SUB-CHRONIC SAFETY ASSESSMENT OF CRUDE METHANOL EXTRACT OF *Solanum aethiopicum* (L.) FRUIT IN RATS

Supported by tetfund

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Received: May 26, 2020 **Accepted:** September 05, 2020

Abstract: Safety assessment of medicinal plant is vital and necessary during the course of drug development. Establishing the toxicity profile of a medicinal plant approves or disapproves next step during bioassay screening. *Solanum aethiopicum* (L.), family *Solanaceae* is popularly known as garden egg. In Nigeria, it is called *gauta* by Hausa tribe, *igbagba* by Yoruba tribe and *afufa* by Igbo tribe. The primary goal of this research was to establish safety profile of crude methanol extract of *Solanum aethiopicum* fruits in rats. Acute toxicity was determined using 5 female rats weighing (180 – 220 g). They were given single dose of 5, 50, 300, 2000 and 5000 mg/kg of extract and observed for 48 h for sign of toxicity and death. Sub-Chronic toxicity test was carried out using 40 rats (20 males, 20 females). Group I were given distilled water and II-IV received 250, 500 and 1000 mg/kg of extract for 28 days. They were observed for behavioural changes, then blood sample taken for haematological and biochemical analysis. Acute toxicity result shows the oral LD_{50} of crude methanol extract of *Solanum aethiopicum*is above 5000 mg/kg in rat. In addition, sub-Chronic toxicity testing indicated no observable behavioural changes and all haematological parameters shows no statistically significant changes. In addition, no significant changes observed in urea, creatinine and electrolytes level. Lastly, no significant change observed in all biochemical parameters except aspartate aminotransferase. *Solanum aethiopicum* fruit extract is generally safe because it does not alter kidney or liver function, does not affect internal organ growth and no any sign of toxicity during short term consumption. Further research should be conducted to establish its chronic toxicological data.

Keywords: Acute, sub-chronic, *Solanum aethiopicum*, OECD, wistar-rats

Introduction

Plants as source of medicine are getting wider acceptance because of their natural source, ease of access, convenience to use and cost effectiveness. Precisely, about 80% of world population use traditional medicine to treat their ailments (WHO, 2013; Edewor-Kuponiyi, 2013; Ameh *et al*., 2010).

Generally, large numbers of herbal medicine consumed for short term disease treatment are considered natural and safe in our communities. In addition, several medicinal plants previously tested scientifically were reported to be safe even above 5000 mg/kg dose (Al-Afifi *et al*., 2018; Porwal *et al*., 2017; Igbe *et al*., 2015; Khan *et al*., 2016; Patric-Iwuanyanwu *et al*., 2012). However, according to the experts opinion, natural does not implies absent of toxicity. In many instances, medicinal plants were found to cause side effects, adverse effects and even drug interactions (Nath and Yadav, 2015; Ernst, 2002). Also, chronic toxicity studies data from six month to one year experiment on the use and consumption of medicinal plants are scarcely available (Eran *et al*., 2016; NRC, 2006).

Solanum aethiopicum (L.), family *Solanaceae* is popularly known as garden egg or Ethiopian eggplant, Ethiopian nightshade, mock tomato or bitter tomato. In Nigeria, it is called *gauta* by Hausa, *igbagba* by Yoruba and *afufa* by Igbo tribes (Eletta *et al*., 2017; Eze and Kanu, 2014; Anosike*et al.* 2012; Chinedu *et al*. , 2011; Osei *et al*., 2010; Burkill, 2000). *Solanum aethiopicum* fruit is used as food and can be eaten raw. The fruit is given to the visitors as a special gift to celebrate marriages and new born babies. *Solanum aethiopicum* is used traditionally to treat mental disorders. In addition, the fruits are used as an ingredients in making traditional vegetable soup (Eletta *et al*., 2017; Eze and Kanu, 2014; Anosike *et al.*, 2012; Chinedu *et al*., 2011; Osei *et al*., 2010; Burkill, 2000). Previous experiments conducted using *Solanum aethiopicum* fruit extract reported anti-inflammatory (Anosike *et al*., 2012). Also, the fruit and leaf decrease body weight and blood glucose (Emiloju and Chinedu, 2016; Okafor *et al*., 2016). In addition, the fruit reduces cholesterol level (Chinedu *et al*., 2013). The leaf has antifungal activity (Watanabe *et al*., 2001). The leaf of *Solanum aethiopicum* possesses laxative activity (Saba *et al*., 2002). The fruit have shown antiulcer property (Chioma *et al*., 2011). The fruit also has antioxidant activity (Eletta *et al*., 2017).

Materials and Methods

Experimental animals

Wistar rats (180 – 220 g) each of either sex were purchased and maintained at the Animal House of Department of Pharmacology, Bayero University, Kano. They were kept in air-conditioned area maintained below room temperature (22± 3ºC) and light regulated at (12 h light/12 h dark circle). The relative humidity was maintained at 50± 6%. The animals were fed on Vital Feed (Buruku, Jos) and water *ad libitum.*

Plant materials

The whole plant material was collected from Fallau Town, Dawakin Kudu Local Government, Kano State. The identification and authentication was done by Bahauddeen Said Adam, Department of Plant Biology, Bayero University, Kano. Voucher number was collected as BUKHAN 0501 and kept for future references.

Ethical approval

The study was conducted after obtaining ethical clearance from ethical committee of the College Health Sciences, Bayero University, Kano: Ref No: BUK/CHS/REC/69.

Extraction

The fruits were first washed, shade dried, and grinded into a coarse powder using motar and pestle. The powdered fruit (2 Kg) was macerated using 6L of 70% methanol v/v with occasional shaking for 7 days and filtered using Whatman No. 10 filter paper. The filtrate was evaporated to dryness *in vacou* at 40ºC to yield residue (Deng *et al*., 2007).

Acute toxicity studies

This test was conducted according to the method of Organization for Economic Cooperation and Development, guidelines 420 of 2001 (OECD, 2001). Five Wistar rats (180-220g) were selected and divided into five groups of one rat each. The animals were made to fast overnight by withdrawing food but not water. The f procedure involved

administration of 5, 50, 300, 2000 and 5000 mg/kg of crude methanol of *Solanum aethiopicum* orally. The animals were observed for signs of toxicity and mortality within 48 hours. They were further observed for up 14 days for the late signs of toxicity. The whole experiment was conducted between **900** and **1600** h (OECD, 2001).

Sub-chronic toxicity studies

This test was conducted according to the OECD method, guidelines 407 of 2008 (OECD, 2008). Forty Wistar rats (20 males and 20 females) weighing (180-220g) were selected and divided into four groups of 10 rats each. The doses were selected based on the result of acute toxicity testing. The highest dose was 20% of LD_{50} which is 1000 mg/Kg, then 500, 250 mg/Kg and the last group received distilled water 1 ml/Kg. The administration was done every 24 h for 28 days (OECD, 2008). The rats were observed for signs of toxicity and behavioural changes on daily basis. Food and water intake were recorded every day. In addition, body weight was measured weekly. At the 29th day, the rats were anesthetized and sacrificed. Blood sample was collected for haematological and biochemical analysis using auto-analyser. Subsequently, the rats were dissected and internal organs such as brain, liver, heart, kidneys, lungs and spleen were removed and weighed (OECD, 2008).

Result and Discussion

Acute toxicity studies

The result of acute toxicity testing shows that the LD_{50} of crude methanol extract was found to be above 5000 mg/Kg.

Sub-chronic toxicity studies

Weekly body weight

Based on the data recorded, there was no statistically significant increase in body weight in experimental groups compared to control throughout the 28 days. However, statistically significant increase in weight was observed in medium dose group at week 4 ($p<0.05$) compared to week 0. The effect of crude methanol extract of *Solanum aethiopicum* fruits on weekly body weight is shown in Table 1.

Haematological parameters

Result shows there was no statistically significant increase or decrease in any haematological parameters compared to distilled water group. The effect of crude methanol extract of *Solanum aethiopicum* fruits on haematological parameters is shown in Table 2.

Table 1: Effect of crude methanol extract of *Solanum aethiopicum* fruits on weekly body weight of animals

Treatment (mg/Kg)	Weekly Body Weight (Mean ± S.E.M)				
	Week 0	Week 1	Week 2	Week 3	Week 4
D/W 1	131.5 ± 15.4	157.8 ± 16.4	174.5 ± 18.1	182.5 ± 19.1	188.0 ± 20.2
CRE 250	151.4 ± 13.4	155.8 ± 14.6	170.6 ± 16.1	180.6 ± 17.1	186.2 ± 18.1
CRE 500	149.3 ± 12.6	164.5 ± 13.4	173.8 ± 14.7	181.5 ± 15.6	192.3 ± 16.5^{a}
CRE 1000	156.3 ± 15.4	161.8 ± 16.4	176.8 ± 18.1	188.3 ± 19.1	187.8 ± 20.2

Data is presented as Mean ± S.E.M. at $^{*}p< 0.05$, $^{**}p< 0.01$, and $^{***}p< 0.001$ compared to D/W(ml/Kg). Also at $^{a}p< 0.05$, $^{b}p< 0.01$, and$^{c}p< 0.001$ compared to week 0 using Repeated Measure ANOVA and Bonferoni Post Hoc's Test for multiple comparism. D/W = Distilled Water, CRE = Crude Extract.

Table 2: Effect of crude methanol extract of *Solanum aethiopicum* fruits on haematological parameters of animals

Treatment (mg/Kg)	HGB (g/dl)	RBC (10^6)	WBC (10^3)	PLT(10^3)	PCT (%)	MCV (fL)	MCHC (g/dl)
D/W 1	14.4 ± 0.47	4.9 ± 0.21	4.7 ± 0.21	225.5 ± 15.96	0.16 ± 0.02	88.18 ± 0.49	33.9 ± 1.01
CRE 250	13.6 ± 1.14	4.3 ± 0.08	3.4 ± 0.23	236.9 ± 23.77	0.18 ± 0.03	90.5 ± 2.76	34.0 ± 1.30
CRE 500	10.9 ± 0.55	4.5 ± 0.21	3.3 ± 0.23	274.5 ± 18.98	0.14 ± 0.03	92.1 ± 2.47	33.6 ± 2.47
CRE 1000	15.6 ± 0.90	4.8 ± 0.25	4.7 ± 0.33	273.5 ± 51.56	0.15 ± 0.04	89.7 ± 0.43	35.6 ± 2.79

Data is presented as Mean ± S.E.M. at $^{*}p< 0.05$, $^{**}p< 0.01$, and $^{***}p< 0.001$ compared to D/W (ml/Kg) using One Way ANOVA and Dunnest's Post Hoc's Test. D/W = Distilled Water, CRE = Crude Extract. Also HGB=Haemoglobin, RBC=red blood cells, WBC= white blood cells, PLT=platelets, PCT= plateletcrit, MCV= mean corpuscular volume, and MCHC= mean corpuscular haemoglobin concentration

Table 3: Effect of crude methanol extract of *Solanum aethiopicum* fruits on kidney function test

Treatment (mg/Kg)	UREA (g/dl)	Creatinin (Meq/l)	Na (mmol/l)	K (mmol/l)	Cl (mg/dl)	BC (mg/dl)
D/W 1	23.0 ± 4.43	135.9 ± 1.87	8.9 ± 1.04	0.65 ± 0.09	24.5 ± 2.63	86.8 ± 3.57
CRE 250	17.8 ± 2.66	132.1 ± 6.61	11.8 ± 1.19	0.80 ± 0.11	22.8 ± 2.46	85.5 ± 2.72
CRE 500	28.3 ± 1.65	115.4 ± 8.03	12.5 ± 1.03	0.88 ± 0.08	19.5 ± 0.87	87.0 ± 1.41
CRE 1000	29.0 ± 2.38	140.1 ± 0.31	9.7 ± 0.65	0.88 ± 0.09	18.0 ± 1.22	87.5 ± 3.17

Data is presented as Mean ± S.E.M. at $^{*}p< 0.05$, $^{**}p< 0.01$, and $^{***}p< 0.001$ compared to D/W(ml/Kg) using One Way ANOVA and Dunnest's Post Hoc's Test. D/W = Distilled Water, CRE = Crude Extract. Also, Na= sodium, K= potassium, and Cl= chlorine, BC=Bicarbonate

Table 4: Effect of crude methanol extract of *Solanum aethiopicum* fruits on liver function test

Treatment (mg/Kg)	ALT (IU/l)	AST (IU/l)	ALP (IU/l)	ALB (g/dl)	TP (g/dl)	DB (mmol/l)	TB (mmol/l)	AST/ALT
D/W 1	19.8 ± 3.68	31.3 ± 5.81	23.1 ± 3.00	2.4 ± 0.46	5.8 ± 0.84	5.7 ± 0.27	7.8 ± 0.51	1.6 ± 0.17
CRE 250	25.3 ± 3.47	53.0 ± 5.05*	34.8 ± 10.48	2.9 ± 0.09	5.4 ± 0.29	5.1 ± 0.37	7.5 ± 0.51	2.2 ± 0.18
CRE 500	20.3 ± 2.36	43.0 ± 2.74	32.4 ± 5.33	2.7 ± 0.23	5.4 ± 0.39	5.3 ± 0.39	8.1 ± 0.41	2.2 ± 0.13
CRE 1000	26.0 ± 3.24	44.3 ± 2.50	17.4± 5.44	2.3. ± 0.32	6.5 ± 0.41	5.3 ± 0.43	7.6 ± 0.26	1.8 ± 0.21

Data is presented as Mean ± S.E.M. at $^{*}p< 0.05$, $^{**}p< 0.01$, and $^{***}p< 0.001$ compared to D/W (ml/Kg) using One Way ANOVA and Dunnest's Post Hoc's Test. D/W = Distilled Water, CRE = Crude Extract, ALT= alanine aminotransferase, AST= aspartate aminotransferase, ALP= alkaline phosphatase, ALB= albumin, TP=total protein, DB=direct bilirubin, and TB= total bilirubin

Kidney function test

Kidney function test data obtained did not show any statistically significant change in all biochemical parameters that determine kidney functions. The effect of crude methanol extract of *Solanum aethiopicum* fruits on kidney function test of animals is shown in Table 3.

Liver function test

In this test, only aspartate aminotransferase shows statistically significant increase ($p<0.05$) in low dose extract group compared to distilled water. Notably, all other biochemical parameters were normal. The effect of crude methanol extract of *Solanum aethiopicum* fruits on liver function test of animals is shown in Table 4.

Relative body organ weight

Body organs harvested include brain, liver, heart, lungs, kidney and spleen. There was no statistically significant difference observed in relative organs weight between experimental and control groups. The relative organ weight is calculated using formula and the result shown in Table 5.

$$\text{Relative organ weight (\%)} = \frac{\text{Absolute organ weight (Kg)}}{\text{Weight of rats on sacrifice day (Kg)}} \text{x}100$$

Table 5: Effect of crude methanol extract of *Solanum aethiopicum* fruits on relative body organ weight

Treatment	Relative organ weight (%)					
(mg/Kg)	Brain	Liver	Heart	Lungs	Kidney	Spleen
D/W 1	0.60±0.14	3.20±0.17	0.36±0.02	0.95±0.13	0.60±0.02	0.44±0.05
CRE 250	0.73±0.04	3.07±0.15	0.38±0.02	0.91±0.21	0.55±0.04	0.70±0.25
CRE 500	0.58±0.04	2.79±0.15	0.31±0.02	0.71±0.07	0.54±0.02	0.43±0.03
CRE 1000	0.74±0.09	3.70±0.45	0.36±0.02	0.81±0.09	0.56±0.06	0.48±0.05

Data is presented as Mean ± S.E.M. at $^{*}p< 0.05$, $^{**}p< 0.01$, and $^{***}p< 0.001$ compared to D/W (ml/Kg) using One Way ANOVA and Dunnest's Post Hoc's Test. D/W = Distilled Water, CRE = Crude Extract

Preclinical examination of all groups of rats on daily basis revealed no physical changes and no behavioural modification. In addition, the crude methanol extract of *Solanum aethiopicum* was found to be safe even at 5000 mg/Kg based on the result of LD_{50}.

This observation was extended to 14 days but there was no sign of toxicity, coma or death recorded. Increase in weekly body weight was noticed which is in accordance with nutritional value of the plant and the rats were within the active growth period. Other researchers also reported increase in body weight after administration of different plant extract (Porwal *et al*., 2017; Bah *et al*., 2017; Donkor *et al*., 2014).

Generally, decrease in HGB, RBC, PLT, PCT, MCV, MCHC, monocytes, basophils or reticulocytes is an indication of anaemia and or bone marrow toxicity (Wolford *et al*., 1986). In this study, *Solanum aethiopicum* fruit extracts did not cause alteration of any of the above haematological parameters. Other researchers also revealed similar findings with different plant extracts (Al-Afifi *et al*., 2018; Porwal *et al*., 2017; Igbe *et al*., 2015; Uma *et al*., 2013). Universally, increase in WBC, neutrophils or lymphocyte is an indication of phagocytosis which signifies presence of infection. In this experiment, *Solanum aethiopicum* did not shows any changes in these parameters which mean absence of infection.

During kidney function test, increase in the blood concentration of urea, uric acid, creatinine, and other electrolytes is an indication of possible kidney damage (Moss and Handerson, 1999; Kluwe, 1981). In this study, all biochemical parameters that determine kidney functions were which implies the plant is safe in kidney. Similar results were obtained in experiments involving other (Al-Afifi *et al*., 2018; Porwal *et al*., 2017; Khan *et al*., 2016; Donkor *et al*., 2014; Uma *et al*., 2013). In liver function test, increase in AST, ALT, ALP and total protein suggests liver damage (Ozer *et al*., 2008; Brautbar and Williams, 2002). Administration of *Solanum aethiopicum* fruit extract produces normal range of above parameters with exception of elevation of AST at low dose. Since AST is a minor determinant of liver damage, and its elevation involved only the lowest dose of extract, it implies the plant extract does not cause any significant change in liver function or damage. The most important marker of liver damage is the elevation of ALT (Ozer *et al*., 2008; Brautbar and Williams, 2002). Lastly, there was no statistically significant difference in relative organ weight at low, medium and high dose extracts compared to distilled water. This is an indication that *Solanum aethiopicum* fruit extract does not affect normal growth of internal organs.

Conclusion

Crude methanol extract of *Solanum aethiopicum* fruits was found to be safe in acute toxicity testing even at very a high dose. Twenty eight 28 days toxicity experiment using low, medium and high dose extracts reveals no sign of toxicity or death. Similarly, it does not affect kidney or liver function and the animal relative body organ weight remains intact. By and large the plant has demonstrated wide margin of safety when administered for short duration. This justifies the use of *Solanum aethiopicum* fruit as food and traditionally in long term treatment of mental disorder and other chronic diseases.

Recommendations

Based on the finding of this research, safety study on crude methanol extract of *Solanum aethiopicum* should be extended to long term chronic toxicity studies.

Acknowledgment

We are very grateful to all staff of Department of Pharmacology and Therapeutics, Faculty of Pharmaceutical Sciences, Bayero University, Kano State, Nigeria.

Conflict of Interest

Authors have declared that there is no conflict of interest reported in this work.

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