MALAYSIAN JOURNAL OF BIOCHEMISTRY & MOLECULAR BIOLOGY

The Official Publication of The Malaysian Society For Biochemistry & Molecular Biology (MSBMB)
http://mjbmb.org

PRECLINICAL SUB-ACUTE TOXICITY ASSESSMENT OF A CRUDE POLYSACCHARIDE EXTRACT ISOLATED FROM THE STEM OF *Solanum nigrum*: A PRELIMINARY ANALYSIS

Ahmad Naeem Mohd Nafi, Muhammad Imran Kamil Mazian, Nur Syahirah Izzati Rani, Siti Hajar Musa and Faizan Naeem Razali *

Faculty of Pharmacy and Health Sciences, Universiti Kuala Lumpur-Royal College of Medicine Perak, (UniKL-RCMP), 30450 Ipoh, Perak, Malaysia

*Corresponding Author: faizan@unikl.edu.my

Keywords:

Solanum nigrum, plant polysaccharide, sub-acute toxicity, toxicology, OECD guideline

Abstract

The polysaccharide extracted from Solanum nigrum was proven to possess an immunomodulatory effect and able to suppress the progression of tumor cells by proxy. However, data on the toxicity profile is still limited. The present preclinical study was conducted to investigate the sub-acute toxicity potential of the crude polysaccharide sample in accordance with OECD 407 guidelines. Twelve female BALB/c mice were randomly divided into 3 groups, 4 mice per group (n=4). Mice in groups B and C were daily administered with crude polysaccharide samples at concentrations of 100 and 500 mg/kg/bw, (300 µL), respectively for 14 days through oral. Mice in group A served as control. Mortality and clinical signs associated with toxicity were observed within the 14 days of a treatment session. Mice body weight were recorded starting at day 0 until day 14 prior to sacrificing at day 15. The crude polysaccharide sample showed a positive indication towards phenol sulfuric test with the total estimation of carbohydrate content around 46%. At the end of sub-acute toxicity assessment, mice in groups A, B and C recorded the mortality rate of 0, 25 and 50%, respectively with no bizarre behavioral indication associated with toxicity. The polysaccharide treatment also revealed no significant elevation in mice glucose serum levels. The present findings suggested that the treatment of crude polysaccharide sample exerted a mild sub-acute toxicity effect when orally administered with a maximum sample concentration of 500 mg/kg/bw with no significant changes in vital organ weight indexes of treated mice as compared to the control group.

INTRODUCTION

Up until this recent moment, herbal medicines have been utilized and are still being used in developing countries as the primary source of medicinal treatment. Multiple scientific studies have been conducted to investigate the potential of terrestrial plants extracts for the preparation of evident-based treatments against various diseases [1]. Even though many believe that the utilization of medicinal plant products recognized as 'natural' as treatments for diseases, it does not mean that it is entirely safe to be consumed. In medicinal production, secondary metabolites that have been extracted from plants such as saponins, terpenoids, cyanogenic, tannins, toxic amino acids, glycosides, and alkaloids were widely used as a part of active ingredients in the pharmaceutical formulation [2]. A toxicology study need to be conducted as it provides knowledge on the plant in terms of their toxicity capability and precaution that need to be taken for the utilization pharmaceutical industry [3].

Solanum nigrum is an herbal plant that widely grows and is distributed throughout temperate climate zones to the tropical region of Asia and the Southern hemisphere, from

sea level to altitudes over 3,500 meters [4]. *S. nigrum* is commonly used as traditional folk medicine and is believed to have various biological activities such as anti-cancer, anti-septic, anti-dysenteric and wound healing properties [5]. In the previous studies, the *S. nigrum* polysaccharide fraction, SN-ppF3 was proven to have immunomodulatory activities where it could classically activate macrophage cells through the NF-kB transduction signaling pathway and indirectly suppressed the proliferation of breast cancer cells in tumor-induced BALB/c mice [6–8]. These documented health benefits of the *S. nigrum* plant products can be utilized in various pharmaceutical applications. However, information regarding toxicity capability is still limited. Although natural source phytoconstituents are familiar to have little to no toxicity effect, the continuous consumption of the compounds could draw follow-up health implications. Thus, this study was carried out to deduce the sub-acute toxicity information of the *S. nigrum* crude polysaccharide extract at the preclinical level.

MATERIALS AND METHODS

Materials

General chemicals used in this study were analytical standard grade and were purchased from Sigma-Aldrich company unless specifically stated. Information on the instruments used was provided in the paragraph.

Methods

Sample Preparation

Fresh plants of *Solanum nigrum* L. *nigrum* were purchased from the local market located at Bangi, Selangor. The *S. nigrum* plant was previously identified and authenticated by Dr. Sugumaran Manickam from Institute of Biological Sciences, Faculty of Science, University of Malaya, Kuala Lumpur, Malaysia, and a voucher specimen was deposited at the Rimba Ilmu Herbarium (Herbarium number: KLU 47872)**.** The crude polysaccharide samples were extracted according to the previously described method [6]. Briefly, polysaccharide was extracted from dried, ground stems of *S. nigrum* by refluxing the sample with 2 L of petroleum ether (60°C–80°C), followed with 2 L of 80% ethanol. The residue was then boiled in 2 L of 95°C water for 5 hours. The polysaccharide in the filtrate of the boiled mixture was precipitated out with an equal volume of 70% ethanol, overnight at 4°C. Next, the crude polysaccharide was collected through centrifugation at 2,300 ×*g* for 5 minutes. After that, the crude polysaccharide sample was washed twice with 95% ethanol. The crude polysaccharide was allowed to dry in a desiccator for approximately 7 days or until it completely dried. The crude polysaccharide was stored at 4°C for further use.

Estimation of Carbohydrate Content

The phenol-sulfuric acid assay was carried out to detect and estimate the carbohydrate content in the crude polysaccharide samples. Exactly 1,000 µL of 1 mg/mL of crude polysaccharide sample was pipetted into a glass test tube. Then, the sample was mixed with 1,500 µL sulfuric acid and 300 µL of 5% phenol. The solution was heated in a 95°C water bath for 5 mins. The presence of carbohydrates was indicated as the appearance of a yellow color solution. To determine the amount of carbohydrate content, the solution was assessed through a spectrophotometer at 490 nm wavelength. Then, the absorbance value was extrapolated in a standard curve of D-glucose [9]. The chemical test was conducted at least in triplicates (n=3).

Estimation of Protein Content

The protein content in the crude polysaccharide sample was estimated by using the Bradford assay. Basically, 1,000 µL of a 1 mg/mL crude polysaccharide sample was mixed with 500 µL of Bradford reagent in a glass test tube. The solution was incubated in a dark condition for 10 mins allowing the reaction to happen. The presence of protein was indicated as the appearance of the blue color solution. A spectrophotometer was used to measure the spectrum wavelength set at 595 nm. The protein content in the crude polysaccharide of *S. nigrum* was estimated by extrapolating the absorbance value in a standard curve of bovine serum albumin (BSA). The chemical test was conducted at least in triplicates (n=3).

Preparation of Preclinical Analyses

Twelve female BALB/c mice (4–5 weeks old) weighing around 28–30 g were purchased from Sinar Scientific Sdn. Bhd. located in Sri Kembangan, Selangor. Mice were acclimatized for at least 7 days prior to the experiment. Mice were kept in cages under controlled environmental conditions of temperature 25 ± 1°C and 12 hours/12 light/ dark cycle, and supplied with standard food pellets and tap water, *ad libitum*. The principles and guidelines for animal care, research and animal sacrificed protocols were in accordance with animal ethical clearance approved by the Universiti Kuala Lumpur Institute of Medical Science Technology (UniKL-MESTECH) Animal Ethics Committee (AEC/MESTECH-UNIKL/2020/001/MAY-2020-NOV-2022).

Sub-acute Toxicity Study

The sub-acute toxicity study was carried out in accordance with OECD 407 guidelines. Healthy mice were randomly divided into 3 groups consisting of 4 mice in each group (n=4). Mice in group B were treated with 300 µL of 100 mg/kg crude polysaccharide samples, while mice in group C

were treated 300 µL of 500 mg/kg crude polysaccharide samples. Mice in group A were served as control that received only the vehicle, 300 µL of normal saline. Treatment regimens were given through oral gavage force-feeding daily for 14 consecutive days [10].

Behavioral Observation of the Treated Mice

The morbidity and mortality of the treated mice were observed twice daily. This observation was conducted to detect possible toxicological symptoms as suggested by OECD 407 guideline. Mice were weighed prior to the first dose of treatment administration. The weight of each mouse was recorded daily starting on day 0 until day 14. The physical observation was carried out to indicate any sign associated with toxicity that arises in terms of survival, food intake, fur, skin, eyes, and any bizarre behavior. On day 15, mice were sacrificed through cervical dislocation. Blood and vital organs (kidney and liver) were harvested for the next subsequence toxicology assessments.

Hematology Assessment on Serum Glucose Level

The blood sample was withdrawn through cardiac puncture and a portion of the collected blood was assessed in term of its serum glucose level by using a standard glucometer. The index of serum glucose level for treated mice was calculated and compared to the control group.

Vital Organ Assessment

The harvested liver and kidney were weighted using a weighing balance. The weigh for each vital organ was recorded and the organ weight indexes were calculated based on the following formula:

$$\text{Organ Index (mg/g)} = \frac{\text{Organ weight (mg)}}{\text{Mice body weight at Day 14 (g)}}$$

Next, the calculated organ weight index of mice in group A and B were compared to the control group. The harvested organs were then preserved in 15 mL of 10% formalin solution for further histopathological analysis.

Statistical Analysis

The one-way analysis of variance (ANOVA) was used to analyze all collected data, with the significant difference between data means determined by Duncan's multiple range test at 95% confidence level (p<0.05) with a minimal number of replication (n=3) using SPSS 17.0 Statistic software. All graphs and standard curves were constructed by using GraphPad Prism 5 software.

RESULTS AND DISCUSSION

Percentage of Extraction Yield of Crude Polysaccharide

One of the crucial technical steps in the study was the extraction method as it impacts the yield, purity, chemical composition, molecular weight distribution, microstructure, and bioactivities of polysaccharides [11]. In this study, the Soxhlet extraction method was carried out to obtain crude polysaccharides from the dried stem of *S. nigrum*. Based on Table 1, the yield of crude extract that was obtained through the Soxhlet extraction method was 1.84%.

A previous study that carried out the extraction of crude polysaccharides using different extraction methods obtained inconsistent percentages of extraction yield according to their purpose of the study. The percentage yield achieved in the current study was not comparable to that recently published data, which reported to yield around 3.65% of crude extract obtained through ethanol solvent. The extraction method could be the possible reason for getting a variety of extraction yields. The study suggested that extraction of crude polysaccharide sample using maceration technique with ethanol as the solvent of choice produced higher extraction yield due to high efficiency of separating both non-polar and polar compounds [12].

Table 1. The yield of crude polysaccharide extracts isolated from the stem of *Solanum nigrum*

Weight of dry stem (g)	Weight of crude polysaccharide (g)	Percentage extraction yield (%)
107.44	1.98	1.84

Carbohydrate Content in Crude Polysaccharide Sample

This assay was conducted to detect the significant amount of carbohydrate present in the isolated crude polysaccharide sample. The crude polysaccharide sample was subjected to carbohydrate analysis by using the phenol-sulfuric acid method and the carbohydrate content was estimated based on a standard curve of D-glucose [9]. Positive indication towards phenol-sulfuric assay confirmed that the sample belongs in carbohydrate group of macromolecules as a yellow color solution developed in the reaction [13]. Data in Table 2 demonstrated that the estimated percentage of carbohydrates in the crude polysaccharide sample was 46.3%. The percentage of carbohydrate content obtained was relatively low as compared to the other study, which obtained 63.60% of polysaccharide content in the crude sample. However, the carbohydrate content ranging in between 8.38% to 67.17% is assumed to be majorly composed of carbohydrates and can be categorized under the polysaccharide group for a crude plant sample [14].

Table 2. Estimation of carbohydrate content in *Solanum nigrum* crude polysaccharide sample

Concentration of crude polysaccharide (µg/mL)	Absorbance (OD)	Concentration of carbohydrate (mg/mL)	Mean ± SD (mg/mL)	Percentage of carbohydrate content (%)
1,000	1.027	389.42	462.9 ± 108.1	46.3%
	1.582	587.00		
	1.091	412.21		

Data expressed was mean ± standard deviation (*n*=3)

Protein Content in Crude Polysaccharide Sample

The protein content from the isolated crude polysaccharide was estimated by using the Bradford assay. As a positive indication, the solution turned into blue color indicating the presence of protein [15]. Referring to Table 3, the percentage of protein content was estimated at around 34.6%. The result was slightly higher as compared to protein content that has been reported in a previous study, which was 24.90% in the dried leaves part and 17.63% in the dried seeds part [16]. However, a big difference could be seen as compared to the other reported study that obtained less than 1% of protein content [6]. By comparison, the crude polysaccharide sample was not purely polysaccharide and may contain glycoproteins and some other polar compounds. It was suggested that additional purification techniques such as ion-exchange column chromatography could be performed to ensure the highest purity of polysaccharide sample could be obtained. The crude polysaccharide sample is expected to possess the very least protein amount, concerning protein derivatives such as glycoproteins in most *Solanaceae* plant families could exert significant toxicity capability.

Table 3. Estimation of protein content in *Solanum nigrum* crude polysaccharide sample

Concentration of crude polysaccharide (µg/mL)	Absorbance (OD)	Concentration of protein (mg/mL)	Mean ± SD (mg/mL)	Percentage of protein content (%)
1,000	0.464	0.683	0.495 ± 0.211	34.6%
	0.182	0.268		
	0.364	0.535		

Data expressed was mean ± standard deviation (*n*=3)

Sub-acute Toxicity Evaluation

Behavioral Observation

Throughout 14 days of the treatment session, major clinical signs associated with toxicity were undetected towards all mice. However, mice treated with SN-CP 500 mg/kg showed symptoms of mild weakness, losing appetite and slow motor activities (Table 4). The reported signs were obviously observed starting at the beginning of the second week. Group of SN-CP 100 mg/kg-treated and controlled mice revealed normal behavioral signs as mice were normally active, showed normal appetite and excreting normal feces condition. End of the assessment day, the remaining mice in SN-CP 500 mg/kg-treated group revealed inactive motion with sleepy eyes and loose appetite. Additional observation parameters suggested in OECD 407 guidelines were conducted in this study, but none of the symptoms such as mucous membrane, the occurrence of secretions, changes in gait, posture, and response to handling as well as the presence of bizarre behavior [17] were developed during the sub-acute toxicity study. Some documented studies revealed that most crude plant extracts tested for acute toxicity were not causing significant changes toward animal behavior throughout the treatment periods [10, 18–19].

Treatment Effect on Survival and Body Weight of Mice

Daily oral treatment for sub-acute toxicity study was conducted towards the respective group starting from day 0 until day 14. All treated and control mice survived the next 24 hours after the first treatment administration. Based on Table 5, the group of mice treated with 100 mg/kg exerted a 25% of mortality rate as there was one mouse found dead on day 6 of the treatment session. For the group of mice treated with 500 mg/kg, the mortality rate was 50% as two mice were found dead on day 6 and day 9 of the treatment session. It was suggested that the crude polysaccharide was proven to be non-toxic at a dose of 100 mg/kg with a 25% rate of mortality. Mild toxicity was indicated for the treatment dose of 500 mg/kg with a 50% rate of mortality. However, the severe harmful effects of the treatment samples at both doses could be expected if continuous treatments are performed for another 14 days of the consecutive treatment period. In comparison to other studies, the current finding is uncommon as most of crude plant extracts tested for sub-

acute toxicity study imparted a mortality rate of 0% [10, 18–19]. It was reported that most *Solanum* genus plants contained solanine [20–21], a toxic glycoalkaloid that probably contributes to the mild toxicity effect.

Table 4. Behavioral observations for control and polysaccharide-treated groups

Groups	SN-CP 100 mg/kg		SN-CP 500 mg/kg		Control	
*Observation (After)	24 hours	14 days	24 hours	14 days	24 hours	14 days
Activity	Active	$^{+}$Active	Active	$^{++}$Inactive	Active	Active
Eyes	Normal	$^{+}$Normal	Normal	$^{++}$Sleepy	Normal	Normal
Appetite	Normal	$^{+}$Normal	Normal	$^{++}$Low	Normal	Normal
Fur	Normal	$^{+}$Normal	Normal	$^{++}$Normal	Normal	Normal
Breathing	Normal	$^{+}$Normal	Normal	$^{++}$Normal	Normal	Normal
Salivation	Absent	$^{+}$Absent	Absent	$^{++}$Absent	Absent	Absent
Diarrhea	Absent	$^{+}$Absent	Absent	$^{++}$Absent	Absent	Absent

Dead mice were detected starting on day 6 and 9. The observation was continued to day 14 for the remaining alive tested mice, $^{+}n=3$ and $^{++}n=2$. *Clinical signs suggested in OECD 407 Guideline. SN-CP: *S. nigrum* crude polysaccharide

Table 5. Sub-acute toxicity study on mortality of treated mice

Group	Dose (mg/kg/bw)	Number of mice in cage	Number of dead mice	*Mortality rate (%)
Control	-	4	0	0
SN-CP	100	4	1	25
SN-CP	500	4	2	50

*Mortality rate (%) = (Number of dead mice after treatment / number of treated mice) × 100. SN-CP = *S. nigrum* crude polysaccharide

At the end of the study (Day 14), there is no significant difference (p<0.05) between the bodyweight of mice in treated groups (100 mg/kg and 500 mg/kg) and the control group were observed. However, data revealed significant differences (p<0.05) in the mice's body weight on day 6 and day 7 (Figure 1) as compared to the control group. Bodyweight correspondently reflects the health status of the treated mice. Sharp or moderate decrease of body weight indicates health deterioration due to sub-acute treatment effect. The present oral treatment of *S. nigrum* crude polysaccharide with doses 100 mg/kg and 500 mg/kg do not severely affect bodyweight of the treated BALB/c mice as no significant changes were observed.

Treatment with crude plant extract is usually uncommon. The present study implies if no toxicity is associated, the crude sample will be used to proceed with further utilization to be developed into food supplement formulation, concerning a series of purification steps significantly reduces the sample quantity. Even the current finding exposed mild toxicity effect, the further purification process is needed to eliminate possible toxic components and relatively minimize toxicity implication.

Vital Organ Assessment

The vital organs were harvested after 14 days of treatment and the data in Table 6 has shown no significant difference (p<0.05) of the organ weight indexes for mice in the crude polysaccharide-treated group as compared to the control group. Significant changes towards organ weight index indicate toxicity implication as the treatment could cause organ damage and a series of inflammation. It was reported that significant increment of mice's liver weight index when exposed to toxicants [22–23]. The present findings suggested that oral treatment of crude *s. nigrum* polysaccharide was not causing deterioration of vital organs (liver and kidney) weight indexes. However, further histopathology assessment is necessary to be conducted to confirm the preliminary claim.

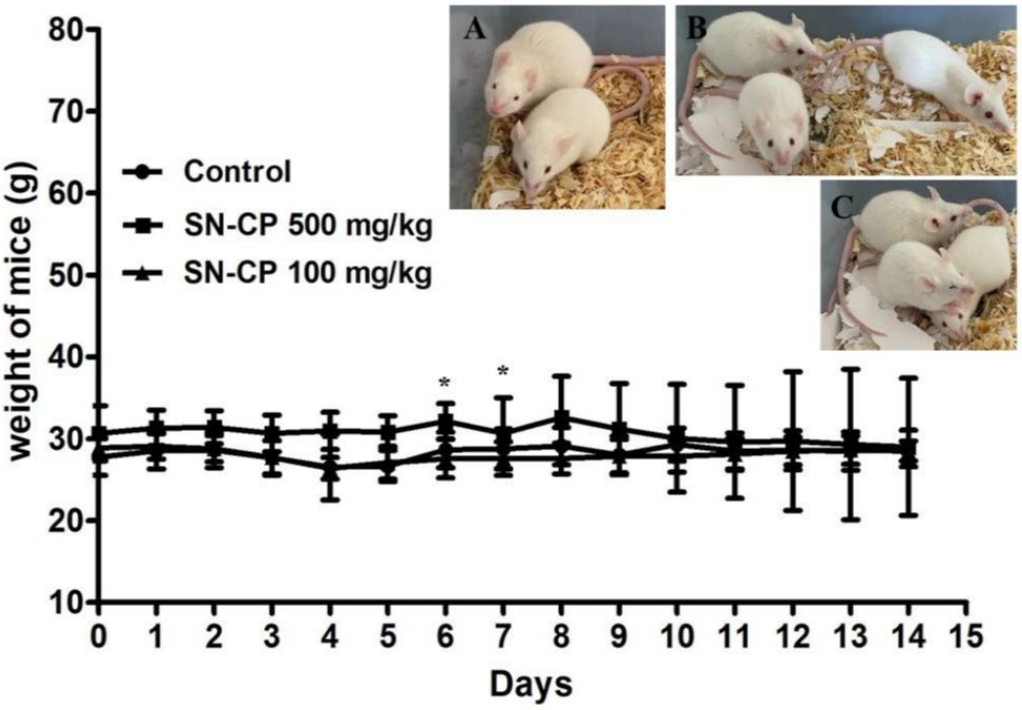

Figure 1. Mice body weight was daily measured for 14 consecutive days. Asterisk signs (*) indicate a significant difference between control and 500 mg/kg/bw of the tested sample at $p<0.05$ ($n=4$). SN-CP: *S. nigrum* crude polysaccharide. Photographs are (A) control, (B) SN-CP 100 mg/kg and (C) SN-CP 500 mg/kg treated mice

Table 6. Vital organ weight indexes of crude polysaccharide treated mice

Group	Dose (mg/kg)	**Body weight (g)	*Liver weight index (mg/g)	*Kidney weight index (mg/g)
Control	-	28.81 ± 2.22^{a}	45.565 ± 8.861^{b}	12.585 ± 0.844^{c}
SN-CP	100	28.51 ± 1.21^{a}	39.810 ± 4.767^{b}	10.487 ± 1.815^{c}
SN-CP	500	29.02 ± 8.37^{a}	43.755 ± 23.837^{b}	14.175 ± 7.502^{c}

*Organ body index (mg/g) = organ weight (mg) / body weight (g). Data expressed were mean ± standard deviation ($n=4$). Superscripted letters (a, b and c) across columns indicated no significant differences at $p<0.05$. **Body weight of mice was taken at the last day of the treatment session. SN-CP: *S. nigrum* crude polysaccharide

Blood Glucose Level of Mice

It was reported earlier that the polysaccharide sample composed of high carbohydrate content (Table 2) and prolonged consumption perhaps affecting the regulation of serum glucose level. Mice serum glucose level was measured by a glucometer at day 15, and data in Table 7 has shown no significant differences ($p<0.05$) of serum glucose level index of treated groups when compared to the control group. It was suggested the treatment of crude polysaccharide samples at doses of 100 mg/kg and 500 mg/kg did not affect the regulation of serum glucose levels. However, as the current study was conducted on healthy test subjects with no diabetic history, further investigation is necessary to be performed onto diabetic test subjects to further determine the effect of crude polysaccharide sample on serum glucose regulation.

Table 7. Serum glucose index of polysaccharide-treated mice

Group	Treatment dose (mg/kg)	*Serum glucose level index (mmol/L/g)
Control	-	0.24 ± 3.39^{d}
SN-CP	100	0.24 ± 2.79^{d}
SN-CP	500	0.28 ± 0.07^{d}

*Index of serum glucose to mice body weight at day 15. Data expressed were mean ± standard deviation. Superscripted letter (d) across rows indicated no significant differences at $p<0.05$ ($n=4$). SN-CP: *S. nigrum* crude polysaccharide

CONCLUSION

In the present study, findings showed that crude polysaccharide of *S. nigrum* at dose 100 mg/kg/bw was determined as non-toxic as one mouse reported dead (at day 6), while dose 500 mg/kg/bw was determined as mild toxic as two mice reported dead in the study (day 6 and day 9). Both treatment doses recorded mortality rate not exceeding 50%. Mild toxicity was assumed caused by the high level of protein content in the sample, and probably due to the presence of a toxic molecule such as solanine. It was suggested that further purification steps are necessary to be carried out to eliminate possible components associated with toxicity.

ACKNOWLEDGMENT

The authors were grateful to the Ministry of Higher Education (MOHE), Malaysia for funding this present research under the Fundamental Research Grant Scheme (FRGS) (Ref: FRGS/1/2019/STG04/UNIKL/02/1). The authors also thank Universiti Kuala Lumpur, Royal College of Medicine Perak (UniKL-RCMP) for providing research facilities.

CONFLICTS OF INTEREST

The authors wish to confirm that there is no conflict of interest associated with this publication.

REFERENCES

1. Sivaraj, R., Rahman, P.K., Rajiv, P., Narendhran, S. and Venckatesh, R. (2014) Biosynthesis and characterization of *Acalypha indica* mediated copper oxide nanoparticles and evaluation of its antimicrobial and anticancer activity. Spectrochimica Acta Part A: Molecular and Biomolecular Spectroscopy 129, 255-258.

2. Dai, J. and Mumper, R.J. (2010) Plant phenolics: extraction, analysis and their antioxidant and anticancer properties. Molecules 15(10), 7313-7352.

3. Mounanga, M.B., Mewono, L. and Angone, S.A. (2015) Toxicity studies of medicinal plants used in sub-Saharan Africa. Journal of Ethnopharmacology 174, 618-627.

4. Huang, H.C., Syu, K.Y., and Lin, J.K. (2010) Chemical composition of *Solanum nigrum* linn extract and induction of autophagy by leaf water extract and its major flavonoids in AU565 breast cancer cells. Journal of Agricultural and Food Chemistry 58(15), 8699-8708.

5. Edmonds, J.M. and Chweya, J.A. (1997) in Black nightshades: *Solanum nigrum* L. and related species, in: Promoting the conservation and use of underutilized and neglected crops, Vol. 15. Rome: Institute of Plant Genetics and Crop Plant Research, Gatersleben / International Plant Genetic Resources Institute.

6. Razali, F.N., Ismail, A., Abidin, N.Z. and Shuib, A.S. (2014) Stimulatory effects of polysaccharide fraction from *Solanum nigrum* on RAW 264.7 murine macrophage cells. PloS One 9(10), 1-8.

7. Razali, F.N., Sinniah, S.K., Hussin, H., Abidin, N.Z. and Shuib, A.S. (2016) Tumor suppression effect of *Solanum nigrum* polysaccharide fraction on breast cancer via immunomodulation. International Journal of Biological Macromolecules 92, 185-193.

8. Razali, F.N., Shuib, A.S. and Abidin, N.Z. (2019) Prediction of signaling pathway induced by *Solanum nigrum* polysaccharide fraction, SN-ppF3 in activating RAW 264.7 macrophage cells. Malaysian Journal of Biochemistry and Molecular Biology 22(2), 6-11.

9. Chen, J.R., Yang, Z.Q., Hu, T.J., Yan, Z.T., Niu, T.X., Wang, L., Cui, D.A. and Wang, M. (2010) Immunomodulatory activity in vitro and in vivo of polysaccharide from *Potentilla anserina*. Fitoterapia 81(8), 1117-1124.

10. Roy, S., Ukil, B. and Lyndem, L.M. (2016) Acute and sub-acute toxicity studies on the effect of *Senna alata* in Swiss Albino mice. Cogent Biology 2(1), 1-11.

11. Xu, Z., Feng, S., Qu, J., Yuan, M., Yang, R., Zhou, L., Chen, T. and Ding, C. (2019) The effect of extraction methods on preliminary structural properties and antioxidant activities of polysaccharides from *Lactarius vividus*. Processes 7(8), 482-496.

12. Mazher, M., Anjum, M., Mushtaq, W., Noshad, Q. and Malik, N.Z. (2017). Antifungal assay of *Solanum nigrum* Linn. fruit, leaves and stems extracts in different solvents. International Journal of Biosciences 10(4), 380-385.

13. Nielsen, S.S. (2017) in Total carbohydrate by phenol-sulfuric acid method. In Food analysis laboratory manual. Springer, Cham, pp. 137-141.

14. Hameed, I. and Hussain, F. (2015) Proximate and elemental analysis of five selected medicinal plants of family Solanaceae. Pakistan Journal of Pharmaceutical Sciences 28(4), 1203-1215.

15. Kielkopf, C.L., Bauer, W. and Urbatsch, I.L. (2020) Bradford assay for determining protein concentration. Cold Spring Harbor Protocols 2020(4), pdb-prod102269

16. Akubugwo, I.E., Obasi, A.N. and Ginika, S.C. (2007) Nutritional potential of the leaves and seeds of black nightshade-*Solanum nigrum* L. *Var virginicum* from Afikpo Nigeria. Pakistan Journal of Nutrition 6(4), 323-326.

17. Organisation for Economic Co-Operation and Development (OECD) 407. (2008) OECD Guidelines for the Testing of Chemicals, 1-13.

18. Loha, M., Mulu, A., Abay, S.M., Ergete, W. and Geleta, B. (2019) Acute and subacute toxicity of methanol extract of *Syzygium guineense* leaves on the histology of the liver and kidney and biochemical compositions of blood in rats. Evidence-Based Complementary and Alternative Medicine, 1-15.

19. Kifayatullah, M., Mustafa, M.S., Sengupta, P., Sarker, M.M.R., Das, A. and Das, S.K. (2015) Evaluation of the acute and sub-acute toxicity of the ethanolic extract of *Pericampylus glaucus* (Lam.) Merr. in BALB/c mice. Journal of Acute Disease 4(4), 309-315.

20. Chen, Z., Li, C., Yuan, A., Gu, T., Zhang, F., Fan, X., Wu, X., Xiong, X. and Yang, Q. (2021) α-Solanine causes cellular dysfunction of human trophoblast cells via apoptosis and autophagy. Toxins 13(1), 67-78.

21. Barceloux, D.G. (2009) Potatoes, tomatoes, and solanine toxicity (*Solanum tuberosum* L., *Solanum lycopersicum* L.). Disease a Month 55(6), 391-402.

22. Chebaibi, M., Bousta, D., Chbani, L., Iken, I. and Achour, S. (2019). Evaluation of acute toxicity of plants' mixture used in traditional treatment of kidney diseases in Morocco. Pharmacognosy Research 11(2), 155-161.

23. Kaware, M. (2013). Changes in liver and body weight of mice exposed to toxicant. International Research Journal of Science and Engineering 3(1), 92-95.