



Evaluation of the Hepatoprotective Effect of Melon (*Citrullus colocynthis*) Seed Milk on Carbon Tetrachloride-Induced Hepatotoxicity in Rats

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Abstract:

This research investigated the effect of melon seed milk on Carbon tetrachloride (CCL₄)-induced hepatotoxicity in rats. Twenty-five (25) male albino rats of weight range 120-250 g were randomly grouped into five: 100 mg/kg milk - treated group, 200 mg/kg milk -treated group, silymarin-treated group, hepatotoxic group and normal control group. *Citrullus colocynthis* seed milk was administered at the doses of 100 mg/kg and 200 mg/kg body weight in CCL₄-induced albino rats and compared with the silymarin-treated group. Preliminary phytochemical screening of *Citrullus colocynthis* seed milk revealed the presence of phenols, tannins, flavonoids, alkaloids,

steroids and glycosides. Acute toxicity test was carried out and it was observed that, melon seed milk was not toxic to the animals, even though some showed mild effects for some minutes. Biochemical parameters such as Alkaline phosphatase (ALP), Aspartate aminotransferase (AST) and Alanine aminotransferase (ALT) were analyzed at the end of the 14th day experimental period. The levels of these biochemical parameters in the CCL₄-induced rats were observed as follows: ALP (41.48 U/I), AST (48.25 U/I), and ALT (19.56 U/I), when compared with the normal group having: ALP (20.24 U/I), AST (29.36 U/I), and ALT (11.73 U/I). *Citrullus colocynthis* seed milk at the doses of 100 mg/kg and 200 mg /kg exhibited significant ($p < 0.05$) reduction in the biochemical parameters (ALP, AST and ALT). The result from this study revealed that, *Citrullus colocynthis* seed milk has potent hepato-protective effect against CCL₄- induced hepatotoxicity thus, may be used in the treatment of hepatotoxicity.

Keywords: hepatotoxicity, *Citrullus colocynthis*, phytochemicals, biochemical parameters, seed milk.

Introduction

Liver is one of the main organs involved in the metabolism of drugs and toxic chemicals and is the first target organ for almost all chemicals that entered our body (Sahreen et al., 2011; Salama et al., 2015). Most of the xenobiotics enter the body

through gastrointestinal tract and after absorption enter the liver through portal vein. The liver has a high concentration of toxin-metabolizing enzymes which can convert xenobiotics to compounds with low toxicity and excrete them. However, sometimes toxic



substances are converted to active metabolites during metabolism which can exacerbate liver damage and cause changes in the macroscopic structure of specific molecules such as bile acid transporters, families of nuclear receptors, intracellular lipids, proteins, and nucleic acids (Cullen, 2005). Improper performance of these molecules activates some secondary paths which finally lead to planned events such as apoptosis, necrosis, autophagy, mitochondrial defects, and immune responses (Nikoletopoulou et al., 2013).

Despite the considerable advancements in medicine and modern pharmacology, drugs used for the treatment of liver damage have many side effects and exacerbate the disease. Therefore, it is necessary to find a compound that has hepatoprotective and anti-hyper-lipidemic effects without causing other side effects (Angulo, 2002; Adams et al., 2005; Argo and Caldwell, 2009).

Citrullus colocynthis also known as Egusi, melon, Bitter apple, Colocynth, is a member of the cucurbit species of the *Cucurbitaceae* family. The plant is native to regions in Africa, temperate Asia, tropical Asia and Europe (USDA, 2019). It is widely cultivated in West Africa, the Middle East, Southwest Asia and other African countries for its nutritional, medicinal and environmental value (Uruakpa, 2004; USDA2019).

Citrullus colocynthis has a significant history of medicinal, pharmaceutical, nutraceutical and nutritional use. Several parts of the plant have been used for medicinal purposes and in recent times many of its attributed properties have been validated in animal and human models. The seed extract of *Citrullus colocynthis* has a significant history of medicinal, pharmaceutical, nutraceutical and nutritional use. The seed extract of *Citrullus colocynthis* has been demonstrated to possess anti-ulcer and anti-oxidant potential and its free radical scavenging action is being postulated to be due to its flavonoid content (Gill et al., 2011; Benariba et al., 2013), while the fruit has been reported to contain a variety of bioactive chemical constituents responsible for its anti-oxidant, cathartic, carminative, anthelmintic, cytotoxic, anti-diabetic, anti-lipidaemic, insecticide, anti-

microbial, anti-pyretic and anti-inflammatory activities (Benariba et al, 2013; Hussain, 2014; Telli et al, 2016). *In-vitro* and animal model studies validate the ethno-pharmacological use of the plant extracts as an anti-microbial (Marzouk et al., 2009) and analgesic agent (Marzouk et al., 2010), while several comparative studies have bolstered its use in promoting hair growth (Roy et al., 2007). Other studies supported its potential in pest control as a green solution to managing dengue vector and lymphatic filariasis vector, *Culex quinquefasciatus* (Rahuman, & Venkatesan, 2008), as alternative biofuel (Giwa et al., 2010), and as an environmental tool to prevent erosion has been demonstrated to possess anti-ulcer and anti-oxidant potentials, and its free radical scavenging action is been postulated to be due to its flavonoid content (Gill et al., 2011; Benariba et al., 2013), while the fruit has been reported to contain a variety of bioactive chemical constituents responsible for its anti-oxidant, cathartic, carminative, anthelmintic, cytotoxic, anti-diabetic, anti-lipidaemic, insecticide, anti-microbial, anti-pyretic and anti-inflammatory activities (Benariba et al, 2009; Hussain, 2014; Telli et al, 2016). *In-vitro* and animal model studies validate the ethno-pharmacological use of the plant extracts as an anti-microbial (Marzouk et al., 2009) and analgesic agent (Marzouk et al., 2010), while several comparative studies have bolstered its use in promoting hair growth (Roy et al., 2007).

Materials and Methods

Plant Collection and Authentication

Hand de-shelled melon seeds were bought from Wurukum market Makurdi and brought to the Department of Botany, Federal University of Agriculture, Makurdi for identification and authentication by a Taxonomist.

Preparation of *Citrullus colocynthis* Seed Milk (Melon Milk)

Hand de-shelled melon seeds were washed and air dried under room temperature. The dried melon seeds were ground using a blender. 10 kg of the ground melon was dissolved in 50 ml of

boiled water and stirred to obtain uniform mixture. The solution was then filtered to produce pure melon milk ready for use.

Qualitative Phytochemical Screening

Preliminary qualitative phytochemical screening was carried out following standard methods (Sofowora, 1993; Trease and Evans, 1989; Bhupal et al., 2015).

Test for Glycoside

An amount, 4 ml of *Citrullus colocynthis* milk was measured. To it was added 1-2 ml of Ammonium Hydroxide and shaken. Appearance of cherry-red colour indicated the presence of glycosides.

Salkowski's Test

A quantity, 2 ml of *Citrullus colocynthis* milk was mixed with 2 ml of chloroform. Then, 2ml of concentrated H_2SO_4 was added carefully and shaken gently. A reddish brown colour indicated the presence of steroidal ring, i.e., glycine portion of the glycoside.

Test for Polyphenols and Tannins

A measured volume of *Citrullus colocynthis* milk was mixed with 2 ml of 2% solution of $FeCl_3$. A blue-green or blue-black colouration indicated the presence of polyphenols and tannins.

Test for Flavonoids

Dilute ammonia (5 ml) was added to a portion of the milk. Then, concentrated sulphuric acid (1 ml) was added. A yellow colouration indicated the presence of flavonoids.

Test for Saponins

A portion of *Citrullus colocynthis* milk was mixed with 5 ml of distilled water in a test tube and it was shaken vigorously for 30 seconds. The formation of stable foam (1 cm height) even after 30 minutes was taken as an indication for the presence of saponins.

Test for Steroids

Few drops of *Citrullus colocynthis* milk were mixed with 2 ml of chloroform and concentrated H_2SO_4 was added sidewise. A red colour produced in the lower chloroform layer

indicated the presence of steroids. Another test was performed by mixing melon milk with 2 ml of chloroform. Then 2 ml of each of concentrated H_2SO_4 and acetic acid were poured into the mixture. The development of a greenish colouration indicated the presence of steroids.

Test for Alkaloids

A 4 g quantity of ground melon was dissolved in dilute HCl and filtered. Filtrate was treated with Mayer's reagent (potassium mercuric iodide). Formation of a yellowish precipitate indicates the presence of alkaloids.

Animal Protocol

Twenty – five (25) male albino rats weighing 120-250 kg were used for this experiment. The rats were obtained from a reputable breeder in the High-Level district of Makurdi in Benue State and brought to the Animal House of the Department of Veterinary Physiology, Pharmacology and Biochemistry for Fourteen-day acclimatization prior to experiment. They were maintained in well-ventilated cages at ambient temperature in hygienic conditions and allowed free access to standard feed and water *ad lib*.

Experimentation

The Twenty - five (25) albino rats used for this investigation were randomly divided into five groups of five rats each as shown below:

Group 1: Hepatotoxicity was induced with Carbon tetrachloride (1.25 mg/kg body weight) and treated with *Citrullus colocynthis* milk at 100 mg/kg body weight.

Group 2: Hepatotoxicity was induced with Carbon tetrachloride and treated with *Citrullus colocynthis* milk at 200 mg/kg body weight.

Group 3: Hepatotoxicity was induced with Carbon tetrachloride (1.25 mg/kg body weight) and treated with silymarin (2.25 mg /kg body weight). This served as standard control group.

Group 4: Hepatotoxicity was induced with Carbon tetrachloride (1.25 mg/kg body weight) and untreated. The rats were given normal feed and normal saline.

Group 5: Hepatotoxicity was not induced in this group and it was not treated. They were given normal feed and normal saline.

Acute Oral Toxicity Test

The acute oral toxicity test was carried out according to OECD guidelines for acute oral toxicity studies (2003). The limit was set at 2500 mg/kg. One animal was dosed at the test dose of 2500 mg/kg and observed for 48 hours. Toxicity signs such as dullness, mild raising of hairs and weakness were observed for some minutes.

Hepatotoxicity Induction with Carbon Tetrachloride in the Experimental Animals

Hepatotoxicity was induced by intraperitoneal injection of CCL₄ at single dosage of 1.25 mg/kg body weight into albino rats of weight ranging from 120-250 g.

Blood Collection and Preparation of Sample

At the end of 14 days experimental period, venous blood was collected from the experimental animals by cardiac puncture using syringe into anti-coagulant free test tubes, centrifuged at the speed of 2500 revolution per minute (rpm) for 15 minutes and the resultant serum samples were collected into plain sample bottles for further biochemical analyses such as; Alanine aminotransferase (ALT/SGPT), Alkaline Phosphatase (ALP) and Aspartate aminotransferase

Determination of Biochemical Parameters (AST/SGOT)

The determination of biochemical parameters such as ALT/SGPT, AST/SGOT and ALP were done using RANDOX diagnostic test kits.

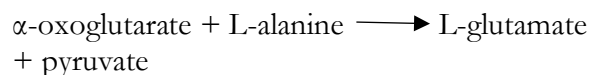
Determination of Alanine Aminotransferase (ALT)

ALT was determined using RANDOX test kits/reagents.

Principle

Alanine Aminotransferase is measured by monitoring the concentration of pyruvate hydrazine formed with 2,4-dinitrophenylhydrazine.

ALT



Procedure

One test tube was labelled blank. Twenty-five (25) other test tubes, five (5) for each group were appropriately labelled test for all groups, i.e. Group A, B, C, D and E.

To the test tube labelled blank and the other twenty-five labelled test tubes, 500uL of working reagent 1 (R1) was added to each. 100 uL of distilled water was added to the reagent blank (already carrying 500 uL of R1), while 100 uL of the sample was added to each of the 25 labelled tests tubes according to the labelling on the tubes (i.e., each animal serum to its own labelled tube). The content of each test tube was mixed by shaking gently and incubated for 30 minutes at 37°C.

After 30 minutes incubation period, 500 uL of working reagent 2 (R2) was added to the test tube labeled blank as well as the twenty-five (25) other test tubes. The content of each test tube was mixed by shaking gently and incubated for 20 minutes at 25°C.

5000 uL of Sodium hydroxide was added to the reagent blank as well as the twenty-five (25) other test tubes (containing R1, R2 and sample). The content of each test tube was mixed by shaking gently and incubated for 5 minutes. The absorbance of sample (A_{sample}) against reagent blank was taken at the wavelength of 546nm.

Calculation of ALT activity

$$C_{\text{test}} = \frac{A_{\text{test}}}{A_{\text{standart}}} \cdot C_{\text{standart}} \quad (1)$$

Where:

C_{test} - Concentration of sample/Test

A_{test} - Absorbance of Sample/Test

A_{standart} - Absorbance of Standard

C_{standart} - Concentration of Standart

Note: that reference Standard $C_{\text{Standard}} = 52 \text{ U/I}$ and reference $A_{\text{Standard}} = 0.300$

Determination of Alkaline Phosphatase (ALP)

Alkaline phosphatase was determined using RANDOX test kits/reagent

Principle

The alkaline phosphatase acts upon the AMP-buffered Sodium thymolphthalein monophosphate. The addition of an alkaline reagent stops enzyme activity and simultaneously develops a blue chromogen, which is measured photometrically.

Procedure

Two test tubes were labelled reagent blank and standard respectively. Twenty-five (25) other test tubes, five (5) for each group were appropriately labelled according to their groups i.e. Group 1, 2, 3, 4 and 5.

500 uL of Alkaline Phosphatase substrate was added into all the labelled test tubes and incubated for 3 minutes at 37°C . 50uL of standard control and sample was added to the test tube labelled standard and the other twenty-five (25) labelled test tubes respectively. De-ionized water was used as blank. The content of all the test tubes was mixed by gently shaking and incubated for 10 min. at 37°C .

2.5mL of Alkaline Phosphatase colour developer was added and mixed well by gently shaking.

Absorbance of sample against reagent blank was read at the wavelength of 590nm.

Calculation of ALP activity

$$C_{\text{test}} = \frac{A_{\text{test}}}{A_{\text{standard}}} \cdot C_{\text{standard}} \quad (1)$$

Where:

C_{test} - Concentration of sample/Test

A_{test} - Absorbance of Sample/Test

A_{standard} - Absorbance of Standard

C_{standard} - Concentration of Standart

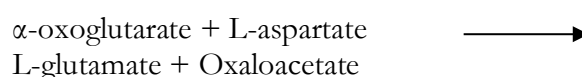
Note: that reference Standard $C_{\text{Standard}} = 50 \text{ U/I}$ and reference $A_{\text{Standard}} = 0.2195$

Determination of Aspartate Aminotransferase (AST)

Principle

AST is measured by monitoring the concentration of oxaloacetate hydrozone formed with 2, 4-dinitrophenylhydrazine.

AST



Procedure

One test tube was labelled blank. Twenty-five other test tubes, five (5) for each group were appropriately labelled test for all groups, i.e. Group 1, 2, 3, 4 and 5.

To the test tube labelled blank and the other twenty-five labelled test tubes, 500uL of working reagent 1 (R1) was added to each. 100 uL of distilled water was added to the reagent blank (already carrying 500 uL of R1), while 100 uL of the sample was added to each of the 20 labelled tests tubes according to the labelling on the tubes (i.e. each animal serum to its own labelled tube). The content of each test tube was mixed by shaking gently after and incubated for 30 min. at 37°C .

After 30 min.incubation period, 500 uL of working reagent 2 (R2) was added to the test tube labelled blank as well as the twenty-five (25) other test tubes. The content of each test tube was mixed by shaking gently and incubated for 20 min. at 25°C .

5000 uL of Sodium Hydroxide was added to the reagent blank as well as the twenty-five (25) other test tubes (containing R1, R2 and sample). The content of each test tube was mixed by shaking gently and incubated for 5 min. The absorbance of sample (A_{sample}) against reagent blank was taken at the wavelength of 546nm.

Calculation of AST activity

$$C_{\text{test}} = \frac{A_{\text{test}}}{A_{\text{standart}}} \cdot C_{\text{standart}} \quad (1)$$

Where:

C_{test} - Concentration of sample/Test

A_{test} - Absorbance of Sample/Test

A_{standard} - Absorbance of Standard

C_{standard} - Concentration of Standart

Note: that reference Standard $C_{\text{Standard}} = 16 \text{ U/I}$
and reference $A_{\text{Standard}} = 0.050$

Statistical Analysis

Data was expressed as means $\pm$ standard error of mean (SEM) and compared using one-way Analysis of variance (ANOVA) with the aid of the statistical package for social science (SPSS). Statistical significance was accepted at $p < 0.05$

Results

Preliminary Phytochemical screening of melon seed milk indicated the presence of phenol,

tannins, flavonoid, alkaloid, steroid and glycoside as shown in the table below:

Table 1. Phytochemical Constituent of *Citrullus colocynthis* Seed Milk

Phytochemical constituent	Results
Phenols	+
Tannin	+
Flavonoid	+
Saponin	-
Alkaloids	+
Steroid	+
Glycoside	+

Key:

+ indicates presence of the Phytochemical constituents

- indicates absence of the Phytochemical Constituents

Table 2. Effect of *Citrullus colocynthis* Seed milk on CCl_4 - induced hepatotoxicity in Albino rats

Group	ALP	AST	ALT
A	25.96 ± 3.46^b	37.91 ± 1.85^b	15.11 ± 0.62^b
B	28.11 ± 2.30^b	33.50 ± 0.76^c	14.93 ± 1.01^b
C	13.34 ± 2.04^d	17.55 ± 1.76^e	8.43 ± 0.86^d
D	41.48 ± 3.07^a	48.25 ± 4.44^a	19.56 ± 0.94^a
E	20.24 ± 2.65^c	29.36 ± 1.94^d	11.73 ± 1.54^c
P. value	0.000	0.000	0.000

Note: The values are in Mean $\pm$ Standard Error of Mean (SEM)

A = CCl_4 + melon seed milk 100 mg/kg

B = CCl_4 + 200 mg/kg melon seed milk

C = CCl_4 + 2.25 mg/kg *Silymarin*

D = CCl_4 only

E = water + feed

As shown in (Fig1), the administration of CCl_4 in the albino rats elevated ALP level in hepatotoxic group (41.41 ± 3.07) compared to the normal group (20.24 ± 2.65). The elevation was modulated in the groups treated with 100 mg/kg (25.96 ± 3.47) and 200 mg/kg (28.11 ± 2.31) of *Citrullus colocynthis* seed milk respectively. Also, there was a significant ($p < 0.05$) reduction

in the group treated with standard drug (13.35 ± 2.04).

On comparison among the treated groups, the group treated with standard drug showed significant reduction ($p < 0.05$) compared to the groups treated with 100 mg/kg and 200 mg/kg.

From (Fig 2), shows that, the administration of CCl₄ elevated the level of AST in hepatotoxic group (48.25 ± 4.44) compared to the normal group (29.36 ± 1.94). The elevation was modulated in the groups treated with 100 mg/kg (37.92 ± 1.85) and 200 mg/kg (33.50 ± 0.76) of the milk and respectively. There was also a

significant ($p < 0.05$) reduction in the group treated standard drug (17.55 ± 1.75).

Comparison among the treated groups indicated that, only the group treated with standard drug showed significant decrease ($p < 0.05$) compared to the group treated with 100 mg/kg and 200 mg/kg milk.

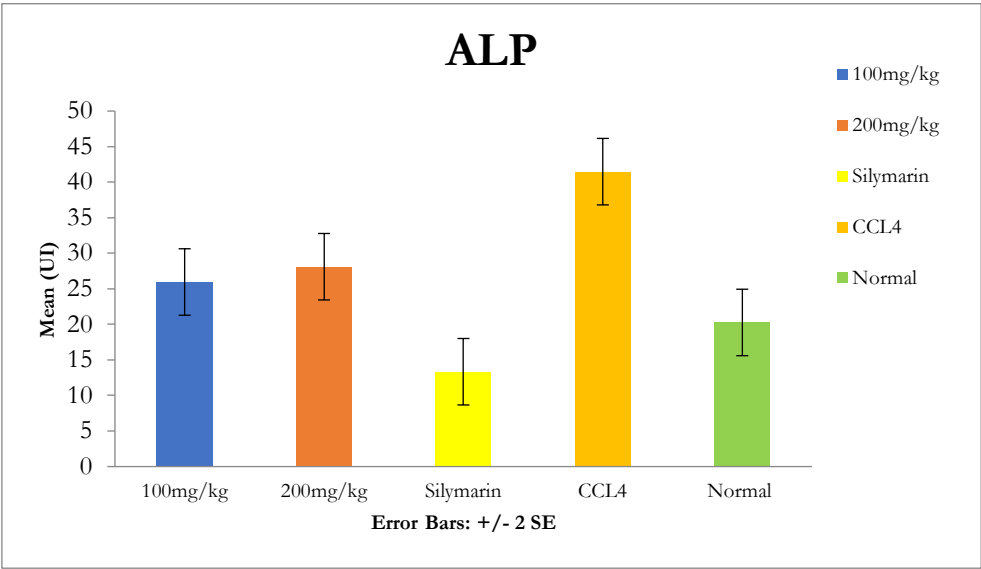


Figure 1. Effect of *Citrullus colocynthis* Seed Milk on ALP in CCl₄-induced Hepatotoxicity

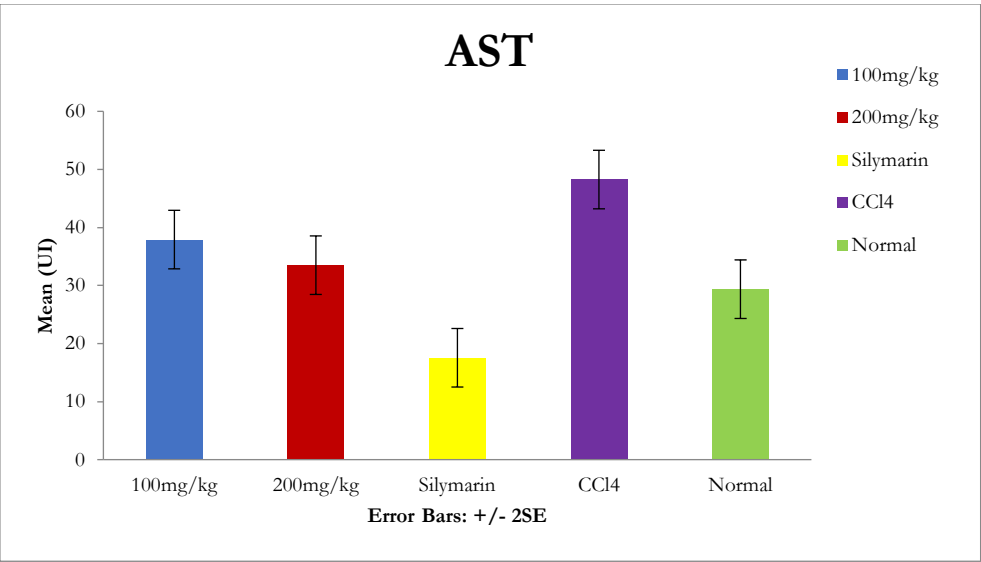


Figure 2. Effect of *Citrullus colocynthis* Seed milk on AST in CCl₄-induced Hepatotoxicity

As shown in Fig. 3 below, the administration of CCl₄ elevated the level of ALT in hepatotoxic

group (19.56 ± 0.94) compared to the normal group (11.73 ± 1.53). The elevation was

modulated in the 100 mg/kg (15.11 ± 0.62) and 200 mg/kg (14.93 ± 1.00) *Citrullus colocynthis* milk treated groups respectively.

Comparison among the treated groups indicated that, there was a significant decrease (even below the normal control group) in the silymarin-treated group compared to the groups treated with 100 mg/kg and 200 mg/kg milk.

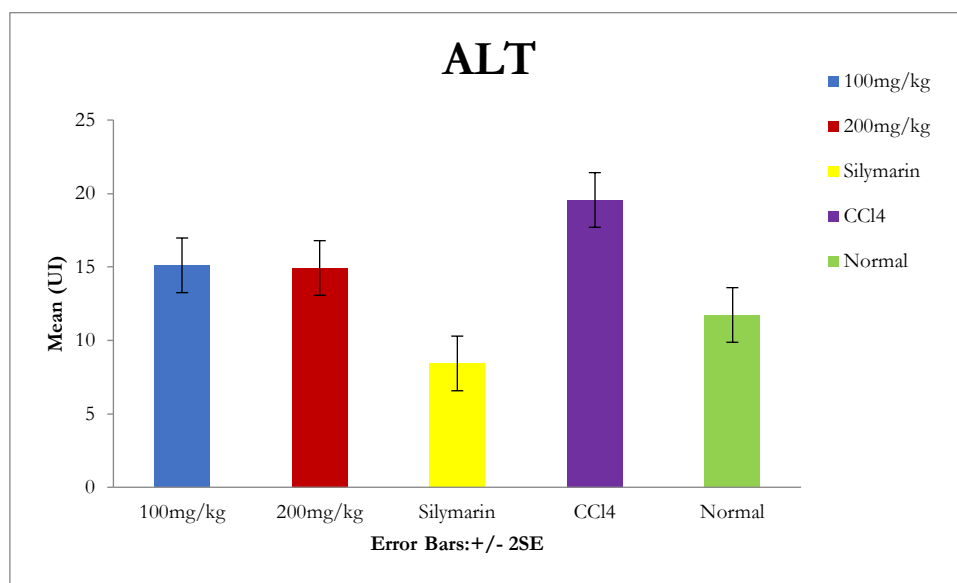


Figure 3. Effect of *Citrullus colocynthis* Seed milk on ALT in CCl₄-induced Hepatotoxicity

Discussion

The liver is a vital organ and its strategic location and multi - dimensional functions support almost every other organ in the body. Liver is also the main organ for metabolism and elimination of drugs (Singh et al., 2012; Swaroop and Gowda, 2013). At the same time, the liver is prone to many diseases like allergy to food and it involves immune system as well. Liver disease is a serious problem in developing countries and a cause of morbidity and mortality throughout the world. Liver ailments are frequently caused by hepatitis A, B and C viruses, carbon tetrachloride (CCl₄), high doses of paracetamol, thioacetamide (TAA) and certain chemotherapeutic agents etc. (Saleem et al., 2010). Morbidity and mortality resulting from liver diseases is a major public health problem worldwide (Zhang et al., 2013).

ALT, AST, ALP and serum bilirubin level are commonly used biochemical parameters to evaluate liver injury. Upon the induction of

CCl₄ hepatotoxicity, the ALT, AST, ALP and bilirubin levels increase in the circulation because they are cytoplasmic in location and are released into circulation after cellular damage (Parmar et al., 2010).

This study investigated the hepatoprotective effect of melon seed milk on CCl₄ - induced hepatotoxicity in albino rats. In this study, preliminary phytochemical analysis of the melon seed milk indicated the presence of phenols, tannin, flavonoid, alkaloids, steroid and glycosides.

The biochemical parameters measured for comparison are Alkaline phosphatase (ALP), Aspartate aminotransferase (AST) and Alanine aminotransferase (ALT). Two different doses of *Citrullus colocynthis* seed milk, 100 mg/kg and 200 mg/kg were given to group A and B of CCl₄ - induced hepatotoxicity for a period of seven days. Standard drug, Silymarin was administered in group C at the dose of 1.25 mg/kg, which served as the standard control group.

Result from this study shows that, the administration of CCL₄ in the rats caused the levels of ALP, AST and ALT to elevate. The result showed a significant ($p < 0.05$) reduction of ALP level in the groups treated with 100 mg/kg, 200 mg/kg, and the group treated with silymarin compared to the normal group. Comparison between the two groups treated with 100 mg/kg and 200 mg/kg of *Citrullus colocynthis* seed milk showed no significant difference. This suggests that, 100 mg/kg dose of melon seed milk is enough to modulate ALP level significantly in liver alteration. This is in correlation with the study conducted by Dar et al., (2012).

The result also indicated that, AST level in the groups treated 100 mg/kg, 200 mg/kg of melon seed milk, as well as the group treated with silymarin was significantly ($p < 0.05$) modulated. Comparison among the treated group showed that, the reduction of AST level was more significant in the group treated with silymarin, compared to the groups treated with 100 mg/kg and 200 mg/kg milk. This implies that, the ability of the milk to restore the elevated levels of AST in CCL₄ - induced albino rat is dose - dependent.

Also, ALT levels in the groups treated with 100 mg/kg, 200 mg/kg of *Citrullus colocynthis* seed milk and that of silymarin - treated group were significantly restored. The result of the treated groups showed that, ALT level in silymarin - treated group was significantly reduced compared to the normal group. There was no significant difference between the two groups treated with 100 mg/kg and 200 mg/kg of *Citrullus colocynthis* seed milk. This implies that, the ability of the milk to restore the elevated levels of ALP in CCL₄ - induced hepatotoxicity is not dose - dependent. This result agrees with the study conducted by Al-Gaithi et al., (2004) and Khalil et al., (2010).

The ability of the milk to restore the elevated levels of liver enzymes could be due to the phytochemicals it contains. The result obtained from this study does not give a definite conclusion on the mechanism through which the *Citrullus colocynthis* seed milk act to modulate the liver damage. However, there are various known mechanisms through which medicinal plants act

to restore liver damage. Through one or more mechanisms, they act on the hepatocytes directly or indirectly and help in proper functioning. The mechanisms include increase in antioxidant level, decrease in oxidants (ROS formation) (Kumar et al., 2011), inhibition of cytochrome P450s (Rofiee et al., 2011), increase and decrease level of Liver enzymes (Peter et al., 2010), reduced peroxidation/Lipid peroxidation (MDA) (Dash et al., 2007) and increase in level of glutathione or reducing equivalents (Das et al., 2012).

Conclusion

This study was able to pre-empt the under-utilized potentials in melon (*Citrullus colocynthis*) seed milk. Hence, further studies should be focused on the seed milk to enable scientists.

understand full scale potentials of melon seed milk. The results of this study showed that, melon seed milk has hepatoprotective and other latent potentials, hence it was able to significantly modulate the elevated levels of liver enzymes in rats at the end of this study, and is also a pointer to other abilities it possesses amongst common debilitating diseases of man.

Ethical approval

The ethical consideration was obtained from the ethical committee of college of veterinary medicine teaching hospital, Federal University of Agriculture, Makurdi, Nigeria.

Recommendation

The experimental period should be extended beyond fourteen days to at least twenty-one (21) days as this would help authenticate and foster confidence in the results obtained from this particular study.

Quantitative phytochemical analysis should be performed on the melon seed milk. This would give a clearer view of the percentage composition of each phytochemical contained in

the extract, hence buttress studies on its anti-diabetic properties.

This research should be carried beyond this limit to see the effect of this plant extract on kidney function as well as diabetic complications like diabetic nephropathy.

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Authors contribution

The authors confirm contributions to the paper as follows: the first author conceptualized and, designed and wrote the paper, the corresponding author proof read and performed the analysis and author number 3 contributed data or analysis tool.

Conflict of Interest

The authors of this work have no conflict of interest whatsoever before, during and after this work.

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