

Research Article

Toxicity Study of *Brassica oleracea* Var. Italica Extracts in Sprague Dawley (SD) Rats

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Available Online: 1st May, 2016

ABSTRACT

Objective: This study was designed to elucidate the acute and sub acute toxicity of the widely used plant *Brassica oleracea* var. Italica in SD rats.

Method: Ethanolic extract isolated from the *Brassica oleracea* var. Italica was taken and their toxic effects were studied. Acute, sub acute and LD₅₀ values were determined in experimental rats according to the Organization for economic cooperation and development guidelines (OECD). In toxicity study, the oral dose (300, 2000 and 4000 mg/kg) of *Brassica oleracea* var. Italica plant extract was administered to three groups in single dose and general behavior, adverse effects and mortality were determined up to 72 hrs and compared to normal group. Dose of 400 and 800 mg/kg of *Brassica oleracea* var. Italica for 28 days was orally administered for 28 days in two animal groups. The body weight, hematological, serum hepatic biochemical parameters were evaluated and compared to normal group by sacrificing all group animals. From the Sub acute administration of *Brassica oleracea* var. Italica, the LD₅₀ values were determined using graphical method.

Result: In studies there was no mortality or any significant changes noticed in the SD rats after the administration of tested plant extract of 300, 2000 and 4000 mg/kg body weight respectively. The experimental animals did not showed any drug related changes in behavior, breathing, skin effects, water consumption, impairment in food intake and temperature. Furthermore *B. oleracea* Var. Italica extract did not produce any remarkable change in biochemical and hematological parameters following the administration of tested crude plant extract of 400 and 8000 mg/kg body weight for 28 consecutive days.

Discussion: After calculation of LD₅₀ values, we found a high therapeutic index value for *Brassica oleracea* var. Italica extracts. Oral administration of the extracts of the leaves of *Brassica oleracea* var. Italica at doses of 300, 2000 and 4000 mg/kg body wt for 28 consecutive days to SD rats did not induce any short term toxicity. Collectively, these data demonstrate that the extracts of the leaves of *Brassica oleracea* var. Italica have a high scope of drug safety.

Keywords: *Brassica oleracea* var. Italica; acute and sub acute toxicity; ethanol extract; hematology; biochemistry.

INTRODUCTION

Medicinal plants, either as an extract, pure compound or as a derivative, offer limitless opportunities for the discovery of new drugs. Most of the naturally occurring products used in folk medicine have solid scientific support in favor of their different biological properties. However, there is very less information available about the possible toxicity that medicinal plants may cause to the consumers¹. In relation to drug discovery and development, all relevant groups such as health authorities, pharmaceutical industry, and patients are concerned which needs to be taken into an account¹. The consumers such as patients and general public are first and foremost interested in fast access to safe and efficient drugs. Plants used in traditional medicine are expected to have low toxicity by the consumers. The latest surveys have indicated that many medicinal plants used as

traditional medicine showed undesirable effects^{2,3}. Therefore emphasizes should be given to elucidate the safe use of any plant for medicinal purposes. The possible toxic effects resulting from the short term and long term use of such medicinal plants have raised an alarm. To increase the confidence in their safety to the consumers especially for use in the development of pharmaceuticals, the data of the acute and sub acute toxicity studies on medicinal plants should be obtained⁴. The most critical part of any medicinal plant extract used animal human is the evaluation of its toxic effects. Hence a systemic approach in evaluating the efficacy and safety profile in such plants is needed.

The present study was aimed to evaluate the safety of *Brassica oleracea* var. Italica extract with acute and sub acute toxicity tests in SD rats. Toxicity is involved in

Table 1: General appearance and behavioral observations of toxicity study for control and treated groups.

Observation	Control group	300 mg/kg extract	2000 mg/kg extract	4000 mg/kg extract
Digestion	Not observed	Not observed	Not observed	Not observed
Body weight	Normal	Not change	Not change	Not change
Temperature	Normal	Normal	Normal	Normal
Food intake	Normal	Normal	Normal	Normal
Urination	Normal	No effect	No effect	No effect
Rate of respiration	Normal	No effect	No Effect	No effect
Change in skin	No effect	No effect	No effect	No effect
Drowsiness	Not present	Not present	Present	Present
Sedation	No effect	No effect	Observed	Observed
Eye color	No effect	No effect	No effect	No effect
Diarrhea	Not present	Not present	Not present	Not present
General physique	Normal	Normal	Lethargy	Lethargy
Coma	Not present	Not present	Not present	Not present
Death	Alive	Alive	Alive	Alive

estimation of LD₅₀. LD₅₀ is the dose which has proved to be lethal to 50% of the tested group of animals. Determination of oral toxicity is an initial screening step in the assessment and evaluation of the toxic characteristics of all compounds⁵. Plants in the family Brassicaceae are among the oldest cultivated plants known to man. Evidence has been unearthed that indicates that a Brassica vegetable was widely cultivated as early as 10,000 years ago⁶. *Brassica oleracea* var. Italica is closely related to cauliflower since both are grown for the clusters of unopened flower buds and tender flower stalks⁷. *Brassica oleracea* var. Italica is popularly used as food and has many traditional claims for herbal medicine. It has been reported to exhibit antimicrobial and anticancer properties but antidiabetic activities are not yet extensively explored. Based on *Brassica oleracea* var. Italica use in traditional practices and the literature references, the present study was undertaken to evaluate the comprehensive acute and sub acute toxicity in the animal models and is reported hereunder.

MATERIALS AND METHODS

Instruments

Several validated instruments have been used in the current research. Buchi rotary evaporator R-144, Buchi water bath B-480 (Buchi Labortechnik AG, Switzerland). Chemical hood (Pacific Vinitex Pte Ltd, Singapore), Refrigerator, Analytical balance (Precisa 40SM-200A, Swiss), Beckman JA 25-25 Rotor (Fullerton, CA, USA), ELX 800 Microplate Readers (Bio-Tek Instruments Inc., USA), RA 50 biochemical analyzer (Bayer Ltd), Haematology analyzer (Sysmex K21, Tokyo, Japan) Water Incubator (Everbloom Medical & Scientific Pte, Ltd., Singapore).

Selection and maintenance of experimental animals

The animals used for in vivo experiments were Sprague Dawley (SD) male rats weighing 150-200 gm), age ranging 6-12 week. The animals were kept in plastic cages (six rats in each cage) of 34×47×18cm³ size. Animals were maintained at room temperature (25±3°C) with relative humidity (60 ± 10%) under 12 hrs night and light cycle. Paddy husk was used as bedding material and

changed twice a week. The animals were kept on overnight fasting before every experiment. The animals used for the experiment were approved by the Animal Ethics Committee of the Lincoln University College, Malaysia.

Plant collection

The whole plants of *Brassica oleracea* var. Italica was collected in the month of June, 2012 from the cultivated field in Selangor, Malaysia. The plant was authenticated by Ms. Tan Ai Lee at Forest Research Institute Malaysia and a voucher specimen herbarium with number (SBID: 018/12) was deposited at the Faculty of Pharmacy, Lincoln University College, Malaysia.

Preparation of the plant extract

After identification of the plant *Brassica oleracea* var. italica was washed with running water to decontaminate from dust particles. After washing with water, the plants were covered with cloth and dried in shade for 20 days at room temperature. After shade drying, the plants were grinded through blender and converted into coarse of powder. The 200gm powder was extracted by using the soxhlet apparatus at a temperature of 78°C using 95% ethanol for 48 hrs. The rotary evaporator under reduced pressure was then used to concentrate the extract. The extracts were collected and preserved in a desiccator until used for further studies.

Acute toxicity study

The acute toxicity was evaluated according to Organization of Economic Cooperation and Development (OECD) guidelines 423⁸ on SD rats, where the limit test dose of 4000 mg/kg was used⁹. All the animals were kept at overnight fasting before to every experiment with free excess to water. The animals were divided into four groups, each comprising 5 animals. The 1st group served as a negative control, while 2nd, 3rd and 4th was considered as tested groups received *Brassica oleracea* var. Italica extract (dissolved in normal saline) at dose of 300mg/kg, 2000mg/kg and 4000mg/kg respectively. Before dose administration, the body weight of each animal was determined and the dose was calculated according to the body weight. The animals were observed for any toxic effect for first 4 hrs after the treatment period. Further animals were investigated for a period of 3 days for any

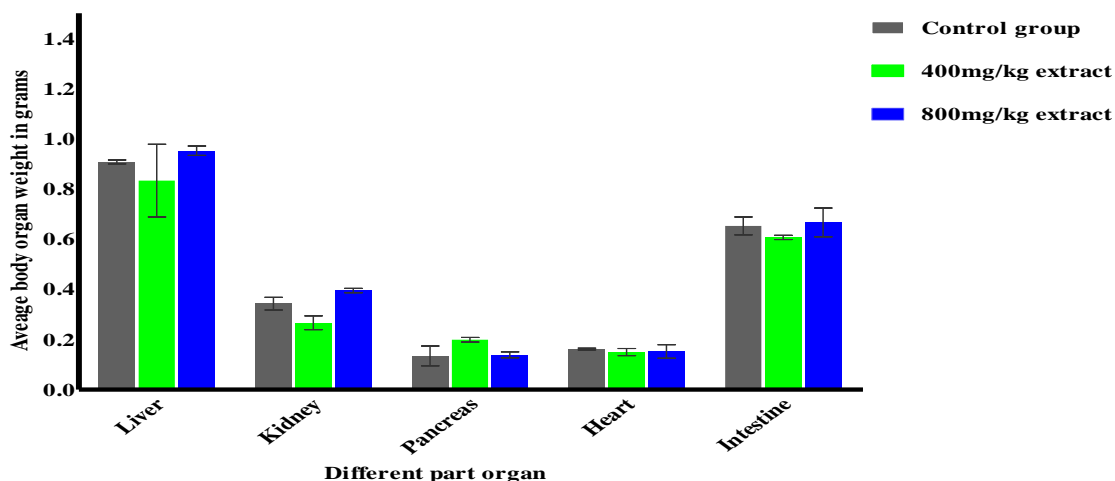


Figure 1: Effect of Oral administration of ethanolic extract of *Brassica oleracea* var. *Italica* on average organ weight (g) of SD rat.

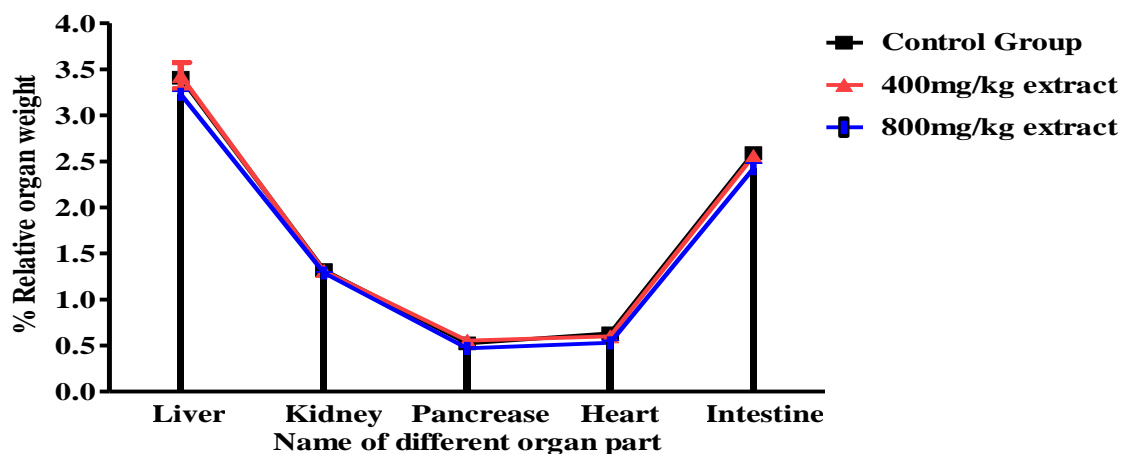


Figure 2: Effect of Oral administration of *Brassica oleracea* var. *Italica* on relative organs weight (g) of SD rats.

toxic effect. Behavioral changes and other parameters such as body weight, urinations, food intake, water intake, respiration, convulsion, tremor, temperature, constipations, changes in eye and skin colors etc.

Subacute toxicity study

The oral sub acute toxicity study was carried out according to OECD guideline 407¹⁰. Adult healthy SD rats were divided into 3 groups of 5 animals and were placed under standard conditions. Group I was considered as control and the other two groups which were considered as tested groups received the plant extract at a dose of 400 and 800 mg/kg. During the study body weight of each animal was evaluated respectively for 28 consecutive days¹¹.

Sacrificing of the animals and Collection of the sample

All animals used were anesthetized Intraperitoneally by injecting a cocktail containing ketamine (60mg/kg) and xylazine (7.5mg/kg)¹. Blood samples for biochemical and haematological analyses were collected by cardiac puncture into non heparinized and EDTA tubes respectively. After cardiac puncture, the rats were euthanized by ketamine/xylazine (80mg/kg/10mg/kg overdose). Biochemical and haematological analyses were performed by RA 50 biochemical analyzer (Bayer Ltd) and Haematology analyzer (Sysmex K21, Tokyo, Japan).

Effect of plant extract on haematological parameters

Red blood cell count, haematocrit, haemoglobin, total leukocyte count, differential leukocyte count and platelet count were determined using an automatic haematology analyzer.

Effect of plant extract on biochemical parameters

Biochemical analysis was performed on serum collected after centrifugation for the determination of aspartate transaminase (AST), alanine transaminase, alkaline phosphatase, High density lipoprotein (HDL), total bilirubin (T-BIL), direct bilirubin (D-BIL), indirect bilirubin (I-BIL), total protein, albumin, calcium, urea and creatinine¹².

Statistical analysis

Statistical analysis was carried out as mean of variance \pm SEM followed by ANOVA test using SPSS and Bonferroni test was performed for multiple comparison tests among the groups. A probability level of $P < 0.001$ was accepted statistically.

RESULTS

Acute toxicity study

The acute toxic effect of ethanolic extract was determined as per the OECD guidelines 423, where the limit test dose of 4000 mg/kg was used. No treatment related toxic

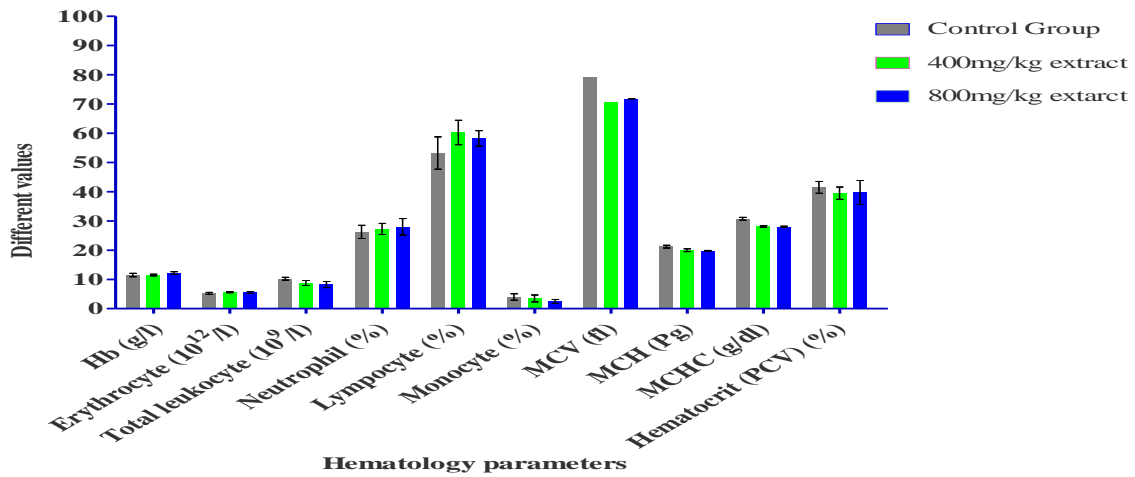


Figure 3: Effect of Oral administration of ethanolic extract of *Brassica oleracea* var. *italica* on Hematological Parameters. Values are expressed as mean ± SEM. P >0.05 when compared to normal control group.

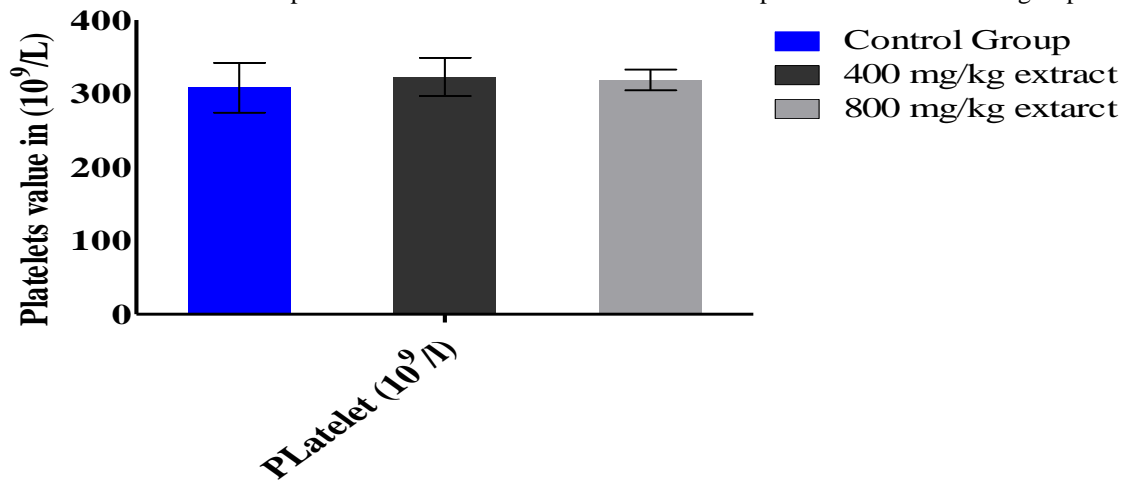


Figure 4: Effect of Oral administration of ethanol extract of *Brassica oleracea* var. *italica* extract on Platelets. Values are expressed as mean ± SEM. P >0.05 when compared to normal control group.

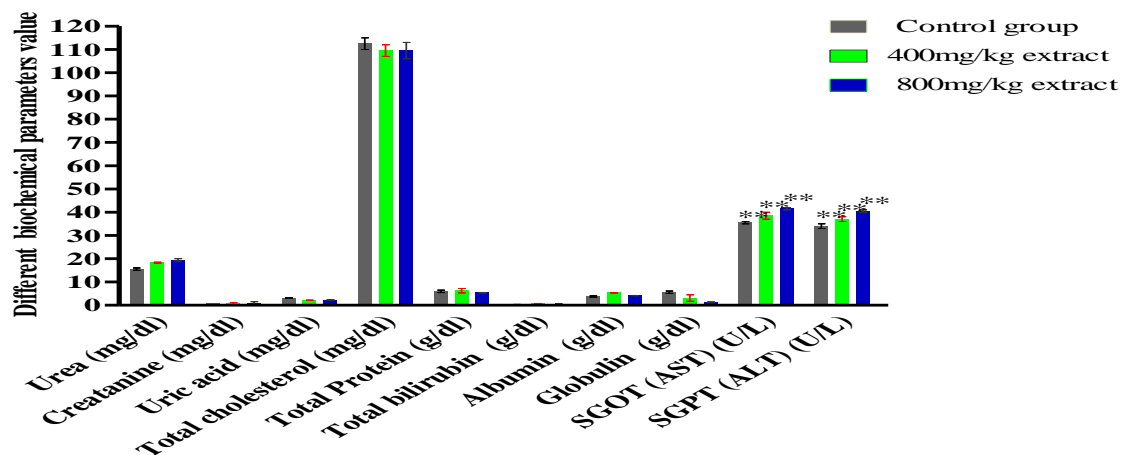


Figure 5: Effect of Oral administration of ethanolic extract of *Brassica oleracea* var. *italica* on Biochemical parameters. Data is expressed as Means ± SEM. *p < 0.01 when compared to control group

symptom or mortality were observed after oral administration of the tested plant extract at a dose of 300, 2000 and 4000 mg/kg. Physically no signs of changes in the skin, eye color, digestion, body weight, temperature, food intake, urination, rate of respiration, drowsiness, sedation, diarrhea, general physique, and coma were

observations. There was no mortality observed at the tested dose nor was the weight loss in the rats affected. No significant differences were observed in the relative organ weights and average organ body weights (Table 1). Therefore, the extract seems to be safe at a dose level of 4000 mg/kg, and the LD₅₀ was considered to be > 4000

mg/kg. However, there were sign of sedation, lethargy and drowsiness after the administration of plant extract at a dose of 2000 mg/kg and 4000 mg/kg compared to control group.

Sub acute toxicity Study

The sub acute toxic study of the tested plant extract was determined as per OECD guidelines 407. All the tested group animals treated with plant extract at a dose of 400 and 800 mg/kg daily survived throughout the 28 days. No clinical toxicity signs were observed in the plant treated group compared to the control group. From the present study it was seen that there was no significant change in the haematological and biochemical parameters in the *Brassica oleracea* var. *Italica* extract treated group compared to the normal control group. Gross examination at autopsy and histopathological evaluations of the various organs stained with haematoxylin and eosin revealed no significant differences.

Effect of plant extract on average and relative organ weight

There was no significant difference in average organs and relative organs weight between control and extract treated group at a dose of 400 and 800 mg/kg. The effect of tested extract on principal organ weights relative to body weights are shown in Fig 1 and Fig 2 respectively. There were no significant differences in the changes of body weight. The results revealed that, the vital organs such as liver, kidney, heart, pancreas and small intestine were not adversely affected throughout the treatment by extract. The average and relative organ weight of tested plant extract and control treated groups shows statistically non significant differences ($P > 0.05$).

Effect of plant extract on hematological parameters

The results of the hematological tests are summarized in Fig 3 & 4. All the tested hematological parameters including hemoglobin, erythrocyte, leukocyte differential leukocyte, packed cell volume, and platelet count were within normal limits compared to control group. No toxicologically significant differences ($p > 0.05$) between treated animals with the plant extract and control were found. There were generally no significant differences noted between control and treated groups for the hematological parameters measured.

Effect of plant extract on Biochemical parameters

The results of the various biochemical tests on the experimentally treated animals with the plant extract and normal group are summarized in Fig 5. Oral administration of the plant treated extract at a dose of 600 and 800mg/kg did not cause significant changes in serum biochemical parameters such as albumin, total protein, globulin, total bilirubin, urea, sodium, creatinine and uric acid levels when compared to control group. However, SGOT (AST) and SGPT were statistically difference in plant treated group at dose of 800 mg/kg body weight when compared to control group SD rats. There was significantly increase in SGOT (AST) and SGPT level $P < 0.05$. Data are expresses as Means \pm SEM. * $P < 0.01$ when compared to control group

DISCUSSION

For centuries medicinal plants have been used for the treatment of various diseases¹³. Estimation and assessment of the toxic characteristics of a natural product extract are usually initial steps in the screening of natural products for the pharmacological activity. Many beneficial effects of *B. oleracea* Var. *Italica* have been explored but detailed knowledge about the toxicology is lacking. Hence, the current study was carried out to estimate and focus on the acute and subacute toxicity of *B. oleracea* in an animal model. During the evaluation of the toxic characteristics of medicinal plants, the determination of LD₅₀ is usually an initial step to be conducted. Data from the toxicity study may provide preliminary information on the mode of toxic action of a substance and helps to find out a dose of a new compound in animal studies. It also helps to determine the LD₅₀ values that provide many indices of potential types of drug activity⁴. Moreover, if a high dose is found to be survivable, no further testing will be conducted¹. This study indicates that *B. oleracea* Var. *Italica* extract does not cause toxicity effects at the dose tested and within an LD₅₀ value of 4000 mg/kg. In principle, the limit test method is not intended for determining a precise LD₅₀ value, but it serves as a suggestion for classifying the crude extract based on the expectation at which dose level the animals are expected to survive¹⁴. Since no toxic effects were found during the toxicity study, further evaluation was carried out to evaluate the subacute toxicity of *B. oleracea* Var. *Italica* extract up to 28 days in SD rats to prepare the complete toxicology data. Subacute studies are used to find out the undesirable effects of continuous or repeated exposure of plant extracts over a portion of the average life span of experimental animals, such as rodents. Sub acute studies provide information on target organ toxicity and are designed to identify no observable adverse effect level¹. Sub acute estimate also helps to decide appropriate dose regimens for longer term studies. Therefore, in this study the subacute toxicity of *B. oleracea* Var. *Italica* extract was evaluated in rats at doses of 400 and 800 mg/kg body weight for 28 days.

Administration of extracts of *B. oleracea* Var. *Italica* for 28 days showed no clinical signs for any toxicity or mortality in the SD rats. In addition, the *B. oleracea* Var. *Italica* extract treated rats did not show any significant change in water or food consumption. Significant decrease in food and water intake is suggested as being accountable for the observed decrement in body weight gain. Loss of appetite is often associated with weight loss due to carbohydrate, protein or fat metabolisms disorders¹⁵. Furthermore, at higher doses crude plant extracts may metabolize to a toxic end product which may interfere with gastric function efficiency¹⁶. During the 28 days study, the food and water intakes were found to be unaffected when compared to a control group in this study. Hence this study suggests that the *B. oleracea* Var. *Italica* extract did not possibly cause any change in protein, carbohydrate or fat metabolism in these experimental animals. The study also shows that the *B. oleracea* Var. *Italica* extract did not adversely obstruct

with the nutritional benefits such as weight gain and stability of the appetite which are predictable for animals that are continually supplied with food and water ad libitum. Also, the extract is safe by oral route in relation to its traditional practice to treat the patients. Therefore, *B. oleracea* Var. *Italica* extract can be considered as non toxic up to the above said dose. Haematological and biochemical analyses were done to evaluate the possible changes in liver and kidney functions influenced by the *B. oleracea* Var. *Italica* extracts. hepatic and renal function tests are very important in the toxicity evaluation of drugs and plant extracts as they are both necessary for the survival of an organism¹⁷. High levels of SHOT, SGPT, and alkaline phosphatase are reported in liver diseases¹⁸. The non significant changes in SGOT and SGPT in experimental rats at all doses suggest that *B. oleracea* Var. *Italica* extract does not affect the liver function in the rats. A decrease in total protein, albumin, and globulin is a sign of the reduced synthetic function of the liver or might be due to impaired hepatocellular function. Low serum albumin content may suggest infection or continuous loss of albumin¹⁹. Thus, the nonsignificant changes in serum total protein, albumin and globulin in the *B. oleracea* Var. *Italica* extract treated and control group further confirms that the extract does not damage the hepatocellular or secretory functions of the liver at any of the doses tested. Renal dysfunction can be assessed by measurements of urea, creatinine and uric acid and their normal levels reflect at reduced of renal problems²⁰. In the present study, changes in plasma urea, creatinine, and uric acid levels in *B. oleracea* Var. *Italica* extract treated groups showed non-significant changes indicating a normal renal function. Assessment of haematological parameters can be used to conclude the degree of the harmful effect of *B. oleracea* Var. *Italica* extract on the blood of an animal model. It can also be used to explain blood relating functions of a plant extract or its products²¹. The non significant effect of the extract on complete blood count (CBC) indicates that the *B. oleracea* Var. *Italica* extract does not affect the morphology, erythropoiesis or osmotic fragility of the red blood cells²². WBC's are the first line of cellular defense that respond to infectious agents, tissue injury, or inflammatory process. No significant changes were observed in the neutrophils, lymphocytes, and monocytes in the *B. oleracea* Var. *Italica* which further justified the nontoxic nature of *B. oleracea* Var. *Italica* extract.

CONCLUSION

In light of these findings, we may conclude that *B. oleracea* Var. *Italica* extract is non toxic in all the doses studied herein and did not produce any toxic signs or evident symptoms in acute and sub acute oral toxicity study. The experimental animals did not showed any drug related changes in behavior, breathing, skin effects, water consumption, impairment in food intake and temperature. Furthermore *B. oleracea* Var. *Italica* extract did not produce any remarkable change in biochemical and hematological parameters. Therefore, the extract seems to

be safe at a dose level of 4000 mg/kg, and the LD₅₀ was considered be > 4000 mg/kg.

Conflict of interest statement

The authors of this paper have no conflict of interests.

ACKNOWLEDGMENTS

The authors are grateful to Faculty of Pharmacy, Lincoln University College, Malaysia for providing materials and facilities during the study. Author also acknowledges the valuable insight of colleagues: Dr. Sathesh Babu, Mr. Mrutyunjay Sisugoswami and Mr. Sreemoy Kanti Das, faculty of pharmacy, Lincoln University College Malaysia.

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