (1g/kg)(b) and high dose treatment (3g/kg) doses (c). Liver cell orientations in the control (d), low dose treatment (1g/kg) (e) and high dose treatment (3g/kg) (f). Liver blood capillary in the control (g), low dose treatment (1g/kg) (h) and high dose treatment (3g/kg) doses (i) (mag 20x)

4. Discussion

Herbal remedies are commonly perceived by the community as natural health treatmentsthat are generally free from side effects and the risks that they might carry. Hence, medicinal plants are presumed by many people to be safe to consume without compromising effects on their health (Harizal*et al.* 2010; Hor*et al*., 2011). Such herbal medicines are especially popular in developing countries, including Malaysia. Herbal cures are often viewed in the same light as food supplements. Dietary supplements are substances that are taken to complement one's normal diet. They can be vitamins, minerals, herbs or plant parts that contain these substances. According to Wang *et al*. (2011) theproper use of medicinal plants as dietary supplements can be useful and important in the maintenance ofhealth. However, many studies have also reported harmful effectsfrom improper use of medicinal plants (Kao *et al*., 1992; Tai *et al.*, 1992; Vanherweghem*et al.*, 1993). The evaluation of toxicological effects of medicinal plants or theirextracts is,therefore, essential before their widespread use in animals and humans (Thamilvaani, 2014). Medicinal herbs are generally self-prescribed by consumers and there is a lack of control and review in terms of dose, manner, and frequency of administration. While the phytochemicals in medicinal herbs occur in nature, they may not be naturally compatible to the human constitution. Any compound that has a therapeutic effect has also the potential to be incorrectly prescribed,leading to over-dosage. (Siti Surian*et al*., 2014)and subsequent health problems, for example, renal toxicity (Mohammad, 2012). This is becoming a matter of concern with herbal medicines gaining popularity as complementary and alternative medicines. With this consideration in mind, the present study aimed to examine the toxicological and biochemical effects of the*Ficuscarica* leaf extract which is commonly used in Malaysia for its medicinal properties. The results of this study could help to allay concerns over the safety of productsderived from this plant. Herbal toxicity depends upon the absorbed dose, the route of exposure and duration of exposure, i.e. acute or chronic. The 28-day toxicity test that has been adopted for sub-acute oral toxicity assessment in this study is commonly used to obtain evidence on safety prior to the commercialization of pharmaceutical products (Arts *et al.*, 2004; Bautista *et al*., 2004).

In the present study on hematology parameters, *viz.*RBC, HGB, PLT and WBC,andserum biochemical parameters, *viz.*serum total protein, alkaline phosphatase (ALP), aspartate aminotransferase (AST), alanine aminotransferase (ALT), creatinine and total bilirubin,no significant differences were observed between the fig leaf extract-treated mice and control mice ofboth sexes. The present study confirmed the safety of *F.carica* leaf extract with respect to sub-acute toxicity in mice. Similar oral toxicity assessment had previously been carried out on the methanolic extract of *Hibiscus rosa-sinensis* L. leaves in mice. Signs of toxicity were not observed during the 14 day observation period with the administration of 400 mg/kg doses of the extract (Purobi and Arun, 2015). As in the present study, there were no obvious adverse effects on the hematological, biochemical parameters studied, or on histopathology of the liver and kidney. On the other hand, when the dosage was increased to 800 mg/kg, hepato-renal toxicity, as evidenced by elevated levels of alanine aminotransferase, aspartate aminotransferase, total and indirect bilirubin, urea and creatinine, was observed. In another study, no abnormal histological changes were observed (after 28 days)in any of the animal groups treated with petroleum ether extract of *W. volubilis* in sub-acute toxicity evaluations (as assessed byserum biochemical parameters such as ALT, ALP, bilirubin, total protein, total cholesterol, triglycerides, glucose, urea, creatinine, sodium and potassium and α-amylase, and histopathology of isolated organs), indicating that the petroleum ether extract of *W. volubilis* was non-toxic (Velmani et la, 2014).

The kidneys are protected through adequate consumption of water and avoiding excess protein intake (Mohammad,2012). According to Halijah*et al.*, (2003), the kidneyis the organ most liable to be adversely affected by plant-based compounds that affect human health. Nevertheless, the inherent properties of the herb are not the only source of herb-associated renal disorders, as mistakes in dosage and identification, and contaminants within the mixture are all issues of concern. Strict controls on the presence of adulterants in herbal medicines and proper labeling of dosages must be maintained to ensure the safety of consumers of herbal medicines (Mohammad, 2012). Liver function deficiency can be detected from elevated activities in serum alanine transaminase, alkaline phosphatase and bilirubin because these molecules leak into the blood stream as a consequence of liver damage (Wannang, 2007). Similarly, increase blood creatinine and urea is a good indicator of any negative impact on kidney functions (Hassan *et al*., 2007).

The liver forms bile from water, electrolytes, and other molecules including cholesterol, bilirubin, bile acids, and phospholipids. Adults produce 400-800 ml of bile per day; it is stored in the gallbladder until needed (Bowen, 2007). The high prevalence of tubular bile in the kidneys of patients with jaundice and renal injury would suggest potential nephrotoxicity of bilirubin (van Slambrouck *et al.*, 2013).

In the present study, histopathology evaluation of *F.carica* leaf extract on the liver and kidney of mice found no adverse effect on the morphology of the organs. As indicated earlier, all animals in the present study treated with 1g/kg extract or 3g/kg elicited no morphological change in kidneys and liver tissues that might indicate sub-acute toxicity. According to the Loomis and Hayes classification (1996), a chemical substance with a LD_{50} within the range of 5000–15000 mg/kg is considered as practically non-toxic. Nevertheless, exceptions to this guideline have been noted. In a study conducted by Alade*et al.* (2009) on*Bauhinia. Monandra* leaf extract, proximal tubularepithelial necrosis and a variation in the lung between the controls andtreated rats were found even at a dose of 4000 mg/kg. Akanmu *et al.*(2004) found histological changes (widening of proximal tubules) in the kidneys of rats treated with 1000 mg/kg of Cassia *fistula* pod extract.

5. Conclusion

In the present study, the 28-day sub-acute toxicological evaluation showed that treatment of mice with *F.carica*leaf extracts did not elicit any significant toxicological or histopathological change in the kidney and liver tissues.

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