

Assessment of Potential Toxicological Effects of Cinnamon Bark Aqueous Extract in Rats

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Abstract: Cinnamon stick is world widely used in cooking, traditional medicine, perfumery and aesthetic industries. Many studies have demonstrated the potential of cinnamon extracts in diabetes treatment. Although it has been reported as safe in general cooking recipe and categorized as GRAS by USDA, sub-acute toxicity procedure was conducted in this study to determine the effect of cinnamon extract on histopathological changes as well as the haematological parameters of blood. Water extraction was done for dried cinnamon. Twenty-four female Sprague Dawley rats were used in this study. The oral route was selected because it is the most likely route of human exposure through the consumption of herbs. The concentrations studied were 0.1, 0.5 and 2.0g/kg cinnamon aqueous extract (CE). There were no statistically significant effects of all concentrations of CE on behaviour, mortality, water intake, food consumption, weight gain, internal organs weight (liver and kidney) and heamatological parameters during treatment and post-treatment periods except: 1) the slight decreased in kidney and liver weight of rats treated with 0.5g/kg and 2) slight decreased in liver weight of rats treated with 2.0g/kg, during post-treatment period. Hence, these toxicity studies suggest that the CE is low to moderate in toxicity and CE below 0.5 g/kg dose level is safe to be used in the efficacy study especially for diabetes treatment.

Key words: Aqueous extract, cinnamon, histopathology, haematology.

1. Introduction

Herbal medicine has enjoyed tremendous popularity worldwide. It has been used as an alternative road to wellness since antiquity even before the synthetic pharmaceutical drugs were introduced. However, there are others who regard herbal medicine with skepticism especially those in mainstream healthcare delivery systems. It is perceived as unsafe, under-developed and not well documented in addition to the possible side-effects and adverse reactions to the herbal medicines. Nonetheless, herbalists beg to differ. Traditional medicine now plays a greater role in modern medicine whereas a lot of readily available medicinal plants offer great potential for the discovery of new drugs while many others are yet to be unearthed [1]-[8].

Currently, there is a growing academic and industrial involvement in traditional medicine. In order to

ensure that people will unequivocally embrace herbal medicine, researchers are executing thorough scientific investigations to provide evidences for its efficacy, safety and toxicity as well as the mechanisms of action. Plants are generally perceived to be safe, but there are plant materials which are hazardous to human being indicating the importance of toxicity study prior to human consumption [9]-[11].

Cinnamon is one of the must-have ingredients in our spice rack. Not only it possesses the unique characteristic as a flavouring agent, but also is famously known for its medicinal values. Barceloux (2009) shared several traditional uses of cinnamon, for instance as an astringent, germicide and antispasmodic [12]. According to Hwa *et al.*, (2011), cinnamon was one of the foremost spices used in the early treatment for chronic bronchitis. In addition to that, other traditional practices for cinnamon include the treatment of impotence, frigidity, dyspnea, eye inflammation, rheumatism, vaginitis as well as wounds and toothaches [13].

Nowadays, numerous discoveries were made on cinnamon potential through modern research and cutting-edge technologies. Findings showed that cinnamon has significant activities as a hypoglycemic and cholesterol lowering agent [14] and mentioned polyphenolic polymer isolated from cinnamon to have insulin-like activity and antioxidant activity *in vitro* [15], [16]. Recent studies from Anand *et al.* (2010) found that *in vitro* incubation of cinnamon extract led to enhanced insulin release while Patel *et al.* (2012) listed cinnamon as an antidiabetic medicinal plant with insulin mimetic property [17], [18]. Apart from its antidiabetic property, it has shown to be promoting wound healing, antimicrobial agent, and anti-inflammatory compound [19]-[21].

Evidences exist, though very few, on safety concerns and toxicity of cinnamon consumption. Dugoua *et al.* (2007) reported that cinnamon is safe to be used in medicinal amounts but may be of concern when it is used in excessive amounts or in long term while Newal *et al.* (1996) specified that the amount of cinnamaldehyde consumption should not exceed 700µg/kg [22]-[24]. Research conducted by Shah *et al.* (1998) stated that there was no significant acute and chronic mortality during their toxicity study on the ethanolic extract of *Cinnamomum zeylanicum* bark [22]. According to the United States Food and Drug Administration (USFDA), *Cinnamomum spp.*, including common and cassia cinnamon are generally recognized as safe when used in amounts commonly found in food. Anderson (2008) also reported that there have been no adverse events accounted in all human studies involving cinnamon, or aqueous extracts of cinnamon [25].

Therefore, this study was undertaken to examine the possible toxicity effect of oral administration of the aqueous extract of cinnamon (CE) in rats in order to determine the 50% of lethal dose (LD50) value to be used as future references. Results of this study will enable us to project safety levels of cinnamon in animals and human. Sub-acute toxicity procedure was conducted in this study to determine the effect of cinnamon extract on haematological parameters of blood as well as the histopathological changes. The oral route was selected for toxicity studies because it is the most likely route of human exposure through the consumption of herbs.

2. Materials and Methods

2.1. Chemicals

Diethyl ether and hydrochloric acid were purchased from CheMar. Xylene, acetic acid, glycerol and ethanol were obtained from MERCK. Potassium acetate, Wright's stain and paraffin plasticized pellets were obtained from BDH. Formaldehyde was purchased from HmbG chemicals. Harris hematoxylin solution and eosin Y were purchased from Sigma-Aldrich.

2.2. Plant Material and Preparation for Extract

The plant material used in this study was the dried stem bark of cinnamon. The barks were purchased from Dru Era, Ceylon. Cinnamon bark was supplied by Nasuha Spices & Herbs Farm near Johor, Malaysia (GPS Coordinates: GPS 1.940900N 102.741300E) using the standard management procedures for the species. Plant sample for voucher specimen was collected by author on Dec 12 2012 and was deposited to UTM Herbarium with voucher number UTM122009-1067. The cinnamon bark (26.5kg) was then dried, grinded later extracted with 110L of water (1:4) for 8h at 120°C by turbo extractor through hydro distillation process. The water extract from the turbo extraction was proceeded to spray dried process to form powder. Dry yield was 2.09% (w/w).

2.3. Preparation of Experimental Animals

Twenty-four female Sprague Dawley rats weighing 200-260g were used in this study. The animals were individually housed in cages under a 12-h light and 12-h dark cycle in a temperature controlled room (25-27°C). Prior to the experiments, the rats were acclimated for a week and were allowed to free access of standard laboratory animal diet and water ad libitum.

2.4. Experimental Design for Sub-acute Oral Toxicity Study (Repeated Dose 14 Days)

The Fixed dose procedure was followed as described in OECD Guidelines 420 (2002) for oral toxicity study in order to determine the LD50. Groups of twenty-four female rats were divided into four groups which were fed with the following diet (Table 1):

- Group Control: Normal rats received distilled water (control)
- Group CE 0.1g/kg: Normal rats received 0.1 g/kg powdered cinnamon extract (CE)
- Group CE 0.5g/kg: Normal rats received 0.5 g/kg powdered cinnamon extract (CE)
- Group CE 2.0 g/kg: Normal rats received 2.0 g/kg powdered cinnamon extract (CE)

Treatment rats were treated once daily by oral administration using sterile ball-tipped gavages needle for 14 days respectively. Close observation was made during the first four hour to examine any adverse effect symptom such as abnormal behaviour, abnormal posture, diarrhea, blood in urine and increase in heart beat caused by CE consumption [26].

Food consumption, water intake and the body weight gained was recorded at day 0, 3, 7 and 14. Blood was withdrawn from tail vein by sterile needle on day 0, 7 and 14 for hematology. Half of survived rats ($n=3$) from each group were sacrificed on day 14 of sub-acute toxicity study to obtain liver and kidney for their relative weight (Table 1). Tissues for microscopic examination were fixed and preserved in 10% neutral buffered formalin, processed and trimmed, embedded in paraffin, sectioned to a thickness of 4-6µm, and stained with hematoxylin and eosin. Complete histopathologic examinations were performed for any abnormalities and toxicological signs.

Table 1. Designated Group for Toxicity Study

Day/Dose	Control	CE 0.1g/kg	CE 0.5g/kg	CE 2.0g/kg
Day 14 (sub-acute toxicity study)	3 rats	3 rats	3 rats	3 rats
Day 28 (recovery period)	3 rats	3 rats	3 rats	3 rats

2.5. Experimental Design for Fourteen Days Recovery Period Study

The Twenty- Half of the survived rats ($n=3$) were returned back to their own cage and being kept for another 14 days for further observation (Table 1). During the period, all groups of rats were not treated with any CE extracts and were fed with food pellets and water ad libitum. Occurrence of toxicity symptom was observed twice daily. The animals were weighed at day 21 and 28 during recovery period. All rats were

euthanized at day 28 to harvest liver and kidney for their relative weight. The organs were further analyzed via histological examination for any abnormalities.

2.6. Statistical Analysis

Data of body weight were evaluated using Sigma Plot 11.0. Values were expressed as means \pm SE. Statistical significance of treatments was determined using the Tukey's test. Food consumption, water intake and relative organ weight's data were evaluated using Sigma Plot 11.0. Values were expressed as means \pm SE. Dunnet's test was used to evaluate the results statistically. Data for differential white blood cell (WBC) count were performed using SPSS statistical version 15. Values were expressed as means \pm SE. Non-parametric Mann-Whitney U-test was used to evaluate the results statistically.

3. Results and Discussion

3.1. Survival Rates, Feed Consumption, Water Intake, Alterations in Body Weight and Relative Organ Weights

There was neither any mortality nor abnormalities in general behaviour observed in all groups throughout the study. Therefore, the approximate LD of cinnamon aqueous extract in Sprague Dawley (SD) rats was higher than 2.0 g/kg. This result was in line with the study by Shah *et al.* (1998), showing no sign of toxicity in rats when low dosage of CE were administered [22].

In order to examine the development of secondary stage of toxicity caused by chemical analysis, parameters such as food consumption and water intake were considered. Changes in food consumption and water intake may relate to the alterations in body weight as well as in organ weight.

No significant differences were observed among all the four groups in food consumption during the 28 days experimental period (Fig. 1a). However, at day 7, groups treated with 0.1 and 2.0 g/kg of CE showed lower food consumption than control group. These results were aligned with the body weight changes results, which showed significant lower in body weights of CE 0.1 and CE 2.0 groups compared to controls at 7 days of experimental period. It may be due to poor palatability of the feed. It is believed that CE did not play a role in food consumption alteration because they showed no dose-related effect.

The overall water consumption of animals receiving the CE extract was generally similar to that of the vehicle control. This result suggested that consumption of all CE dosages was not affecting the changes of water intake during the study period (Fig. 1b).

Changes in body and internal organ weights could be used as an indicator for unspecific adverse side effects. Generally, weight loss is a simple and sensitive index of toxicity after an exposure to toxic substance [27], [28]. From (Fig. 1c.), the result showed no significant difference in the body weight changes for all groups throughout the study. Hence, it indicated that the comparable amounts of CE consumed within the experimental period did not cause toxicity.

Relative liver and kidney weights of the experimental rats were calculated based on Abdullah *et al.* (2009) method and are presented in (Fig. 2a and b) [29]. No statistical differences were observed between the relative organ weight of liver and kidney in the orally treated groups compared to the control group during 14-day sub-acute toxicity study (Fig. 2a and b). The oral administration of 0.5 g/kg CE significantly decreased the relative liver and kidney weights compared to control group after fourteen days recovery period (Fig. 2a and b). This result was similar with the work done by Shah *et al.* (1998), where *C. zeylanicum* treatment caused reduction in the relative liver weight [22]. Besides, the rats fed with 2.0 g/kg of CE showed a significant decrease in relative kidney weight as compared to control group but not in relative liver weight (Fig. 2a and b).

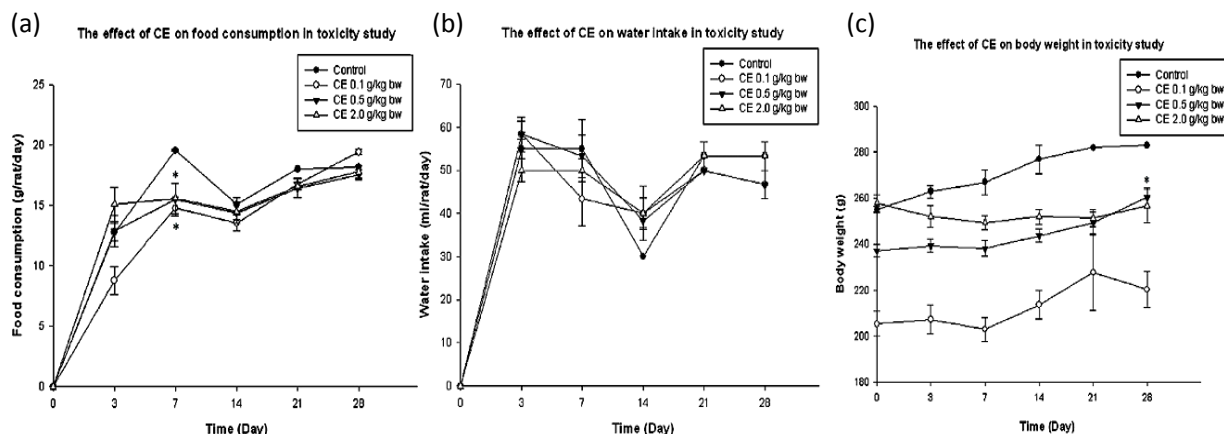


Fig. 1. (a) The effect of CE on food consumption in normal rats. (b) The effect of CE on water intake in normal rats. (c) The effect of CE on body weight in normal rats.

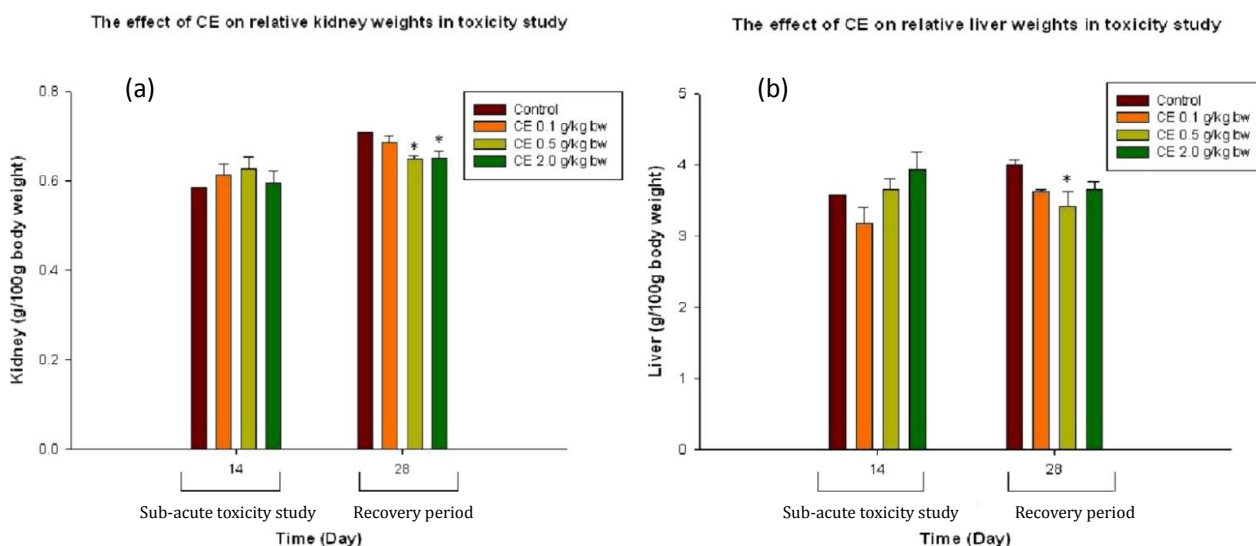


Fig. 2. The effect of CE on relative organ weight in normal rats in sub-acute toxicity study and recovery period. (a) Relative kidney weights in each group. (b) Relative liver weights in each group.

3.2. Haematological Examination

The effects of repeated-dose oral administration of the CE on the differential white blood cells (WBC) count parameters were presented in Fig. 3. The differences in the values of differential WBC count parameters between the control group and the experimental rats were statistically insignificant. Thus, this result did not appear to be related to the treatment with CE because they showed no dose-response relationship.

3.3. Histological Examinations

Histopathological changes in liver and kidney of the control and experimental rats during 14 days sub-acute toxicity study as well as fourteen days recovery period were compiled in below (Fig. 4). However, since diethyl ether was used in this study to euthanize the animals, its application may have affected the findings.

Fig. 4a revealed no apparent changes in the morphological structure of the liver in normal rats since

significant changes occurred after administration of cinnamon aqueous extract (CE).Thrombus in the sinusoidal liver tissue of rat treated with 0.1 g/kg CE indicated the presence of toxic substances though other parts of the liver tissue were normal (Fig. 4b). Fig. 4c showed sinusoids were more experienced thrombosis in rat's liver tissue treated with 0.5 g/kg CE. Moreover, nucleuses of the hepatocytes began to lose chromatin. From Fig. 4d., fibrinous material in the endothelium of portal vein showed that injuries were caused by toxic substances. Thrombus formation began to occur in the portal vein itself and the sinusoid cells started to show a presence of fibrinous material. However, no fatty liver had occurred.

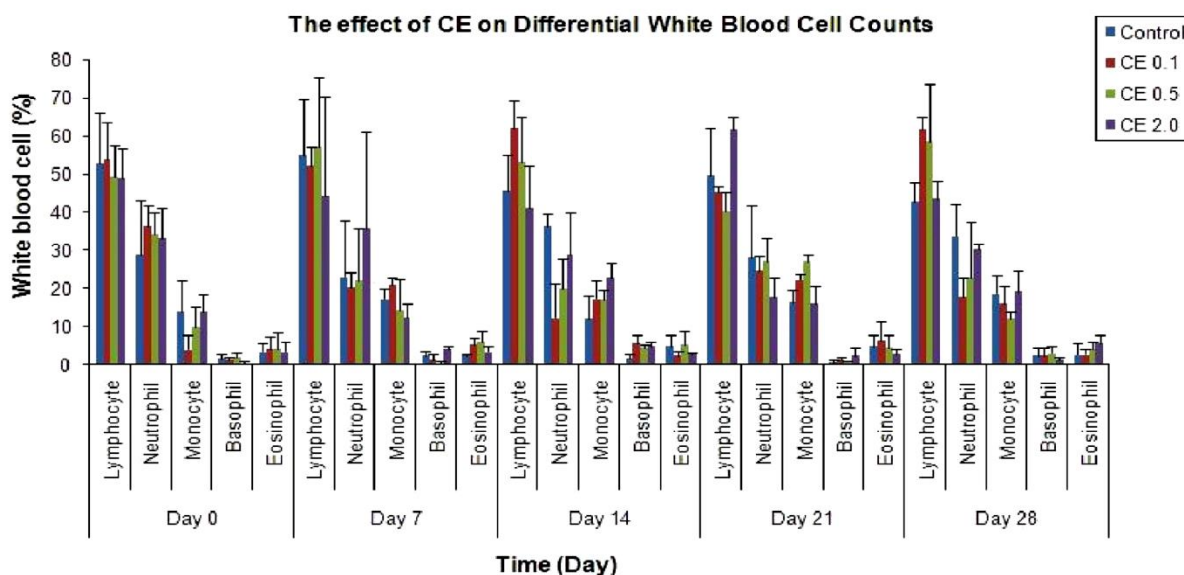


Fig. 3. The effect of CE on differential count of white blood cell in normal rats.

Fig. 4e showed that hepatocytes began to lose their nucleus in control rats. This was a symptom of fatty liver in which the nucleus was pushed to the peripheral and lost. Swelling of hepatocytes and presence of fibrinous material and thrombus were clearly shown in the liver tissue of rats treated with 0.1 g/kg CE (Fig. 4f). In addition, Fig. 4g exhibited a clear fibrinous material of sinusoids and swelling of hepatocytes with exception of the thrombus presence while in rats treated with 2.0 g/kg CE, the situation was more serious because of fibrinous material met the hepatic portal vein lumen and sinusoidal (Fig. 4h).

Fig. 4i to 4p showed the histopathological changes in the kidney of control and experimental rats after 14-day sub-acute toxicity study along with the findings for fourteen days recovery period. Fig. 4i illustrated normal architecture of kidney of the control rats. Bowman capsules space narrowing indicated swelling of the glomerulus in the rat administered with of 0.1 g/kg CE. Convoluted proximal tubules also showed that cells were having hypertrophy (Fig. 4j). Fig. 4k showed thrombus presence in the small blood vessels that supplied nephron tissues in the kidney section of the rat given 0.5 g/kg CE. The cells lining on proximal and distal convoluted tubules were experiencing hypertrophy. Besides, glomerulus had fibrinous materials which showed sensitivity towards toxic substances in the rats supplemented with 2.0g/kg CE (Fig. 4l).

Fig. 4m illustrated normal appearance of kidney from control rats. Fibrinous materials can be observed in the proximal convoluted tubules and hypertrophy of the cells lining the tubules in kidney tissue of the rat treated with 0.1 g/kg CE (Fig. 4n). Mild fibrinous materials were observed in the glomerulus area of rats treated with 0.5g/kg CE (Fig. 4o). Early development of glomerulonephritis was spotted in kidney of rats treated with 2.0 g/kg CE (Fig. 4p).

Thus, these results revealed serious signs of toxicity in the liver and kidney of the rats treated with 2.0

g/kg CE. Although pathological changes were observed in liver and kidney of the rats supplemented with 0.1 and 0.5 g/kg of CE, there were no relevance of serious signs in the histopathological examination. In consequence, these results suggested that administration of CE below 0.5 g/kg dose level is safer to be employed for further study.

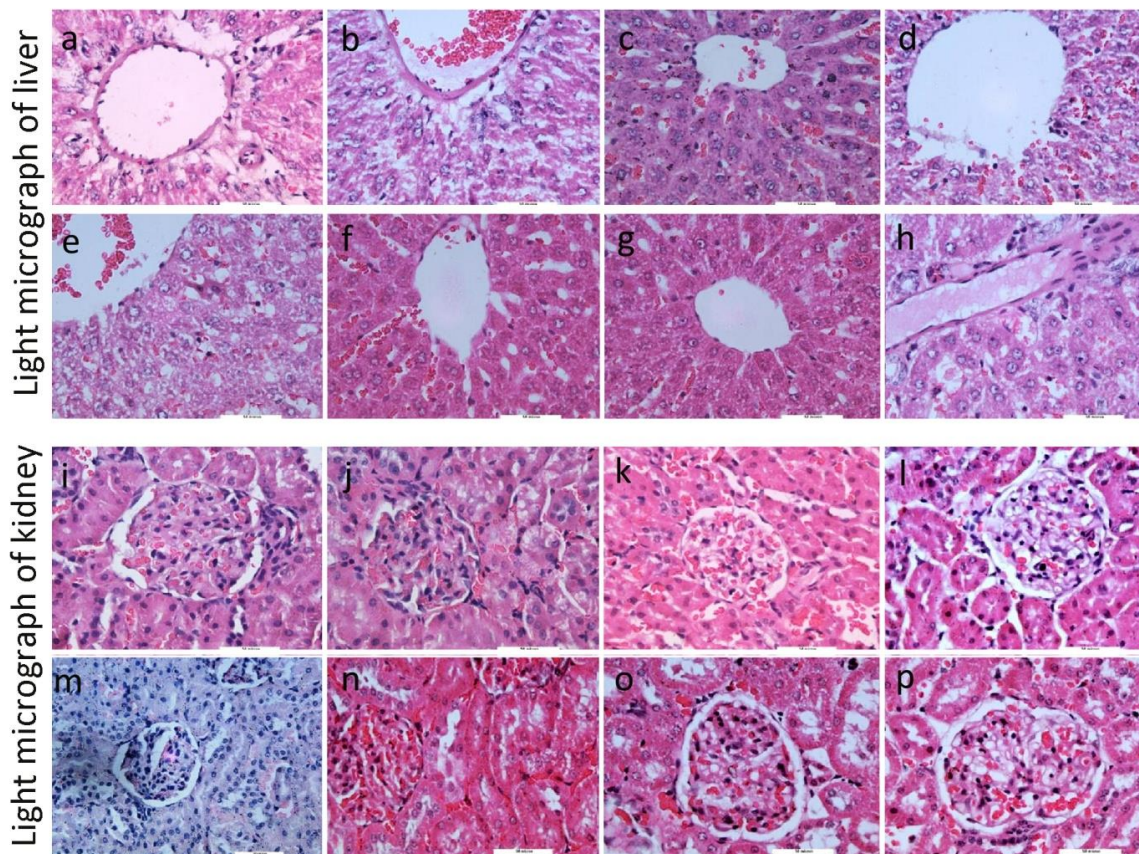


Fig. 4. Light micrograph of liver and kidney during 14 days sub-acute toxicity study.

4. Conclusion

Haematological and histopathological data of rats fed with cinnamon bark aqueous extract were evaluated in this acute oral toxicity studies to select suitable starting dose for human phase I study. These toxicity studies suggest that the CE is low to moderate in toxicity, evidenced by high LD50 values with no lethality. The CE below 0.5 g/kg dose level is safer to be used in the efficacy study. With the low toxicity of the CE, it offers a wide margin of safety for beneficial doses. Nevertheless, further in-depth toxicity studies are needed to confirm the findings. In this respect, the present work provides some indications at what doses further efficacy studies can be conducted.

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