

# EPHROPROTECTIVE EFFECTS OF ETHANOLIC EXTRACT OF *Solanum Melongena* AGAINST CARBONTETRACHLORIDE (CCl<sub>4</sub>) INDUCED TOXICITY IN RATS

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## Abstract

Nephroprotective effects of ethanolic extract of solanum melongena against carbontetrachloride (ccl<sub>4</sub>) induced toxicity in rats was studied. Serum urea, Na<sup>+</sup>K, chloride, Bicarbonaate were determined using Diacetyl monoxime method, Flame photometry method, Shales and Scale method and Trimetric method. The result for changes in body weight following oral administration of the extract (Solanum melongena) for induced toxicity on rats ranged; 157-127mg, 0.39 – 10.28mg, 5.59-5.08mg, 3.44-13.64mg, 0.85-3.90mg and 6.40-1.65mg for the initial, 3days, 6days, 12days and 15 days respectively. Effects of oral administration of Solanum melongena on Indices of renal function and relative organ weight in CCl<sub>4</sub> induced toxicity in rats indicates that creatinine(μmol/l) has the highest value of 143.50±13.86 in group5 and the lowest value of 36.80±4.59 in group1. Urea (mmol/l) has the highest value of 8.34±0.86 and the lowest value of 6.38±0.68. Sodium (mmol/l), Potassium (mmol/l), chloride (mmol/l), Bicarbonate (mmol/l) and Relative kidney have the highest values of 125.50±0.77, 5.46±71.00, 102.20±3.74, 21.00±0.44 and 0.91±0.07 respectively. Administration of carbon tetrachloride has lead to a slight but insignificant increase in the serum level of creatinine, sodium, potassium, chloride and bicarbonate. This indicates that the carbon tetrachloride might not have caused significant damage to the kidneys. Oral administration of ethanolic extract of Solanum melongena for fourteen days increased significantly the serum creatinine levels. Oral administration of the extract alone also led to similar increase in the creatinine level suggesting possible nephrotoxicity of the extract.

**Keywords:** key words, nephrotoxicity, ethanolic, toxicity, carbontetrachloride

## 1. Introduction

Natural products have been and have remains the cornerstone of health care, throughout ages, nature has provided humans with essential source of foods, medicines and raw materials from natural products. Plants in particular are good source of medicine from ancient time and today they continue to play a dominant role in primary health care. Currently, the use of herbal remedies has increased because of their effectiveness and safety (Gabriela *et al.*, 2009). One of the important medicinal plant species which has been reported in the maintenance of health is the *Solanum melongena* known as eggplant commonly called Yalo in the northern part of Nigeria. It belongs to the family of Solanaceae and the plant genus Solanum with over 1,000 species worldwide. It is a small tropical perennial flowering plant, a native to Africa and Asia, growing up to a height of 55 inches, wild type can grow much larger up to 85 inches. The leaves are pubescent and sometimes spiny. Eggplants come in many shapes, size and colors. It needs a lot of water and prefer ordinary garden soil for its production (Bonsu *et al.*, 2008; Manoko & Van der Weerden, 2004). This plant is cold sensitive and requires a long warm season. *Solanum melongena* have been reported to contain protein, minerals such as Calcium (7mg), Phosphorus (22mg), Potassium (217mg), and Magnesium (14mg). It also contains about 6.07g of carbohydrate, 0.18g of Fat, and vitamins such as Vitamin C (1.7mg), Vitamin B<sub>2</sub> (0.034mg), Vitamin B<sub>6</sub> (0.084mg), Vitamin A (8.414mg), Vitamin E (0.3mg) and Niacin (0.59mg).

Eggplants have indigenous medicinal uses, which range from weight reduction to treatment of several ailments including asthma, skin infections and constipation. Various plant parts are used in decoction for curing ailments such as diabetes, leprosy, gonorrhea, cholera, bronchitis, dysuria, dysentery, asthenia and haemorrhoids. (Gill, 1992; Bello *et al.*, 2005).

In other to have standard natural plant products that could be used for medicinal purpose, preliminary studies have to be done so as to evaluate possible risk such as undesirable effect on life (Lienou *et al.*, 2007). This research was carried out to determine the nephroprotective effects of ethanolic extract of *Solanum melongena* against CCl<sub>4</sub> induced toxicity in rats and also to investigate the protective effects of *Solanum melongena* on the kidney from CCl<sub>4</sub> injury.

## 2. Materials and Methods.

### 2.1 Collection and Preparation of Extract

Matured *Solanum melongena* was purchased from Utako market within Abuja metropolis, Nigeria, in June 2016, and was authenticated in the Department of Biological sciences, Ahmadu Bello University (ABU) Zaria, Nigeria. One hundred grams (100g) fresh fruit of *Solanum melongena* were cut into small pieces and then extracted twice for 20 minutes each with 300ml of 95% ethanol, using a household mixer. The residue was filtered through cotton membrane and then the residues were re-extracted under some condition, the ethanol was removed by evaporation on a water bath at 45°C.

### 2.2 Chemicals and Reagents.

Carbon tetrachloride was purchased from riedel-dettaen chemical (32215 UN-N018461). Ethanol (Absolute) AR was obtained from NAFCO Scientific supplies LTD London (prod;10077YN0) Batch 6541987/98. livolin. All other reagents used are of analytical grade and were obtained from the laboratory of departments of Biochemistry, Ahmadu Bello University, Zaria, Kaduna State Nigeria.

### 2.3 Experimental animals and Experimental design

Wister strain albino rats (150g-200g) obtained from National Veterinary Research Institute Jos, Plateau state, Nigeria was used. The animals were housed in plastic cages with free access to food (grower mash) from feed live stock Zaria and water from Ahmadu Bello University (ABU) Zaria tap. The total of 25 rats divided into five groups of 5 rats each was used. Group one serve as the control, the second group received carbon tetrachloride (CCL<sub>4</sub>) alone. The third group received ethanolic extract of *Solanum melongena* and the fourth group received the extract same as group three, the fifth group received livolin (mg/kg). The group one and two received daily saline solution (0.9%) for 14 days, while group 3 and 4 received the extract for 14 days. Group 2, 4 and 5 received a single oral dose of 1.5mg/kg CCL<sub>4</sub> in olive oil (1:1%) for 6 hours. After the last administration of the saline, extract and livolin on the 14<sup>th</sup> day, all the rats were humanly killed at the end of the two weeks, blood was then collected and serum separated by centrifugation for determination of kidney function test. The kidney, liver, spleens of the rats were removed and their relative organ weight was determined.

### 2.4 Biochemical analysis

Serum creatinine and urea were assayed using Jaffe's reaction and diacetyl monooxime method respectively. Serum sodium and potassium were assayed using flame photometry. Serum chlorides were assayed using Schales and Schales method (1941). Biocarbonate was assayed using Trimetric method.

### 2.5 Biochemical assay

#### Determination of serum creatinine (Jaffe's method)

Jaffe's reaction was used to estimate the serum creatinine. Creatinine gives a red color with alkaline solution of picric acid. The serum was deprotonized with sodium tungstate, when picric acid was added to the protein free serum, followed by sodium hydroxide, a red color complex is formed. The red color complex is proportional to the amount of creatinine in the serum. 1ml of sodium tungstate (0.5g/100ml), 1ml of 0.33M sulphuric acid and 1ml of distilled water were all added to a clean test tube containing 1ml serum mixed thoroughly and was centrifuge at 300rpm for 10 min then the reading was taken.

$$\text{Serum creatinine, } (\mu\text{m/l}) \text{ for standard (A)} = \frac{\text{Reading of unknown}}{\text{Reading of standard}} \times 200 \text{ or } 2.26$$

For mg/100ml or for standard (b) x 400 or 4.52 for mg/100ml.

#### Determination of serum urea (Diacetyl monooxime method)

1ml of water and 1ml of trichloro acetic acid was added to 0.2ml of serum, the solution was well mixed and centrifuged. 0.2ml of supernatant was added to 3ml of colour reagent. The colour reagents, blank and standard were then put up by replacing the serum with water or the standard for the first step above. It was then heated in a boiling water bath for 20 minutes, cool to room temperature in cold water, and then the test and standard were read against the blank at 520nm within 15 minutes (Marsh *et al.*, 1965)

#### Determination of Na<sup>+</sup>K<sup>+</sup> (Flame photometry method)

0.1ml of the test sample was diluted in 10ml of distilled water in a test tube labelled as test sample and another 0.1ml of control serum was diluted in 10ml of distilled water in a test tube labelled a control and the machine was standardized by using standard solution of known concentration of 140mmol/l (Na<sup>+</sup>) and 5.0mmol/l (K<sup>+</sup>) and the wave length of 589nm and 404nm was then selected for Na<sup>+</sup> and K<sup>+</sup> respectively and The sample was aspirated into flame, the element (i.e. Na/K<sup>+</sup>) present becomes excited and released energy in the form of photon

(light) which return to its ground state at a wave length characteristic to the element in question and signal was detected by a galvanometer. The amount of light produced is directly proportional to the concentration of the element.

#### Determination of chloride (Schales and Schales method 1941)

Schales and Schales method was used for the determination of chloride in the sample. 0.2ml serum was added to 1.8ml water followed by 60ml of the indicator. The solution was titrated with mercuric nitrate solution using micro burette calibrated to 0.01ml. The titration was then repeated using 2ml of standard chloride solution. The expected titrant volume is about 2.3ml and the end point was better with protein free solution which gives an intense violet blue colour on adding the first drop of excess mercuric nitrate. On adding more titrant the colour became pale yellow until a change to pale violet was observed which denote the end point.

#### Calculation

$$\text{Serum chloride (mmol/l)} = \frac{\text{ml titrant needed for unknown} \times 100}{\text{ml titrant needed for standard}}$$

#### Determination of Bicarbonate (Trimetric method)

The plasma bicarbonate present in the dilute sample was mixed with 0.1N HCl to generate CO<sub>2</sub> which was allowed to escape and the excess HCl was titrated against 0.1N NaOH in the presence of methyl red indicator to produce a golden yellow end point. 2ml of deionized water was added to 0.1ml sample and 1 drop of methyl red was added. Another 2ml of deionized water was diluted with 0.1ml of the control and was titrated until the end point was reached.

#### Statistical analysis

The results were expressed as Mean±SEM. The differences between 2 mean were compared using student-t test.

### 3.0 Result and Discussion

**Table 1: Changes in body weight following oral administration of the extract (*Solanum melongena*) induced toxicity on rats.**

Groups	Initial body Weight (g)	3 <sup>rd</sup> day (g)	6 <sup>th</sup> day (g)	9 <sup>th</sup> day (g)	12 <sup>th</sup> day (g)	15 <sup>th</sup> day (g)
1	157.00±13.80	0.39±1.11	5.59±1.3	3.44±1.54	-0.85±1.48	6.40±2.08
2	138.60±9.34	1.98±1.34*	9.88±1.82*	3.52±1.08*	1.78±0.63*	-22.40±1.43*
3	166.00±9.96	-6.41±3.31*	-0.01±1.	- 1.01±3.10*	17.43±2.62*	0.58±2.21*
4	127.50±6.25	10.28±5.54 <sup>a</sup>	5.08±1.19 <sup>a</sup>	3.64±2.29 <sup>a</sup>	3.90±3.72 <sup>a</sup>	1.65±4.28 <sup>a</sup>
5	127.50±8.95	23.54±7.16 <sup>a</sup>	-0.08±1.96 <sup>a</sup>	15.74±2.32 <sup>a</sup>	41.40±2.69 <sup>a</sup>	4.27±2.36 <sup>a</sup>

\* indicates significant different when compared to control group at P<0.05

<sup>a</sup> indicates significant different when compared to negative group at P<0.05

#### Changes in body weight

The result for changes in body weight following oral administration of the extracts is presented in table 1. At the end of the experiment, the negative control group showed a significant decrease in body weight when compared with the normal control group. Slight increases in the body weight were observed in all the other groups.

**Table 2: Effects of oral administration of *Solanum melongena* on Indices of renal function and relative organ weight in CCl<sub>4</sub> induced toxicity in rats**

Parameters	Group1	Group2	Group3	Group 4	Group 5
Creatinine(μmol/l)	36.80±4.59	41.80±5.00	79.20±12.20*	139.80±8.33 <sup>a</sup>	143.50±13.86
Urea (mmol/l)	7.22±0.50	6.38±0.68	8.34±0.86	7.48±0.42	7.75±0.52 <sup>a</sup>
Sodium(mmol/l)	09.60±8.68	121.00±0.54	112.60±5.95	120.20±1.51	125.50±0.77 <sup>a</sup>
Potassium(mmol/l)	4.20±0.38	5.46±71.00	4.58±0.27	5.04±0.01	5.25±0.11
chloride(mmol/l)	74.40±14.40	102.20±3.74*	84.40±10.50	99.00±3.23	101.70±1.23
Bicarbonate(mmol/l)	2.20±6.66	20.80±0.37	20.20±0.37	21.00±0.44	20.00±0.01
Relative kidney	0.91±0.07	0.63±0.07	0.55±0.09	0.63±0.05	0.60±0.03

#### Organ weight

\* indicates significant different when compared to control group at P<0.05

<sup>a</sup> indicates significant different when compared to negative group at P<0.05

### Indices of renal function

The results of renal function indices and relative organ weight are presented in table 2 above. Following the oral administration of  $\text{CCl}_4$  to group 2 rats, it was observed that there was insignificant increase in serum levels of creatinine, sodium, potassium and bicarbonate when compared with normal control group, but serum chloride was significantly increased at  $P < 0.05$ . In group 3 (extract control group), there was an insignificant increase in serum level of urea, sodium, potassium, chloride and bicarbonate compared to control group, but creatinine level was significantly increased at  $P < 0.05$ . In the fourth group (extract plus  $\text{CCl}_4$ ), there was also insignificant increase in serum urea, sodium, potassium, chloride and bicarbonate when compared to group two, but creatinine level was significantly increased at  $P < 0.05$ . In group 5 ( $\text{CCl}_4$  plus standard drugs), there was a significant increase in serum level of creatinine, urea and sodium, but there was an insignificant increase in serum level of potassium, chloride and bicarbonate at  $P < 0.05$ .

Administration of the extract to experimental rats has led to insignificant decrease in their body weights, which may be due to inability of the extract to increase their feed intakes. In vitro and in vivo studies has indicated that the carbon tetrachloride enhances lipid peroxidation, reduces renal microsomal NADPH, cytochrome P450 and renal reduced/oxidized glutathione ratio (GSH/GSSH) in kidney cortex as well as renal microsomes and mitochondria (Walker *et al.*, 1996). In general, a number of chemicals including various environmental toxicants and even clinical useful drugs can cause severe cellular damages in different organs of the body through metabolic activation to highly reactive substances such as free radicals (Noguchi *et al.*, 1982)

Carbon tetrachloride is one of such widely used environmental toxicants to experimentally induced animal models of acute nephrotoxicity. Carbon tetrachloride is metabolized by cytochrome P450 to trichloromethyl radical ( $\text{CCl}_3\text{-CCl}_3^\cdot$ ) and its highly reactive derivative, the trichloromethyl peroxide radical ( $\text{CCL}_3\text{COO}^\cdot$ ), are assumed to initiate free radical mediated lipid peroxidation leading to accumulation of lipid peroxidation products that causes renal injuries (Aleynik *et al.*, 1997).

Serum creatinine and urea concentration changes with change in glomerular filtration rate (GFR) and are useful in gauging the degree of renal dysfunction, but changes in serum creatinine more reliably reflect changes in glomerular filtration rate than changes in serum urea concentration and useful for detecting or accessing the progression of renal disease (Gerich *et al.*, 2001).

Administration of carbon tetrachloride has lead to a slight but insignificant increase in the serum level of creatinine, sodium, potassium, chloride and bicarbonate. This indicates that the carbon tetrachloride might not have caused significant damage to the kidneys. Oral administration of ethanolic extract of *Solanum melongena* for fourteen days increased significantly the serum creatinine levels. Oral administration of the extract alone also led to similar increase in the creatinine level suggesting possible nephrotoxicity of the extract. Further work is needed to confirm this effect and proffer possible reasons since increase in creatinine level is a strong indicator of kidney damage. The group administered the standard drug also showed a similar significant increase in creatinine levels as did the test groups. Overall it safer not to draw any strong conclusion as the study needs to be repeated to confirm the results.

### 4.0 CONCLUSION

The ethanolic extract of *Solanum melongena* is not nephroprotective against carbontetrachloride induced toxicity at the dose used.

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