Original Paper

Acute and Subacute Toxicities of Hydroethanolic Extract of the Ripe Fruits of *Solanum torvum Sw.* (Solanaceae)

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ABSTRACT

Acute and subacute toxicity of the hydro-ethanolic extract of the ripe fruit of Solanum torvum Sw. was studied by force-feeding albino Wistar rats following the European Community and WHO toxicity guidelines. The results of the acute toxicity study indicated the median lethal dose (LD_{50}) as 19g/kg body weight after 48 hours of treatment, and the significant variation ($P < 0.05$) of serum aspartate aminotransferase (AST), alanine aminotransferase (ALT), total proteins (TP), total bilirubin (TBil), and creatinine at doses of 16 -20g/kg body weight. These results also indicated significant variation of the liver alkaline phosphatase (ALP), AST, ALT, TP, glutathione (GSH), and malondialdehyde (MDA) at higher doses. The results of the subacute toxicity study showed significant variation in the body weight, but no modification ($P > 0.05$) of blood and liver parameters compared to the control group. In both acute and subacute toxicity, histological studies revealed that there were no major pathological changes of the liver and kidneys in treated rats. The results show that this extract is not highly toxic, but consumption of higher doses beyond 16g/kg could cause liver injury. Moderate consumption of small doses up to 1g/kg twice a week for 6 weeks appeared safe.

Key Words: *Solanum torvum Sw., toxicity assay, hydro-ethanolic extract*

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Introduction

Solanum torvum Sw (Solanaceae) is a small tree or shrub of 3-4 meters height, with a woody stem. Leaves are alternate, large, densely shaggy on both sides, and are rough to the touch. The bay like fruit is green coloured, with numerous seeds. The plant grows well in the humid prairies of tropical regions.[1] The herbalists and traditional practitioners of the West of Cameroon use decoction of the fruits in the treatment of male and female infertility, gastritis, bacterial urogenital infections, and infections caused by *Candida albicans*.[2,3] Many traditional practitioners assign to the fruits of this plant, an anti-witchcraft and anti-vampirism properties. The fruits are also used in the folk medicine of Cameroon for the treatment of AIDS. In Nigeria, the decoction of the fruits is given to treat splenomegaly, and it is also used as a remedy for cough in the paediatric age group.[4] In Guatemala, the plant is believed to have antispasmodic, anticonvulsive, and anticoagulant properties.[5]

The antimicrobial activity of this plant has been tested on a number of pyogenic microorganisms of animal and human infections, urogenital infectious agents, and *Herpes simplex virus* types I and II.[6-8] However, in spite of these claims, and the widespread use of this plant, no toxicological studies have so far been reported. This study was therefore carried out to evaluate the acute and subacute toxicity of the hydro-ethanolic extract of the fruits on albino rats. The effects on body weight, liver, as well as on serum and hepatic biochemical parameters were investigated.

Materials and Methods

Plant material: The fruits of *S. torvum* were collected in February 2001 in the Bamenkombo village,

Bamboutos division of the West province of Cameroon. The botanical identification of the plant was confirmed by the National Herbarium of Cameroon where the voucher specimen was conserved under the reference number 49427/HNC.

Preparation of extract and phytochemical analysis: The collected plant material was finely cut, dried, and ground using a grinder. 3 kg of the obtained powder was extracted with 10 litres of the water-ethanol solvent system (40:60 v/v) for 48 hours. The extract was filtered using Whatman filter paper n°1 and concentrated in an incubator at 56°C till total dryness. The hydro-ethanolic extract (HEST) obtained with 8.51 ± 3.15% yield (3 repetitions) was stored at 4°C for the various toxicological studies. Phytochemical analysis was carried out using the previously described method of Harbone for alkaloids, flavonoids, steroids, tannins, glycosides, phenols, polyphenols, saponins, coumarins and anthraquinones.[9] Bioguided purification of the HEST was also done.[10]

Experimental animals: Five weeks (90-100 g) and 10 weeks (140-160 g) old, clinically healthy Wistar rats bred in the Department of Biochemistry, University of Yaoundé I, Cameroon, were used. The animals were kept under standard environmental conditions (temperature: 27±1°C). They were fed with standard diet and water *ad libitum*. The bioassays were conducted in accordance with European Community EEC Directives of 1986 (86/609/eee).

Acute toxicity: The five week-old rats were divided into eight groups of eight animals each (four males and four females). Animals were force-fed with a single dose of the following extract concentrations: 0, 4, 8, 12, 16, 20, 24 and 28 g/kg body weight. The control group received distilled water (0 g/kg). Rats were deprived of food but not water 15 hours prior to administration of the plant extract.

Observations were made and recorded systematically at 1, 2, 4, 24 and 48 h after extract administration. The body weights were recorded from the 1st to the 7th day of observation. Visual observations included change in behaviour pattern, breathing rhythm, as well as characteristics of faeces. The number of survivors was noted after 24 hours, and these were maintained for further 7 days with single daily observation.

At the end of the experiment, all surviving animals were sacrificed, and the liver as well as the serum were carefully collected. The serum was separated from the non-heparinized blood, and assayed for AST, ALT, ALP, TP, total bilirubin (TBil), direct bilirubin (DBil), creatinine (Cr), serum urea (SU), and serum glucose (SGlu). The liver was weighed immediately on an electronic balance and triturated using mortar. The crushed liver was mixed with Tris-HCl buffer 50 mM, KCl 150 mM, pH 7 at a ration 20% (w/v). The supernatant was harvested after centrifugation at 10,000 g for 30 minutes (Centrifuge LD5-2A), and also assayed for liver AST, ALT, ALP, TP, MDA and GSH using standard spectrophotometric methods [Blood and liver proteins: Buiret method; ASAT and ALAT activity: Reitmann-Frankel method; ALP: p-nitrophenol method; TBil and DBil: Jendrassik & Grof method; SU: diacetyl monoxime method; SGlu: O-toluidine method;[11] liver GSH: 2,2'-dithio-5,5'-dinitrobenzoïc acid method;[12] liver MDA: thiobarbituric acid method[13]]. As the result of one survivor at the dose of 24 g/kg could not be compared statistically with the other group,[14] the rat was not sacrificed.

Subacute toxicity: Two groups of 8 rats (4 males and 4 females) received the plant extract at the dose of 1g/kg body weight or water (control group) 2 days weekly for 6 weeks. During the period of administration, the animals were weighed weekly and observed daily to detect signs of toxicity. The rats were fed with a pathologically safe commercial diet during the test period. At the end of the period, all the animals were fasted overnight before anesthetization. Blood samples were collected from the common carotid vessel into non-heparinized centrifuge tubes. The serum was separated and assayed for the previously mentioned indices, as in the case of the acute toxicity study.

After blood collection, animals were sacrificed, and the liver was collected. The 20% (w/v) supernatant of the liver was also prepared and assayed for the same parameters as for the acute toxicity study.

Histopathological study: Histopathological study was done according to the method described by Lamb[15] on the liver and kidneys in both acute and subacute toxicity studies. Briefly, small organ pieces (3-5 mm) were fixed in 10% formal-saline for 24 hours. Samples were dehydrated by passing through 50, 70, 90, and 100% alcohol over a 2-day period, and then cleared in benzene to remove alcohol until the tissue became more or less transparent. Embedding was done by passing the cleared samples through three cups containing molten paraffin at 50°C and then in a cubical block of paraffin made by the "L" moulds. This was followed by section cutting, and staining with eosin.

Statistical analysis: Results were expressed as mean ± standard deviation (SD). Statistical analysis was determined by one-way analysis of variance (ANOVA), and post hoc least significant difference (LDS) test. A P <0.05 was considered statistically significant.

Results

Preliminary phytochemical results indicated the presence of alkaloids, flavonoids and steroids, tannins, glycosides, phenols, polyphenols, and saponins in the HEST. Coumarins and anthraquinones were absent. Bioguided purification of the HEST[10] indicated the presence of known compounds such as solasodine, lupeol acetate, and solanine in the HEST at the respective percentages of 0.5%, 0.018% and 0.16%. Four other alkaloids and three steroids of unelucidated structure were also isolated in the HEST at respective percentages ranging from 0.053% to 0.11% and 0.00022% to 0.045%.

In the acute toxicity study, the animals treated at a dose of 0 to 8 g/kg did not present any behavioural change after administration, and during the 7 days of observation. From 12 g/kg onwards, changes observed 30 minutes after the administration of the plant extract included slow response to external stimuli, prostration, stretching, and sluggishness, all punctuated by diarrhoea. However, no animal's death was noted, and the behaviour became normal after 24 hours. From 16 g/kg, the signs of poisoning became more pronounced. The diarrhoea was more abundant, and 3 of the 8 rats died in the first 2 hours that followed the treatment, and this number was maintained till the end of the observation period. At 20 g/kg and 24 g/kg, there were 4 and 7 dead rats respectively. Mortality was total at the dose of 28 g/kg. The death of the animals was generally preceded by a progressive reduction in motility initially, and later convulsions. The LD_{50} calculated according to the Behrens and Karber method[14] was 19 g/kg body weight.

At 0 to 16 g/kg, the weight of the rats varied minimally, but at 20 g/kg a significant difference (P <0.05) was noted between the rats of the control group and the test animals (**Table 1**). The evolution of the liver weights in the acute toxicity is presented in **Table 2**. Statistical analyses showed a significant difference between the rats treated at the dose of 20 g/kg, and those of the control group. At this dose, a reduction of 10.31% in the liver weight (3.65 ± 0.035 against 4.07 ± 0.24 g) was noted as compared to the animals of the control group.

The evaluation of the blood and hepatic biochemical parameters for the acute toxicity screening is presented in **Table 3** and **Table 4** respectively. The serum ALP, DBil, SGlu and the SU of the treated animals did not vary significantly (P >0.05) when compared to that of the control group. The most important variation for these parameters was that of the DBil; in effect at the dose of 16 g/kg, an increase of about 13.99% (0.057 ± 0.008 against 0.05 ± 0.005 mg/l) was noted as compared to the control group. The SGlu showed a small increase of 0.35% (1.415 ± 0.019 against 1.41 ± 0.015 g/l for the control group) with animals treated with 20 g/kg. Other blood biochemical parameters (AST, ALT, TP, TBil, and Cr) varied significantly (P <0.05).

As for the studied liver biochemical parameters, all showed significant modification (P <0.05) between the treated and control group animals. ALT and MDA were the most sensitive parameters, with 44.86% reduction rate (18.40 ±1.14 against 33.37 ± 0.14 IU/l) and 385.80% increase rate (33.375 ± 11.73 against 6.87 ± 2.32 nmole/g of the liver) respectively with the rats treated at 20 g/kg.

The body weight evolution in the subacute toxicity study is presented in **Table 5**. The ANOVA did not show any significant difference (P >0.05) between the weights of the treated and control rats. Nevertheless, a difference of 7.15 g in the weight gain of the animals treated with 1g/kg body weight was noted when compared to the control group.

The evolution in the liver weights in the subacute toxicity study presented in **Table 6** show that the modifications (P >0.05) were not significant between the treated and non-treated rats. These modifications were not also significant for all the investigated blood and liver biochemical indices in the subacute study (**Table 7** and **Table 8**).

In the acute toxicity study, histological examination revealed no pathological features in both control and treated animals up to a dose of 12g/kg body weight. From 16 to 20g/kg, mild liver pathological findings characterized by abnormal hepatic configuration were observed with treated animals. Nevertheless, no degenerative effect of the kidney cells was observed both in control and treated rats up to a dose of 20g/kg body weight (**Table 9**). In the subacute toxicity study, no hepatic or renal pathological features were observed in control and treated rats (**Table 10**).

Discussion

Hydro-ethanolic extract was employed in this study because of its use in Cameroon folk medicine for the treatment of various ailments. The oral administration of the extract, up to a dose of 8g/kg body weight did not produce behavioural changes. As LD_{50} of 19g/kg body weight was significantly greater than 5g/kg, this extract can be considered as a fairly non-toxic compound as per the OECD guidelines for testing chemicals.[16] Nevertheless, the toxic effects causing death of the animals at higher doses might be due to the presence of toxic compounds. Solanine is the common glucoalkaloid of the genus Solanum known to cause nausea, vomiting, diarrhoea, colic, dizziness, fever, sweating, and tachycardia, followed by the slowing of the heart, dilatation of pupils, convulsions, coma, and death.[17] This compound may be implicated in the observed behavioural changes. The biochemical blood and liver parameters were evaluated.

Table 1 Acute toxicity study - Body weight change in rats

Treatment (g/kg)	Body weight[a]		
	Day 0	Day 7	Weight gain
0 (n=8)	97.10 ± 3.15	117.26 ± 4.32	20.16 ± 1.17
4 (n=8)	94.36 ± 3.36	113.74 ± 6.05	19.38 ± 2.71
8 (n=8)	97.52 ± 2.17	118.76 ± 5.71	21.24 ± 3.54
12 (n=8)	96.08 ± 2.84	112.75 ± 7.88	16.67 ± 5.04
16 (n=5)	98.21 ± 3.05	114.25 ± 8.59	16.04 ± 4.50
20 (n=4)	95.92 ± 3.58	104.15 ± 6.35	8.23 ± 2.77*

*Values are expressed as mean ± SD; [a] n varied from 8 to 4 rats; * Significant difference from control P<0.05*

Table 2 Acute toxicity study - Liver weights (g/100g body weight) of rats

Treatment (g/kg)	0 (n=8)	4 (n=8)	8 (n=8)	12 (n=8)	16 (n=5)	20 (n=4)
Liver weight[a] (g)	4.07 ± 0.23	4.04 ± 0.47	4.09 ± 0.58	4.01 ± 0.26	3.67 ± 0.29	3.65 ± 0.035
Ranged value (g)	3.81-4.51	3.48-4.76	3.20-4.98	3.49-4.55	3.18-4.16	3.60-3.70

Values are expressed as mean ± SD; [a] n varied from 8 to 4 rats; No significant difference from control P>0.05

Table 3 Acute toxicity study - Blood biochemical values

	Treatment (g/kg body weight) [a]					
Biochemical indices	0 (n=8)	4 (n=8)	8 (n=8)	12 (n=8)	16 (n=5)	20 (n=4)[a]
ALP (IU/L)	95.58 ± 12.02	95.06 ± 11.52	97.10 ± 8.02	96.91 ± 9.27	98.6 ± 8.64	98.37 ± 8.04
AST (IU/L)	89.97 ± 7.15	89.99 ± 7.06	91.80 ± 7.77	94.12 ± 6.46	97.24 ± 5.60	104.44* ± 12.87
ALT (IU/L)	36.39 ± 2.18	37.48 ± 2.11	38.6 ± 2.55	40.44 ± 2.04	40.11 ± 1.60	44.46* ± 2.66
Total Protein (mg/ml)	40.18 ± 1.15	40.59 ± 1.415	40.82 ± 1.46	41.79* ± 0.93	38.74* ± 1.19	36.36* ± 2.67
Total Bilirubin (mg/l)	3.87 ± 0.45	3.98 ± 0.24	4.30 ± 0.59	4.76* ± 0.56	4.98* ± 0.41	4.83* ± 0.15
Direct Bilirubin (mg/l)	0.05 ± 0.005	0.054 ± 0.003	0.05 ± 0.003	0.051 ± 0.002	0.057 ± 0.008	0.053 ± 0.002
Creatinine (mg/l)	3.28 ± 0.047	3.18 ± 0.19	3.34 ± 0.08	3.34 ± 0.04	3.68* ± 0.10	3.97* ± 0.16
Glucose (g/l)	1.41 ± 0.015	1.386 ± 0.015	1.391 ± 0.016	1.4 ± 0.014	1.40 ± 0.013	1.415 ± 0.019
Urea (mg/l)	116.63 ± 10.06	119.25 ± 10.56	119.75 ± 11.50	121.38 ± 16.25	123.88 ± 14.83	127.38 ± 19.29

*Values are expressed as mean ± SD; [a] n varied from 8 to 4 rats; * Significant difference from control P<0.05*

Table 4 Acute toxicity study - Liver biochemical values

	Treatment (g/kg body weight) [a]					
Biochemical indices	**0 (n=8)**	**4 (n=8)**	**8 (n=8)**	**12 (n=8)**	**16 (n=5)**	**20 (n=4)**
ALP (IU/ L)	24.1 ± 1.08	26.12 ± 1.6	24.12 ± 2.4	23.15 ± 1.9	23.19 ± 1.8	17.23* ± 1.2
AST (IU/ L)	62.55 ± 0.23	62.62 ± 0.84	63.30 ± 0.51	60.53 ± 0.59	51.37* ± 0.26	53.439* ± 1.03
ALT (IU/ L)	33.37 ± 0.14	33.33 ± 0.14	31.32 ± 0.19	31.33 ± 0.15	24.36* ± 1.07	18.40* ± 1.14
TP (mg/ g of the liver)	130.89 ± 17.32	130.21 ± 13.57	130.33 ± 24.62	111.79* ± 19.86	108.13* ± 24.85	106.04* ± 14.37
GSH (µmole/g of the liver)	5.65 ± 0.25	5.31 ± 0.196	5.27 ± 0.12	5.06* ± 0.17	4.97* ± 0.40	4.78* ± 0.28
MDA (nmole/g of the liver)	6.87 ± 2.32	11.37 ± 4.44	12.75 ± 4.99	12.37* ± 3.84	23.75* ± 7.33	33.375* ± 11.73

*Values are expressed as mean ± SD., [a]n varied from 8 to 4 rats * Significant difference from control P< 0.05*

Table 5 Sub-acute toxicity study - Body weight change in rats

Treatment (g/kg)	Body weight [a]			
	Week 0	**Week 3**	**Week 6**	**Weight gain**
0	146.38 ± 2.08	163.01 ± 4.19	186.83 ± 6.24	40.45 ± 4.16
1	146.07 ± 2.72	150.07 ± 3.28	179.37 ± 7.04	33.30 ± 4.32

Values are expressed as mean ± SD; [a] n = 8 No significant difference from control P>0.05

Table 6 Sub-acute toxicity study - Liver weights (g/100g body weight) of rats

Treatment (g/kg)	0	1
Liver weight (g)	4.91 ± 0.44	4.46 ± 0.43
Ranged value (g)	4.48-5.52	4.07-5.23

Values are expressed as mean ± SD., n=8 No significant difference from control P> 0.05

Table 7 Sub-acute toxicity study - Blood biochemical values

Biochemical indices	Treatment (g/kg body weight)	
	0	**1**
ALP (IU/L)	87.16 ± 6.62	88.26 ± 6.20
AST (IU/L)	90.83 ± 11.59	93.22 ± 4.79
ALT (IU/L)	38.27 ± 3.37	40.93 ± 1.91
Total Protein (mg/ml)	78.01 ± 2.60	82.71 ± 3.02
Total Bilirubin (mg/l)	2.12 ± 0.48	2.44 ± 0.20
Direct Bilirubin (mg/l)	0.047 ± 0.003	0.044 ± 0.009
Creatinine (mg/l)	5.97 ± 0.08	6.14 ± 0.07
Glucose (g/l)	1.45 ± 0.024	1.446 ± 0.031
Urea (mg/l)	140.25 ± 11.23	147.13 ± 10.67

Values are expressed as mean ± SD., n=8 No significant difference from control P> 0.05

Table 8 Sub-acute toxicity study - Liver biochemical values

Biochemical Parameters	Treatment (g/kg body weight)	
	0	1
ALP (IU/L)	26.18 ± 1.24	26.67 ± 1.59
AST (IU/L)	67.48 ± 3.32	66.52 ± 2.16
ALT (IU/L)	34.48 ± 0.83	32.48 ± 1.4
Total Protein (mg/ml)	172.32 ± 21.86	164.25 ± 16.57
GSH (µmole/ g of the liver)	6.32 ± 0.12	6.15 ± 0.20
MDA (nmole/ g of the liver)	15.13 ± 4.65	19.63 ± 6.22

Values are expressed as mean ± SD., n=8 No significant difference from control P> 0.05

Table 9 Acute toxicity study - Histological observations

Treatment (g/kg body weight)	Organ	
	Liver	Kidney
0	Normal histological picture	Normal histological picture
4	Normal histological picture	Normal histological picture
8	Normal histological picture	Normal histological picture
12	Normal histological picture	Normal histological picture
16	Few degenerative cells observed	Normal histological picture
20	Few degenerative cells observed	Normal histological picture

Table 10 Sub-acute toxicity study - Histological observations

Treatment (g/kg body weight)	Organ	
	Liver	Kidney
0	Normal histological picture	Normal histological picture
1	Normal histological picture	Normal histological picture

so as to determine the various target sites of the toxic effects of the plant, and determine the non-toxic dose. As no significant modification of these parameters occurred in both the blood and the liver for doses up to 8g/kg body weight, this dose can be retained as highest non-toxic dose.

The enzyme most often measured to indicate bile duct obstruction is ALP. The tissue sources of ALP include liver cells, osteoblasts, intestinal cells, and placental tissue. Measurement of this enzyme is also done to investigate bone, liver, and gall bladder diseases in which ALP may reach very high levels. In this study, ALP levels varied significantly only in the liver at the dose of 20g/kg body weight. This result showed that the extract may be implicated, though poorly in bile duct obstruction. However when the bilirubin values were considered, it can be noticed that the extract did not enhance bile duct obstruction in the rats, as the first significant modification value in TBil was obtained at the dose of 12g/kg body weight without a variation in DBil value for up to the dose of 20g/kg. Creatinine values of the treated rats increased significantly from the dose of 16g/kg to 20g/kg, but still were not higher than one-fold when compared with the control group. The extract may not be implicated in kidney damage as no significant variation of SU was obtained for up to a dose of 20g/kg .

In the case of the liver, the two enzymes most associated with hepatocellular damage are AST and ALT. AST is highly concentrated in cardiac muscle, liver, skeletal muscle, and kidneys. Small amounts of enzyme are present in the brain, pancreas, and lungs. ALT is found principally in liver with only small amounts being present in other organs. Therefore, ALT estimation is more specific to evaluate liver damage than AST. In general, with liver diseases, serum AST and ALT rise and fall in paral-

lel.[18] In the acute toxicity evaluation of the studied extract, the modification of the AST and ALT values were not significant for up to a dose of 16 and 12g/kg body weight respectively for the blood, and in the liver parameters. This result implies that the injury to the liver as well as to the heart and other organs might only be induced by doses greater than 12g/kg body weight. Their release in the blood might be essentially due to the peroxidation of the cell membrane lipids as shown by the MDA rate, an essential marker of membrane degradation. Generally, the level of AST, ALT, and ALP are higher than that found in the liver, and this dynamic is respected in this study.

The main serum proteins comprise albumin and globulin. Albumin is synthesized entirely in the liver, and is present in greater concentration than globulin in the plasma and serum. Globulin is produced in the liver, and also in the reticuloendothelial system. An increase in serum total protein is not common. When it occurs, it is usually due to haemoconcentration following shock, severe vomiting, or diarrhoea; increase in globulin production is associated with splenomegaly, chronic infections, and liver disease. Decrease in total protein has been found to be associated with high tissue demands as a result of an increase in body need for protein due to liver disease associated with a reduction in protein synthesis, loss of protein from the body in urine as in nephrotic syndrome, malabsorption as in chronic pancreatitis and coeliac disease, and low protein intake. In the present study, no modification of the serum and liver proteins was observed up to a dose of 12g/kg. Beyond this dose, consumption of this plant extract is likely to enhance liver damage, or cause a disorder of protein metabolism.

Glutathione is an endogenous compound playing an important role in drug metabolism. It is the main substrate for most of the body purification enzymes (GSH-S transferase, GSH peroxidase, etc).[19] A significant decrease in the liver concentrations between the treated and control group rats was observed at the dose of 8g/kg. This may be due to its faster use for the inactivation of reactive metabolites formed during the biotransformation of the constituents of the plant extract compared to regeneration speed.

The dose of 1g/kg was used for the study of the subacute toxicity. According to the OECD guidelines, if an acute toxicity test at one dose level of at least 5g/kg body weight produces no observable toxic effect, then the full study using three dose level is considered unnecessary. Therefore the dose of 1g/kg could be given twice a week for up to 6 weeks. After such treatment, no significant variation of the biochemical values tested in the acute toxicity assay were observed.

The results of the histological studies indicate that hepatic damage manifesting as hepatitis in which cellular destruction is limited in extent[20] might be expected only in treatment at higher doses. On the contrary, this plant extract does not seem to induce kidney damage. The results show that prolonged usage of this extract at lower doses is safe even for the liver, while administration of doses beyond 12g/kg could cause liver injury.

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