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Safety Evaluation of *Carex baccans* Nees. (A Traditional Anthelminthic Remedy), in Swiss Albino Mice: An Acute and Sub-acute Toxicity Study

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Abstract

The medicinal properties of *Carex baccans* Nees. (Cyperaceae) has widely been exploited in traditional healing practices for the treatment of various ailments. Though, this is a widely used herb, not much is known about the adverse implications it may have. Therefore to shed some light into this aspect, acute and sub-acute toxicity study of the plant's crude extract and fractions were carried out in Swiss albino mice following Organization for Economic Co-operation and Development 423 and 407 guidelines respectively. Additionally, the phytochemical composition of the crude extract was also determined. Effect of the extract and the fraction was analyzed taking into consideration the body weight, organs weight, blood physiology, key markers of liver and kidney functions, and microscopic analysis of liver, kidney and intestine structural composition using scanning and transmission electron microscopy. The results showed that the crude extract exert no observable signs of acute toxicity even at a dose of 5000 mg/kg body weight of mice, however, the butanol fraction proved to be toxic with a mean lethal dose of 4677.35 ± 103.38 mg/kg. Repeated oral sub-acute test of the mice with crude extract and fraction of the crude extract was also found to exert adverse effects on the animals at high doses (500 mg/kg body weight and above) when compared to control group. These adverse implications were manifested in the body weight, hematology, biochemistry, and the structural and ultrastructural composition of some important organs. Therefore, it may be concluded that the plant *C. baccans,* if taken in its crude native form has a high safety margin at a single dose but not the butanol fraction. Furthermore, repeated exposure to the plant, both in its crude and fractionated (butanol fraction) form at high doses should be discouraged as it may have severe implications.

Keywords: Biochemistry, *Carex baccans* Nees, Hematology, Toxicity, Ultrastructure

1. Introduction

Traditional plant based medicine has remained as the most affordable and easily accessible source of treatment in the primary health care system of resource poor indigenous and rural communities[1]. Though, traditional plant based medicines have been around for ages and are generally believe to be safe, a number of concerns do exist. All treatments have the potential to do good as well as harm; indeed, herbal medicines are the most important and unregulated source of potential health hazards of all the complementary therapies[2]. Different parts of the plant have different phyto compounds with different medicinal properties, therefore, direct consumption of crude extract of plants may have various adverse effects on the health of an organism[3]. Several reports showed that regular consumption of herbal medicine causes toxic effects like neurotoxicity[4], cardiac toxicity[5], hepatotoxicity[6], nephrotoxicity[7], lymphocytosis[8], antifertility[9], DNA fragmentation[10], and even death of consumers[11,12]. Therefore, it is necessary to carry out

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toxicity test of such medicinal plants to validate their safety/safety margin before using them for therapeutic purposes.

Carex baccans Nees. (family-Cyperaceae) is a perennial herb with stout culms and several tricostate leaves native to temperate and tropical Asia. In India, it is found in Maharashtra, Madhya Pradesh, Karnataka, Kerala and Tamil Nadu besides Meghalaya. It mainly grows in moist places and swamps at altitude of 1,500–2,500 m in dense moist to semi-evergreen forests[13].

The roots of *C. baccans* is use in folk medicine in Meghalaya, India, as a remedy for intestinal worms[14]. Other uses of the plant in traditional medicine include– to cure hypertension, fever, leucorrhea, chin-cough, ulcer, measles, fractures, cardiovascular diseases, dysmenorrhea and gynecological problems[15–17]. Considering the importance of this medicinal plant in traditional healing practices, this study aim to evaluate the acute and sub-acute oral toxicity of the crude methanol root extract of *C. baccans* and its most toxic fraction in Swiss albino mice.

2. Material and Methods

2.1 Collection and Identification of Plant Material

Fresh samples of *C. baccans* were collected from different areas in Shillong. Identification of the plant was done with the help of a taxonomist from the Department of Botany, NEHU, Shillong, Meghalaya.

2.2 Preparation of *Carex baccans* Methanol Extract (CbME)

Fresh roots of *C. baccans* were washed in running water and air dried in shade. The root skin was shaved off and pulverized into a coarse powder using a mortar and pestle. The coarse powder was further processed into fine powder using an electric grinder. 250g of the powdered plant material was soaked in 1000 ml of methanol for 4-5 days with periodic agitation. The mixture was filtered through whatman filter paper No. 1 and the filtrate was refluxed in a Soxhlet apparatus at 40^0C. Methanol extract of *C. baccans* root peel was recovered with a yield percentage of 1.8%.

2.3 Preparation of Fractions

The crude plant extract was dissolved in 70 percent methanol and subjected to liquid-liquid partition using a separating funnel in different solvents of increasing polarity viz., n-hexane, diethylether, ethylacetate, n-butanol and the residual aqueous layer was treated as an aqueous fraction.

2.4 Preliminary Phytochemical Screening

The crude methanol extract of *C. baccans* root bark was screened for the presence of major phytochemicals following the method described by Harborne[18], Trease and Evans[19] and Sofowora[20].

2.5 Experimental Animals

Adult Swiss albino mice were procured from Pasteur Institute, Shillong, India. The mice were housed and maintained under standard animal house conditions as per Organization for Economic Co-operation and Development (OECD) guidelines (i.e., 22^0C±3^0C temperature, 12 hours light/12 hours dark and fed with conventional laboratory diet with water ad libitum)[21]. The mice were allowed to acclimatize to laboratory conditions for at least 5 days.. Approval for experimental procedures was obtained from the Institutional Ethics Committee (IEC), North-Eastern Hill University, Meghalaya.

2.6 Acute Toxicity Test

Acute toxicity study was carried out using limit test as per OECD-423 guidelines[21]. About 70 healthy nulliparous and non-pregnant Swiss albino mice of 2-4 months old weighing 25-30 grams were randomly selected. The animals were divided into 3 doses group of 5 numbers; the first group received only vehicle (distilled water) and serve as control group while the second and third group received a single dose of 2000 mg/kg and 5000 mg/kg body weight (bw) of CbME by oral gavaging using a ball tipped intubation tube. The animals were fasted for 3–4 hours, and their weights were recorded before dosing and the animals were fasted further for 2 hours after dosing. The same treatment procedure was carried out using the fractions also. The animals were observed for signs of toxicity (tremors, convulsions, salivation, diarrhea, lethargy, sleep and coma, and mortality) during the first 30 minutes, periodically in the next 24 hours and thereafter daily for 14 days. After 14 days, all the animals that survived had their weight recorded and were sacrificed for gross necropsy findings.

2.7 Sub-acute Toxicity

A repeated dose oral toxicity study was carried out following the guidelines prescribed by OECD-407 guidelines[22]. Mice were divided into four groups of six animals each (3 males and 3 females) randomly. The first group served as control and received only vehicle (distilled water) while the remaining three groups received 250 mg/kg, 500 mg/kg and 1000 mg/kg bw of the CbME by oral gavage daily for 28 days and the same was repeated for the most toxic fraction. Body weight of the mice were recorded before the initial dosing and weekly till the end of the treatment period. The

mice were also monitored for general signs of toxicity and mortality throughout the study period. On the 29th day the mice were sacrificed humanely after overnight fasting.

2.7.1 Relative Organ Weight

Vital organs like liver, heart, kidneys, stomach, spleen and brain were carefully dissected, washed in normal saline, blotted dry and their weights were recorded. The weight of each organ relative to the body weight (before sacrificing) was calculated to obtain the Relative Organ Weight (ROW).

ROW (%) = (Absolute organ weight/Body weight of mice on day 29) x 100

2.7.2 Hematological Assay

On 29th day, blood was collected from the mice by retro-orbital sampling method (after applying appropriate topical anesthesia) and collected in Ethylene diamine tetra acetic Acid (EDTA) coated vials. The blood samples were analyzed for White Blood Cells (WBC) count, differential WBC count, platelet count, Hemoglobin (Hb) level, red blood cells (RBC), Packed Cell Volume (PCV) and mean corpuscles volume. All hematological analysis was done using an automated analyzer.

2.7.3 Biochemical Assay

Blood samples were also collected in non-EDTA vials for serum extraction. In brief, whole blood collected was allowed to stand undisturbed for around 45 minutes and centrifuged at 3000 rpm (at room temperature) for 10 minutes to separate the serum from other components of blood. The serum extracted was used for the analysis of important liver and kidney function markers namely alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (ALP), creatinine (Cr) and urea. All biochemical analysis was carried out using an automated analyzer.

2.7.4 Scanning and Transmission Electron Microscopy

To study the surface alterations, cause by the plant extract/fraction, the small tissue block (1mm thickness) of liver, kidney, and intestine of control and plant extracts-exposed mice were collected, fixed in neutral buffer formalin, dehydrated in acetone grades and followed by air drying in tetramethylsilane following the method of Dey *et al.*[23], and modified by Roy and Tandon[24]. The gold coated specimens were viewed under a scanning elecrtron microscope (JSM-6360JEOL). Similarly, to study the ultrastructural alterations, the small fragments of the liver, kidney and intestine tissues were fixed in 1X Karnovsky's fixative, post-fixed in 1% OsO_4 buffered with 0.1 M sodium cacodylate for 4 hours, dehydrated through graded series of acetone, and embedded in araldite. Ultrathin sections were taken and stained with uranyl acetate followed by lead citrate and were viewed under a transmission electron microscope (JEM-2100).

2.7.5 Statistical Analysis

Experimental data were expressed as mean ± Standard Error of Mean (SEM). The statistical difference between the mean of the acute and sub-acute group was analyzed by one-way analysis of variance (ANOVA) followed by Tukey's multiple comparison post hoc test using Origin Pro 2019. Data were considered to be statistically significant at $p< 0.05$.

3. Results

3.1 Phytochemical Screening Test

Preliminary phytochemical screening of *C. baccans* roots bark indicate the presence of alkaloids, phenols, flavonoids, tannins, steroids, terpenoids and glycosides.

3.2 Acute Toxicity Test

Acute oral toxicity test produced no mortality nor any observable signs of toxicity in any of the test group exposed to even a high dose of 5000 mg/kg bw of CbME during the 14 days of observation. However, of all the fractions of the CbME, only the butanol fraction appears to be toxic and causes death of the test animals within 48 hours after dosing (Table 2); while, the rest of the fractions did not show any sign of toxicity at doses as high as 5000 mg/kg bw. Hence, the *C. baccans* butanol fraction (CbBF) was considered to be the most toxic fraction and its median lethal dose (LD_{50}) was calculated by following the graphical method of Miller and Tainter, 1944[25].

3.3 Sub-acute Toxicity Test

Repeated oral administration of CbME and CbBF for 28 days showed no observable signs of toxicity nor death in the treated mice. However, a significant reduction in body weight of the test animals exposed to 1000 mg/kg bw of CbME was observed and the same effect was observed with the CbBF starting from 500 mg/kg bw(Table 3). However, no significant changes were observed in the relative organ weights of the test animals exposed to both the substances. Macroscopic examinations of the organs of test animals showed no changes in color when compared to control group.

Table 1. Finney's table for conversion of corrected mortality percentage into probit values

%	0	1	2	3	4	5	6	7	8	9
0	-	2.67	2.95	3.12	3.25	3.36	3.45	3.52	3.59	3.66
10	3.72	3.77	3.82	3.87	3.92	3.96	4.01	4.05	4.08	4.12
20	4.16	4.19	4.23	4.26	4.29	4.33	4.36	4.39	4.42	4.45
30	4.48	4.50	4.53	4.56	4.59	4.61	4.64	4.67	4.69	4.72
40	4.75	4.77	4.80	4.82	4.85	4.87	4.90	4.92	4.95	4.97
50	5.00	5.03	5.05	5.08	5.10	5.13	5.15	5.18	5.20	5.23
60	5.25	5.28	5.31	5.33	5.36	5.39	5.41	5.44	5.47	5.50
70	5.52	5.55	5.58	5.61	5.64	5.67	5.71	5.74	5.77	5.81
80	5.84	5.88	5.92	5.95	5.99	6.04	6.08	6.13	6.18	6.23
90	6.28	6.34	6.41	6.48	6.55	6.64	6.75	6.88	7.05	7.33

Table 2. Acute oral toxicity test of the *C. baccans* butanol fraction.

Group	Dose (mg/kg bw)	Log dose	Mortality (%)	Corected mortality (%)	Probit
1	4500	3.65	0	2.5	3.04
2	4600	3.66	20	20	4.16
3	4800	3.68	60	60	5.25
4	5000	3.70	100	97.5	6.96

Corrected 0% mortality = (0.25/n) 100; Corrected 100% mortality = (n – 0.25/n) 100

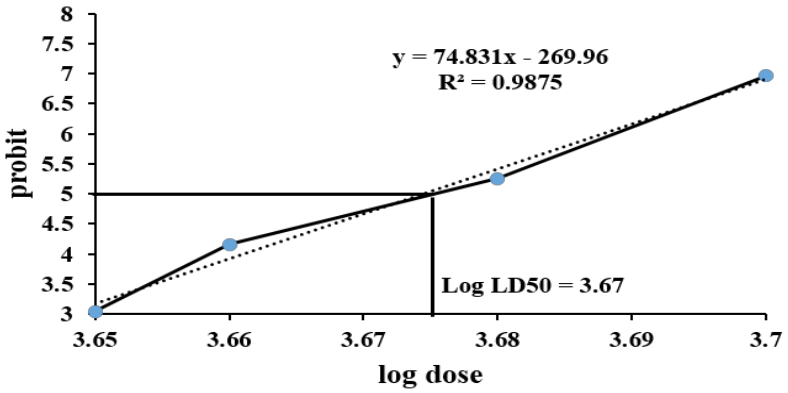


Figure 1. log dose versus probit plot to determine the LD_{50} of CbBF.

Calculation of the median lethal dose: To determine the LD50 the mortality percentage at 0% and 100% were corrected. The probit values of the corresponding mortality percentages were read from Finney's table (Table1) and are plotted against the logarithm of the different doses (Figure 1). The dose that corresponded to 50% mortality or probit 5 was found to be 4677.35 mg/kg.

To calculate the standard error (SE) of LD50, the doses corresponding to probit 4 and 6 were determined from the logarithm dose vs probit value plot and were found out to be 4570.88 and 4897.79 respectively and the standard error was calculated using the formula-

SE of LD_{50} = (log LD_{84} – log LD_{16})/$\sqrt{2n}$ = 103.38

Where, log LD16= logarithmic dose corresponding to probit 4

log LD84= logarithmic dose corresponding to probit 6.

n = number of animals in a group.

Therefore, the median lethal dose (LD_{50}) of CbBF = 4677.35 ± 103.38 mg/kg bw.

Table 3. Effect of repeated exposure of mice to *C. baccans* methanol extract and butanol fractions on the body weight changes during 28 days of sub-acute test.

	Test substance	Control	250 mg/kg bw	500 mg/kg bw	1000 mg/kgbw
Change in body weight (g)	CbME	2.19 ± 0.29	1.36± 0.11	1.42± 0.08	-0.63 ± 0.38*
10	CbBF	2.19± 0.29	1.65 ± 0.17	1.28 ± 0.167*	1.00 ± 0.07*

Results were expressed as Mean ± SEM; number of test animal for each dose group (n) = 6. Asterisk (*) – significant change at $p<0.05$ with 95% confidence interval. CbME – *C. baccans* methanol extract, CbBF – *C. baccans* butanol fraction.

Results on blood parameters analysis are shown in Table 4. The analysis revealed a significant increase in WBC count, accompanied by significant decrease in hemoglobin (Hb) content, RBC count and packed cell volume (PCV) in the group treated with 1000 mg/kg bw of CbME compared to control group. While a significant increase in WBC count and neutrophil levels was observed in the group treated with 1000 mg/kg of CbBF; a significant decrease in Hb level, RBC count and PCV was also observed starting from the dose of 500 mg/kg bw compared to the control group.

Result of the biochemical analysis of some of the liver and kidney function markers is shown in Table 5. The analysis revealed a significant increase in serum ALT activity in the test group exposed to 1000 mg/kg bwCbME, a significant increase in serum AST and ALP activity in the test group exposed to 500 mg/kg and 1000 mg/kg bwCbME compared to control; while the animals exposed to CbBF showed a significant increase in serum levels of ALT, AST and ALP starting from 500 mg/kg bw. Furthermore, the animals treated with CbME showed a significant ($p<0.05$) increase in blood urea level at 500 and 1000 mg/kg bw, and an increased level of serum creatinine were seen only at 1000 mg/kg bw compared to control group, while the animals treated with CbBF showed a significant elevation of serum levels of urea and creatinine starting from 500 mg/kg bw.

Electron microscopic analysis of liver, kidney and small intestine of the mice treated with the crude plant extract and butanol fraction showed marked differences in the tissue organization, cellular and organelle structural integrity when compared to the control set. Scanning electron microscope (SEM) study of the small intestine showed an invariably damaged villi and patches with eroded microvilli in both the test group (Figure 2 B and C) when compared to the control (Figure 2A) and transmission electron microscope (TEM) study also showed damaged columnar epithelium with deteriorated enterocytes (Figure 3 B and C) compared to control (Figure 3 A). SEM images of the liver shows numerous small indentations once occupied by fat droplets on the hepatocytes all along the hepatic plate of both the test group (Figure 2 E and F), while such structures are absent in the control (Figure 2 D). TEM analysis also concurred with the former observation with the presence of multiple spherical bodies indicating the presence of numerous fat droplets inside the hepatic cells of both the test groups. Furthermore, TEM study showed other alterations in the cellular and sub cellular organization of the hepatic cells. The treated group showed an inconspicuous cell membrane, partially disintegrated nuclear membrane, severed endoplasmic reticulum and mitochondria (Figure 3 E and F), whereas, the control (Figure 3 D) showed no such alterations. SEM of kidney showed some aberrations in the tissue of both the crude extract and butanol fraction treated mice such as atrophy of the proximal convoluted tubules (Figure 2 H and I), compared to control (Figure 2 G) and effacement of the podocyte foot processes (Figure 2 K and L) in both the treatment group when compared to control (Figure 2 J). TEM showed abnormal tubules characterized by dilation of the basolateral membrane of proximal convoluted tubules, loss of brush borders, collapsed cuboidal epithelial cells, crenated nucleus, damaged nuclear membrane, mitochondria and severed endoplasmic reticulum in the crude extract treated group (Figure 3H). While the butanol fraction treated group revealed the same effect except the crenation of the nucleus (Figure 3I). TEM study further indicate the retraction and effacement of the foot processes of the podocytes, dilation of the glomerular basement membrane and the thinning of the endothelial fenestration in both plant treated group (Figure 3 K and L) which are absence from the control (Figure 3 J).

Table 4. Effect of *C. baccans* methanol extract and its butanol fraction on blood parameters of control and treated mice

		CbME			CbBF		
Parameters	**Control**	**250 mg**	**500 mg**	**1000 mg**	**250 mg**	**500 mg**	**1000 mg**
Hb (g%)	15.88 ± 0.3	15.96 ± 0.27	16.30 ± 0.22	13.30 ± 0.27*	14.78 ± 0.17	14.60 ± 0.33*	14.45 ± 0.41*
RBC (106 Cells/L)	9.54 ± 0.18	9.39 ± 0.10	9.26 ± 0.26	7.92 ± 0.41*	9.10 ± 0.13	8.65 ± 0.16*	7.95 ± 0.30*
PCV (%)	47.51 ± 1.31	46.38 ± 1.09	43.43 ± 2.07	40.32 ± 2.53*	44.78 ± 0.51	41.77 ± 1.85*	39.58 ± 1.51*
MCV (fl)	47.96 ± 0.64	49.31 ± 0.98	48.15 ± 1.24	48.47 ± 1.71	51.68 ± 1.50	57.16 ± 0.65	58.65 ± 0.68
WBC (103 Cells/ cmm)	5.08 ± 0.22	4.95 ± 0.16	6.15 ± 0.65	7.53 ± 0.28*	4.83 ± 0.22	5.00 ± 0.16	6.15 ± 0.28*
Neutrophils (%)	5.83 ± 0.47	5.66 ± 0.33	6.16 ± 0.47	6.66 ± 0.21	6.50 ± 0.22	6.33 ± 0.21	8.50 ± 0.22*
Lymphocytes (%)	85.66 ± 1.6	85.00 ± 0.63	86.33 ± 1.33	88.17 ± 0.31	87.00 ± 0.58	88.33 ± 0.49	86.00 ± 0.45
Eosinophils (%)	4.66 ± 0.50	5.33 ± 0.49	4.83 ± 0.61	3.17 ± 0.31	3.83 ± 0.40	3.17 ± 0.48	3.16 ± 0.31
Monocytes (%)	3.83 ± 0.83	4.00 ± 0.26	2.66 ± 0.33	2.16 ± 0.17	2.67 ± 0.21	2.17 ± 0.17	2.33 ± 0.21
Platelets (105 Cells/L)	7.40 ± 0.03	7.50 ± 0.01	7.90 ± 0.04	6.60 ± 0.01	8.00 ± 0.01	8.30 ± 0.03	7.50 ± 0.03

Results were expressed as Mean ± SEM, number of test animal for each dose group (n) = 6. Asterisk (*) –significant change at p<0.05 with 95% confidence interval. CbME- *Carex baccans* methanol extract, CbBF- *Carex baccans* butanol fraction, Hb-hemoglobin, RBC-red blood cells PCV- pack cell volume, MCV- mean cell volume, WBC- white blood cells.

Table 5. Effect of *C. baccans* and its butanol fraction on liver and kidney functions in treated mice in comparison to a control group

		CbME			CbBF		
Parameters	**Control**	**250 mg**	**500 mg**	**1000 mg**	**250 mg**	**500 mg**	**1000 mg**
ALT (U/L)	16.79 ± 0.75	17.23 ± 0.50	18.85 ± 0.27	21.03 ± 1.03*	21.55 ± 0.99	39.12 ±1.26*	51.14 ± 2.15*
AST (U/L)	161.27 ± 1.62	162.35 ± 1.15	169.76 ± 1.76*	174.55 ± 1.13*	154.45 ± 2.74	173.91 ±1.18*	180.52 ± 4.46*
ALP (U/L)	91.21 ± 2.31	99 ± 2.95	196.77 ± 6.40*	270.52 ± 5.97*	95.5 ± 0.96	164.36 ± 1.56*	205.34 ± 4.95*
Urea (g/dl)	38.86 ± 0.72	41.63 ± 1.60	61.07 ± 1.52*	73.85 ± 1.61*	42.55 ±0.99	60.66 ± 1.77*	64.55 ± 0.83*
sCr (g/dl)	0.37± 0.01	0.41 ± 0.01	0.42 ± 0.02	0.42 ± 0.01*	0.45 ± 0.01	0.53 ± 0.04*	0.60 ± 0.01*

Results were expressed as Mean ± SEM, number of test animal for each dose group (n)= 6. Asterisk (*) – significant change at $p<0.05$ with 95% confidence interval. CbME- *Carex baccans* methanol extract, CbBF- *Carex baccans* butanol fraction, ALT – alanine aminotransferase, AST – aspartate aminotransferase, ALP – alkaline phosphatase, sCr – serum creatinine

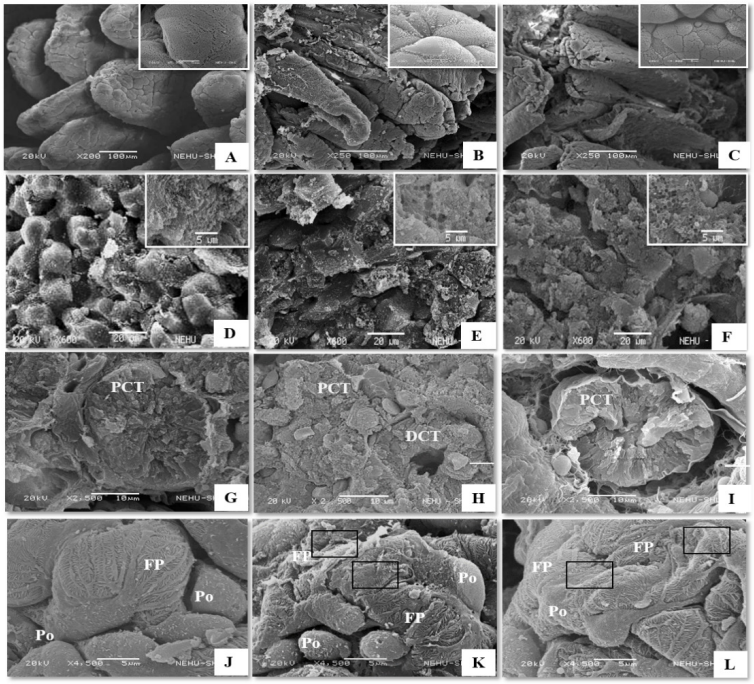


Figure 2. Scanning electron micrographs of small intestine (**A-C**), liver (**D-F**), kidney (**G-L**). A, D, G and J showing control group; B, E, H and K showing *Carex baccans* crude extract treated group; and C, F,I and L showing butanol fraction treated group. Highlighted rectangular area (K and L) showing part of the glomeruli with eroded foot processes of podocytes. PCT: Proximal convoluted tubules, DCT: Distal convoluted tubules, Po: Podocytes, FP: Foot processes.

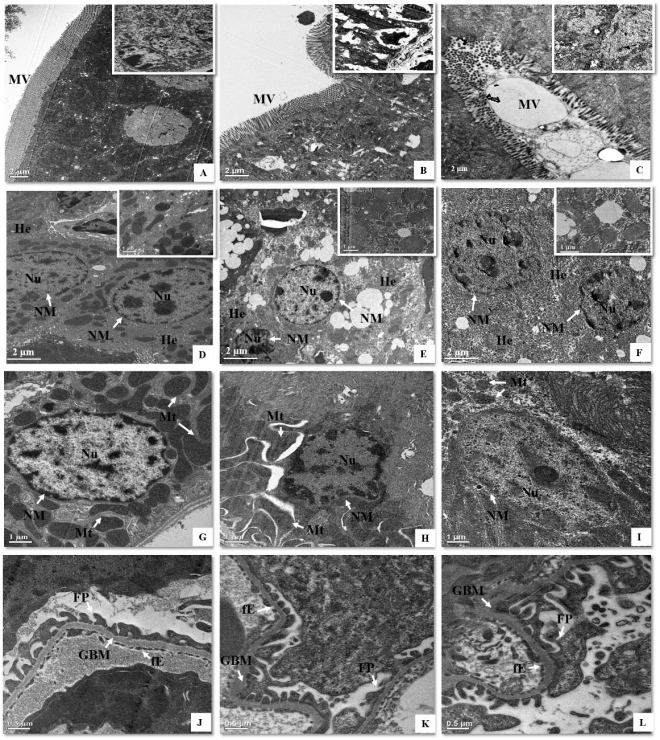


Figure 3. Transmission electron micrographs of the small intestine epithelium **(A-C)**; liver **(D-F)**; kidney **(G-L)**. A, D, G and J showing control group; B, E, H and K showing *Carex baccans* crude extract treated group; and C, F, I and L showing butanol fraction treated group. Inset picture showing magnified view.MV: Microv i, He: Hepatocyte, Nu: Nucleus, NM: Nuclear membrane, Mt: Mitochondria, FP: Foot processes, GBM: Glomerular basal membrane, fE: Fenestrated endothelium.

4. Discussions

Preliminary phytochemical screening of the CbME indicated that the plant possesses an array of phytochemicals which often serve as drug precursors and drug prototype[26]. In fact, the medicinal properties of the plant may be attributed to these phytochemicals and the interaction that exist between them. In the current studies, the oral acute toxicity study of the plant showed that the crude extract has a high safety margin evident by the lack of mortality or observable signs of toxicity in the test animal even up to a dose as high as 5000 mg/kg bw; further testing was stopped in accordance to OECD-423 limit test guidelines[21]. However, the butanol fraction of the plant causes mortality of the test animals within 48 hours after administration of 5000 mg/kg bw. Hence, the butanol fraction of *C. baccans* crude extract was considered toxic and the median lethal dose was calculated to be 4,677.35 ± 103.38 g/kgbw. One of the reasons that the butanol fraction causes mortality while the crude extract did not might be due to the neutralizing effect that the phytochemicals has on one another, as the pharmacologic properties of a medicinal plant is often a result of the actions and interactions of the plethora of secondary metabolites present and the synergistic action that exist amongst them[27].

The repeated oral administrations of the plant extract have detrimental effects on the test animals and the effect followed a dose dependent pattern. This effect was manifested in the weight of the test animals whereby weight loss was observed in animals exposed to higher doses of the plant and its butanol fraction. Some phytochemicals (alkaloids, tannins and others) are known to cause loss of appetite and weight through mechanism which involves the central nervous system or parietal nervous system or both. For instance, p-octopamine and synephrines both alkaloids cause loss of appetite and weight loss through their adrenergic agonist activity[28], while tannins such as, protanthocyanidins inhibit food intake and digestion[29]. Similarly, flavone glycosides and numerous other flavonoid compounds were reported to have appetite suppression effect through central and peripheral nervous system[30].

The study also suggests that higher doses of the plant causes an elevation of leukocyte and neutrophils which often is a symptoms of underlying stress or disease or an infection. Elevated levels of neutrophils are indications of an early acute phase reaction of the innate immune response, they bind to the endothelium and release cytokines, reactive oxygen species and proteases which serves as an early marker of kidney diseases[31]. Furthermore, there is a suggestion of red blood cells destruction which is corroborated by low hemoglobin content, low red blood cells count and reduced percentage of red cells in circulation as highlighted by the low packed cell volume. These anomalies in blood parameters might be related to the disturbances effected by the plant on the kidneys, as suggested by the elevated levels of both creatinine and urea in the serum and by the electron microscopic evaluation of the kidneys. Also, the kidney secretes the hormone erythropoietin (EPO) which promotes the formation of red cells by the bone marrow; an underlying kidney disease might have retarded the production of EPO and hence reduced the number of circulating red cells[32].

The plant extract also showed a dose dependent effect on liver chemistry whereby serum activities of ALT, AST and ALP were observed to be significantly increased at higher doses in both the crude extract and fraction treated group. Elevated levels of transaminases are a hallmark of hepatocellular injury while elevated levels of ALP may be a result of biliary obstructions due to cholestatic and/or infiltrative liver damage[33]. Additionally, serum levels of urea and creatinine also followed the same pattern as the liver functions counterparts. Serum concentrations of urea and creatinine are relatively useful biomarkers of renal function[34]. Elevation in serum levels of urea and creatinine are often seen in acute renal diseases or failure[35]. Though, serum creatinine is a poor indicator of renal injury particularly at the early stages[36], it may be used to indicate the efficiency of glomerular filtration and renal tubular function to some extent.

Ultra-structural studies of the small intestines, liver and kidney of the test animals using scanning and transmission electron microscopy further highlighted the adverse impact that the plant has at the cellular and subcellular levels. The small intestine represents the most optimal site for drug absorption particularly across the villi surface. The small intestinal lumen is a very robust structure with a very high regenerative capability and regenerates its epithelial lining every 5–7 days to withstand the normal wear and tear[37]. Effects of the plant on the small intestine include damage to the columnar epithelium and subsequent loss of microvilli. These columnar epithelial cells line the inner surface of the tubular organs such as the small intestines which is the site where majority of absorption and metabolism of xenobiotic compounds take place. Damage to these intestinal epithelial cells may lead to mal absorption of nutrients and the metabolism of the exogenous material. In the case of the liver, the cells lack a distinctive cell membrane separating and even the nucleus have an uneven and crenated appearance with extensively damage nuclear membrane, characteristic of a damage and dying cell. Besides the deviation in the overall cell structure and nuclear integrity, presence of fat droplets accumulation

in the liver indicates an interference in lipid metabolism of the liver. The causes for such disruptions may be an endoplasmic reticulum stress, as the endoplasmic reticulum play a vital role in lipid metabolism and hepatic steatosis[38]. Besides fragmented ER, the hepatic cells also showed other anomalies such as severed mitochondrial membrane, lysed cristae due to phospholipids membrane interference[39]. The results also highlighted the effect the plant has on the kidney. The effects include alterations in the morphology of the podocytes characterized by the retraction and swelling of the foot processes resulting in their effacement, dilation of the glomerular basement membrane and disappearance of endothelial fenestration all these could lead to an impaired glomerular filtration rate and may lead to renal complications. A number of toxins and drugs are known to cause glomerular complications which are mainly associated with damages to the glomerular endothelial cells, podocytes or deposition of immune complex within the glomerular capillary basement membrane[40,41]. Furthermore, loss of brush borders and sloughing of cuboidal epithelial cell into the PCT lumen also indicate the possibility of tubular necrosis[42] which may be attributed to the extensive cellular uptake of toxins by the apical and basolateral transport system. Accumulation of such toxins in the cell cytoplasm at high concentration may facilitates the degradation of phospholipids membrane, oxidative stress and mitochondrial injury that eventually leads to proximal tubular cell apoptosis/necrosis[43].

5. Conclusion

This study highlighted and validated the danger of prolonged exposure to the plant *C. baccans*, and particularly its butanol fraction which exhibit lethal consequences even for single use at high concentration. This indicates that the plant may possess some phyto-toxins or the biotransformation of the plant's active ingredients may lead to the formation of a potent toxin resulting in the manifestation of a number of adverse effects. The adverse effect of the plant on the test animals range from weight loss, leukocytosis, thrombocytosis, hepatic and renal complications. Therefore, the uses of the plant for healing purposes via oral route of administration should be limited to low doses to avoid any adverse effect.

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