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RESEARCH ARTICLE

HEPATOPROTECTIVE AND TOXICOLOGICAL ASSESSMENT OF BETA VULGARIS AQUEOUS ROOT EXTRACT USING MALE ALBINO RAT

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ABSTRACT

Beta vulgaris root is claimed by traditional medicine to treat a wide variety of ailments such as fevers and constipation, liver diseases, amongst other ailments. The objectives of this study were to assess the possible hepatoprotective effect of the extract on carbon tetrachloride (CCl_4) - induced hepatotoxicity and to assess the possible general toxicity of the root, in albino rat. The hepatoprotective assessment was determined biochemically (using Liver Function Test, LFTs), morphologically (histopathological) in albino rat. In general toxicological assessment, the effects of the extract on haematological, biochemical, morphological and in organo-body ratio assessment were performed. The extract was found to possess profound therapeutic ability as it decreased bilirubin levels from 5.2±0.1 µmol/L in the group treated with CCl_4 only to 4.2±0.5 µmol/L in the group that received CCl4 and 1000 mg/kg of extract. Additionally, alanine aminotransferase (ALT) levels decreased from 219.7±30.02 U/L in CCl_4 only treated group to 40.25 ± 2.39 U/L in groups treated with CCl_4 and 1000 mg/kg respectively. This effect was clearly evident in histopathological studies of livers of rats. The therapeutic ability of the aqueous extract was comparable to Silymarin, a standard hepatoprotective agent. The extract was also found to be effective in both prophylactic and concomitant administrations. This reduction was comparable to the control (316.6 ± 28.2 minutes), and more significant than in the group that received CCl_4 and Silymarin (432.6±42.0 minutes). Acute toxicity studies preliminarily carried out on the aqueous extract revealed no lethality. With the exception of food intake (that dose-dependently increased), no physical, physiological and behavioural effects on mice were observed in the study. The $LD_{50}$of the aqueous extract was found to exceed 5000 mg/kg body weight. Sub-acute toxicity studies on the extract also showed no significant physical, physiological and behavioural effects. Haematological and biochemical studies also revealed no effect on rats administered with doses from 300 mg/kg to 1500 mg/kg body weight of extract. This effect was clearly confirmed by histopathological studies, as they showed no pathological difference in the target organs, livers and kidneys of treated rats when compared to those of the control rat group. It is concluded that the aqueous leaf extract of *Beta vulgaris* root is safe at doses of 50-1500mg/kg in albino rats. It is also hepatoprotective against CCl_4 induced liver damage. This hepatoprotective ability should have a role in the traditional use of the extract in the management of hepatitis related jaundice.

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INTRODUCTION

Beta vulgaris (beet) is a plant which is included in Betoideae subfamily in the Amaranthaceae family. It is the economically most important crop of the large order Caryophyllales. It has several cultivar groups: the sugar beet, of greatest importance to produce table sugar; the root vegetable known as the beetroot or garden beet; the leaf vegetables chard and spinach beet; and mangelwurzel, which is a fodder crop. Three subspecies are typically recognised. All cultivars fall into the subspecies *Beta vulgaris* subsp. *vulgaris*. The wild ancestor of the cultivated beets is the sea beet (*Beta vulgaris* subsp. *maritima*).The claimed therapeutic use of beetroot includes its antitumor, carminative, emmenagogue, and hemostatic and renal protective properties and is a potential herb used in cardiovascular conditions (Gamal *et al.,* 2014). Beetroot is known tobe a powerful antioxidant (Devasagayam *et al.,* 2004). In ancient times, beetroot was believed to help enhance human sex hormones and as an aphrodisiac. The juice of beetroot is also consumed as a natural remedy for sexual weakness and to expel kidney and bladder stones (Sharma *et al.,* 2008). In recent years, beetroot has gained popularity to be a natural food to boost the energy in athletes. The beetroot leaves were recommended by the Father of Medicine "Hippocrates" for faster healing of wounds (Singh *et al.,* 2011). Recent reports indicate that *Beta vulgaris* extracts (root) possess antihypertensive, hypoglycemic, antioxidant (Sharma *et al.,* 2011), anti-inflammatory, and hepatoprotective activities (Singh *et al.,* 2011). Previously, red beetroot extract has been demonstrated to be an effective multiorgan tumor

suppressing agent in laboratory animals (Chakole *et al.,* 2011). Recent studies have also postulated that renal inflammation, which is characterized by infiltration of inflammatory cells such as monocytes/macrophages and subsequent release of proinflammatory cytokines and activation of NF-κB in response to oxidative stress, is involved in this process. Furthermore, induced apoptosis/necrosis of renal tubular epithelial cells (Ormsbee *et al.,* 2013). From above literature, *Beta vulgaris* is known to possess potent antioxidant, anti-inflammatory properties.

MATERIALS AND METHODS

Plant materials and preparation of extracts collection of plant: *Beta vulgaris* were obtained from Shoprite, Umuahia Abia State Nigeria. The plant was identified and authenticated at taxonomist in the department of Plant Sciences and Biotechnology, Abia State University, Uturu, Nigeria. Avoucher specimen (ABSUU/PSB/ 084) was deposited at the Herbarium of the department of Plant Sciences and Biotechnology, Abia State University, Uturu, Nigeria.

Preparation of plant leaf extract of Beta vulgaris: The plant leaves were washed with distilled water to remove dirt and contaminants. The leaves were dried under the sun to a fine powder. Two hundred (200g) of the powdered leaf sample was soaked in 1000ml of distilled water and allowed to stand for 48 hours with occasional stirring to allow for proper extraction (Trease and Evans, 1983). 1ml of the filtrate was pipetted into a pre-weighed 100cm^3 beaker and evaporated to dryness on a boiling water bath. Sample suspensions were freshly prepared with 2% Tragacanth in distilled water, which served as drug vehicle, and for its controls. Suspensions were administered orally (p.o.). Volumes of extract administered did not exceed 2ml/kg body weight of the animal. Prepared suspensions were kept at -2 to + 8 ^{0}C for a maximum of one week.

Measurement of body weight of the animals: Body weight of animals was measured on days 0, 7,14, 21, and 28. Body weight of animals noted was expressed as mean body weight (g).

Preparation of the extract and fractions: The fresh roots of *Beta vulgaris* (1 kg, cut into small pieces) were exhaustively macerated by soaking in 70% (1.5 L) ethanol and the process repeated for three successive days. The obtained alcoholic extract was then concentrated under reduced pressure using rotatory evaporator till complete drying. The resulted extract (*Beta vulgaris* L.) Beet Root Ethanolic Extract (BVEE, 150 g) was later suspended in distilled water and evaluated for nephroprotective activity.

Haematological analysis: Blood samples were collected via veinous puncture into sterile sample tubes containing the anticoagulant, EDTA. Blood haemoglobin concentration (HB), Red blood cell (RBC) count, White blood cell (WBC) count, Haematocrit (HCT), Mean haemoglobin concentration (MHC), Mean corpuscular volume (MCV), Mean corpuscular haemoglobin concentration (MCHC) as well as Platelet (PLT) count were analysed using an automated analyser, Cell Dyne, model 331430, Abbott laboratories, IL USA.

Assessment of serum biochemical parameters: Blood was collected via veinous puncture into sterile sample tubes without anticoagulant, allowed to settle, and separated by centrifuging at 500 rpm for 10 minutes. Supernatant serum was collected and analysed for levels of the liver enzymes, Alanine Aminotransferase (ALT), Aspartate Aminotransferase (AST), Alkaline Phosphatase (ALP) and Gamma Glutamic Transpeptidase (GGT), using an Automated Analyser, ATAC 8000 (Elan Diagnostics, CA USA). Total, direct and indirect bilirubin levels were also determined.

Organo-bodyratios: At the end of the experiment, animals were weighed and euthanized. The target organs, liver, kidneys, spleen and stomach were excised from individual animals and weighed. The ratio of organ to body weights were then computed and statistically analysed.

Hepatoprotective Studies: Liver injury was induced as recommended by Jang *et al,* for one week. This was carried out in 3 phases: therapeutic, prophylactic and concomitant. In all phases, Silymarin was used as a standard for hepatoprotective agent in one of the groups.

Effect of beetroot in carbon tetrachloride induced liver injury in albino rat: In this study, liver injury was induced before administration of extract. Induction of liver injury was done by oral administration of 50 % carbon tetrachloride (CCl4) in olive oil at 2 ml/kg body weight, every other day for 1 week. 20 female rats were divided into 4 groups (n=5). In week 1, liver injury was induced in groups 2, 3 and 4. Group 1, the negative control group, received olive oil (2ml/kg). Forty-eight hours after administration of last dose, administration of extract commenced. Extract was administered daily p.o. 14 days. Groups 2 and 3 received 300mg/kg and 1000mg/kg body weight of *beetroot* extract, while group 4 received Silymarin at 25mg/kg body weight, daily. Volumes administered did not exceed 2ml/kg body weight. Group 1 received 2 % Tragacanth (2ml/kg). Twenty-four hours after the last dose of treatment, blood samples were taken from animals for biochemical analysis. All animals were subsequently euthanised, and the livers excised for histopathological examination.

Prophylactic study of beta VULGARIS L Extract in albino rats of carbon tetrachloride-induced liver injury: Liver injury was induced 1 week after commencement of treatment with extract and standard drug reference. Twenty male rats were divided into 4 groups. Group 1 received 2 % Tragacanth and olive oil (at 2ml/kg) and served as the negative control. In the week 1, groups 2 and 3 received 300 mg/kg and 1000 mg/kg body weight of *beetroot* extract, while group 4 received Silymarin at 25mg/kg body weight orally, per day. Volumes administered did not exceed 2ml/kg body weight. Group 1 received 2 % Tragacanth at 2 ml/kg. Liver injury was induced by oral administration of 50 % carbon tetrachloride in olive oil at 2 ml/kg body weight, every other day for 1 week. Administration of extract and reference drug doses continued in week 2, coupled with the induction of liver injury in groups 2, 3 and 4, and administration of olive oil at 2 ml/kg body weight in group 1 every other day, for a week. Twenty-four hours after the last administration, blood samples were taken from animals for biochemical analysis. The animals were subsequently euthanised, and the livers excised for histopathological examination.

Concomitant effect of Beta vulgaris L Extract and Liver Injury with Carbon Tetrachloride in Rats: This experiment was conducted to assess the hepatoprotective ability of the extract when administered at the same time carbon

tetrachloride injury is incurred. Twenty female rats were grouped into 4 (n = 5). Group 1 served as the negative control and received 2 % Tragacanth and olive oil (2ml/kg). Liver injury was induced concomitantly with daily administration of minimum and high doses of extract, and reference drug for one week. Induction of liver injury was done by oral administration of 50% carbon tetrachloride in olive oil at 2 ml/kg body weight, every other day for 1 week. Groups 2 and 3 received 300 mg/kg and 1000 mg/kg body weight of *beetroot* respectively, whilst group 4 received Silymarin at 25 mg/kg body weight. Volumes administered did not exceed 2ml/kg body weight. Twenty-four hours after the last administration, blood samples were taken from animals for biochemical analysis. All animals were subsequently euthanised, and the livers excised for histopathological examination.

Statistical analysis: Results are expressed as mean ± standard error of the mean (beetroot), and the number of observations is represented by 'n'. One way Analysis of Variance (ANOVA) was used to compare group data, followed by Tukey's multiple comparison test ($p < 0.05$ was considered significant). The statistical package used was Graph pad Prism 5.00.288.

RESULTS AND DISCUSSION

There was no change in the organ – body weight ratios in the animals over the period of treatment. Ratios were comparable to the control, with the exception of the group administered with 300 mg/kg, which showed slightly decreased ratios.

Serum biochemical parameters: After a 14 day administration of doses of *beetroot*, levels of Alanine Aminotransferase (ALT), Aspartate Aminotransferase (AST), Gamma Glutamic Transpeptidase (GGT), Total Bilirubin, Albumin and Total Protein, in all groups treated with the *beetroot* extract (300 mg/kg, 1000 mg/kg and 1500 mg/kg body weight) were comparable to that of the control group (Figure 2). There was no significant change in serum biochemical parameters.

Haematological parameters: There was no significant difference in Blood Haemoglobin Concentration (HB), Red Blood Cell (RBC) Count, Haematocrit (HCT), Mean Haemoglobin Concentration (MHC), Mean Corpuscular Volume (MCV), Mean Corpuscular Haemoglobin Concentration (MCHC).

Table 1. Effect of *Beetroot on* Relative Organ to Body Weights in Rats Treated with Extract for 14 Days

	Organo-body ratios (%)			
Liver	**Kidney**	**Stomach**	**Spleen**	
Control	2.27±0.10	0.79±0.12	0.74±0.07	0.27±0.02
300mg/kg *Beetroot*	1.79±0.06	0.54±0.04	0.51±0.02	0.22±0.01
1000mg/kg *beetroot*	2.01±0.08	0.64±0.04	0.59±0.03	0.24±0.01

Table 2. Effect of *Beta vulgaris* on Serum Biochemical Parameter in Rats Treated with *Beta vulgaris* and Reference Drug for 14 Days, after Administration of CCl_4

Treatments			Level of serum biochemical parameter		
AST U/L	**ALT U/L**	**GGT U/L**	**Total bilirubin µmol/L**	**Albumin(g/L**	**Total protein(g/L)**
Control	138.5±1.5	48.5±0.5***cc	5.0±0.0	3.4±0.0	35.1±0.51
CCl_4 only	139.4±6.5	219.7±30.02	5.5±0.5	5.2±0.1	31.68±0.75*
CCl_4 +300mg/kg	138.7±7.2	44.56±2.01***cc	5.46±0.23	5.9±0.6	33.0±0.83
CCl_4+1000mg/kg	114.9±1.7*cc	40.25±2.39***cc	5.425±0.27	4.2±0.5	35.38±0.57**cc
CCl_4 +Silymarin	111.3±0.7**cc	38.04±0.88***cc	5.28±0.19	5.06±0.16	34.9±0.54*cc
p value	0.0015	<0.0001	0.7929	0.0489	0.0030

Values are represented as mean ± SEM. *CC indicates significance at $p<0.05$ compared to the CCl_4 control.

Table 3. Effect of *Beta vulgaris* on Serum Biochemical parameter Doses were Administered Concomitantly with Carbon Tetrachloride Injury Induction

Treatments			Serum biochemical parameters			
AST(U/L)	**ALT(U/L)**	**ALP(U/L)**	**GGT(U/L)**	**Total bilirubin µmol/L**	**Albumin(g/L**	**Total protein(g/L)**
Control	147.1±6.3	55.04±3.12	791±94.4	1.86±0.38	39.68±0.55	60.9±1.60
CCl_4 control	374.8±64.7	226.5±32.7	1577±158.4*	3.58±0.69	37±0.71	66.28±1.69
CCl_4 + 300mg/kg	176.2±46.1	126.8±44.4	1077±216.7	2.33±0.48	39.84±0.02	67.3±2.73
CCl_4+1000mg/kg	152.1 ±74.4	113.8±55.9	982.1±147.6	2.4±0.59	39.56±1.01	67.96±2.09
CCl_4+1500mg/kg	135.6±83.5	133.6±69.6	1288±182.4	2.32±1.08	41.34±1.20	79.16±9.01
CCl_4+ Ref. drug	177.4±51.7	155.1±43.5	913.7±169.2	2.74±1.28	42.12±0.92	74.26±3.85*cc
p value	0.0626	0.2044	0.0316	0.7770	0.0540	0.0882

Table 4. Effect of *Beta vulgaris* on serum Biochemical Parameter in Rats Treated with *Beta vulgaris* and Silymarin (for 7 day) before and during the Administration of CCl_4

Treatments				Serum biochemical parameter			
AST(U/L)	ALT(U/L)	ALP(U/L)	GGT(U/L)	Total bilirubin (µmol/L)	Indirect bilirubin (µmol/L)	Albumin (g/L)	Total protein (g/L)
Control	120.1±2.8**cc	110.2±0.6	284.5±17.6	5.22±0.4	4.8±0.6	2.40±0.27	40.22±1.52	66.74±1.83
CCl_4 only	135.9±4.0	119.1±0.8	249±0.6*	4.46±0.3	5.68±0.4	3.00±0.08	34.22±1.24	64.14±0.89
CCl_4 +300mg/kg	114.6±1.6***cc	117.6±0.6	251.1±1.2	4.18±0.4	5.85±0.1	3.08±0.08	35.76±1.19	66.24±0.48
CCl_4+1000mg/kg	116.8±1.9***cc	115.3±2.1	240.6±4.5**	4.76±0.1	5.08±0.2	2.82±0.09	36.6±1.89	66.4±0.93
CCl_4+1500mg/kg	118.2±1.6**cc	114.6±2.6	237.6±4.7**	4.98±0.4*	5.1±0.4	2.46±0.20	42.4±1.17**CC	67.5±1.09
CCl_4 +Silymarin	110±4.1***cc	104.3±5.1	220.5±5.7***	3.6±0.4*	5.45±0.5	2.8±0.16	36.5±0.96	67.63±0.81
P value	<0.0001	0.0047	0.0007	0.0398	0.4157	0.0348	0.0031	0.2987

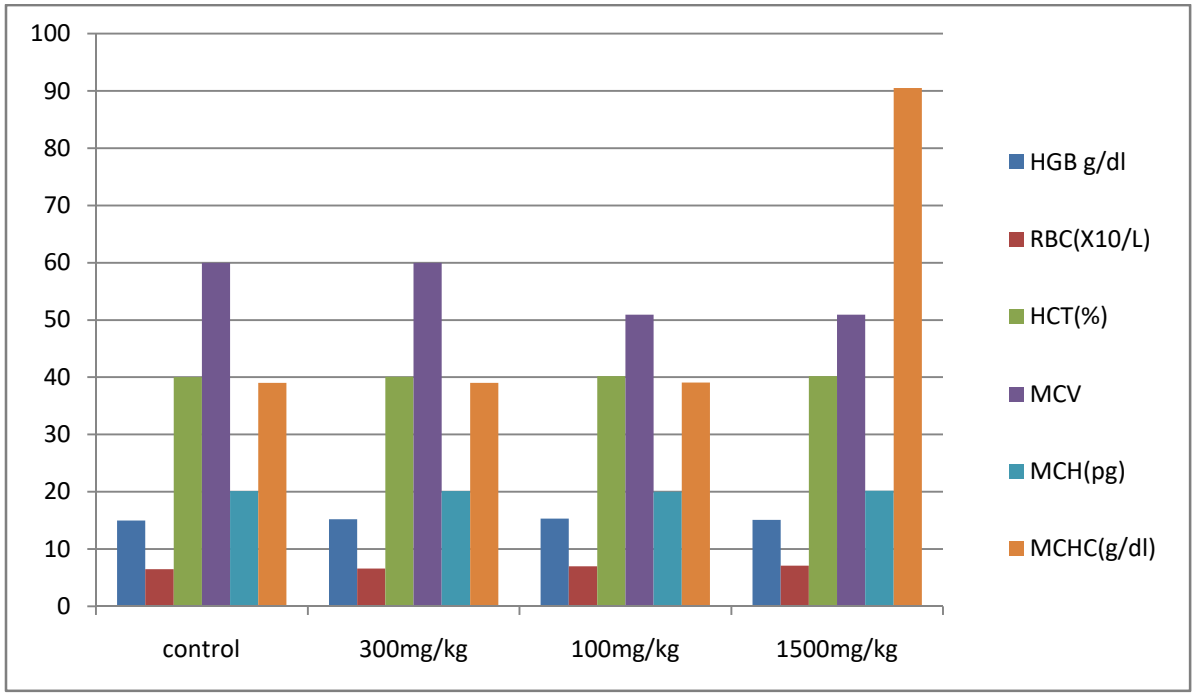
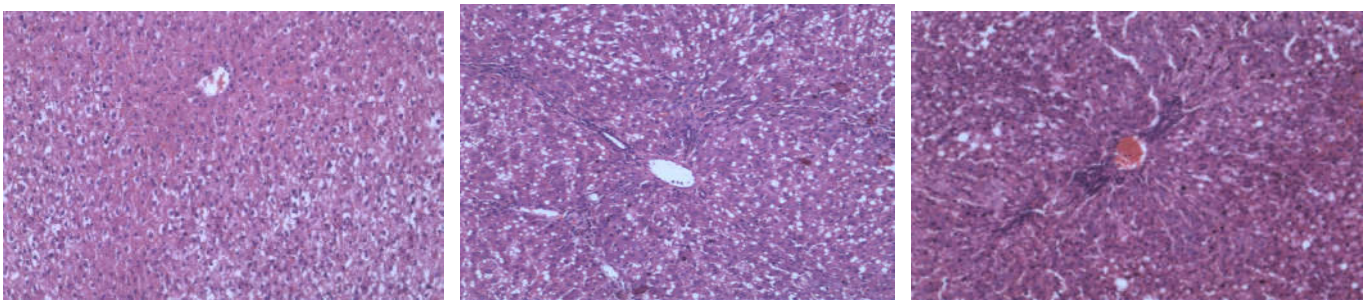


Figure 1. Effect of *Beta vulgaris* root Extracton Haematological parameters in rats. Animals received doses orally for 14 days. The Blood Haemoglobin Concentration (HB) (A), Red Blood Cell (RBC) Count (B), Haematocrit (HCT) (C), Mean Corpuscular Volume (MCV) (D), Mean Haemoglobin Concentration (MHC) (E) and Mean Corpuscular Haemoglobin Concentration (MCHC) (F) levels. Values are represented as mean ± SEM. * indicates significance ($p < 0.05$), and ** indicates significance ($p < 0.01$), compared to control group by Tukey's test

The Blood Haemoglobin Concentration count however showed a significant decrease at doses of 300 mg/kg (22.53 ± 4.61 X 10^9/L at $p < 0.01$) and 1000 mg/kg (30.88 ± 2.47 X 10^9/L at $p < 0.05$) of extract compared to the control (46.65 ±0.35 X 10^9/L) by Tukey's test. The extract however showed a dose dependent decrease in WBC counts compared to control.

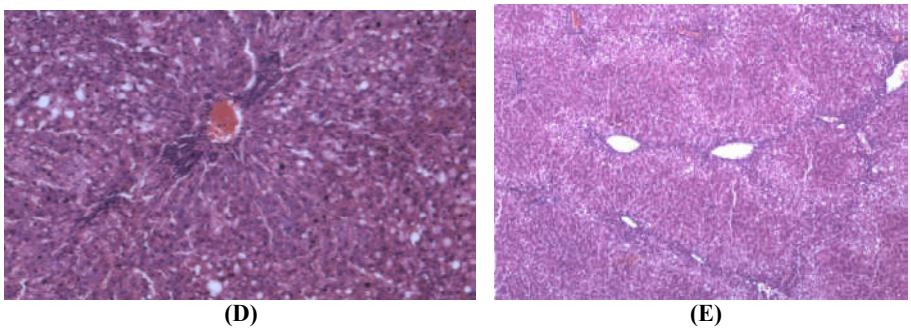
Histopathological examination: Examination of histopathological slides of the liver confirmed no cellular damage in all groups of animals, compared to the control (Plate 1). Cells of liver and kidneys were within normal limits, with only mild reactive changes in cells of rats that received 300 mg/kg body weight of extract. Kidney cells observed also showed no cellular damage. Cells were within normal limits, with mild reactive changes in cells of group rats that received 300mg/kg body weight of extract. Plate 1 shows high power photomicrographs of kidney cells of various rat groups. Treatment of diseases associated with the liver is very vital, and must be done with importance and extensive care. Many herbal remedies for liver diseases are known but only a few of them have been pharmacologically assessed for their efficacy. It is very important to assess natural products for their efficacy in the treatments they are used for. It is especially very important to assess remedies for liver diseases due to the liver's fragility and relation to other vital organs, and yet its numerous vital roles detrimental to the survival of a person. In recent times, due to economic factors, people are in need of available, easily accessible and less costly medication, even with the slightest knowledge of efficacy, and minimum idea of toxicity. It is believed by most people that since herbal remedies are natural, they are non-toxic. Toxicity of natural remedies have however been reported. Even scientifically proven hepatoprotective plant was found to contain hepatotoxins as well (Mac Gregor *et al.,* 1989; Oshima *et al.,* 1995). Thus, work on hepatoprotective herbal remedies remain a challenge (Schuppan *et al.,* 1999).

The claimed therapeutic use of beetroot includes its antitumor, carminative, emmenagogue, and hemostatic and renal protective properties and is a potential herb used in cardiovascular conditions, though there have not been reports of toxicity of the extract. The fact still lies that herbal remedies are not adequately monitored. We were also prompted by the claim of herbalists that extract of *Betavulgaris* root is effective in the management of jaundice, hemostatic and renal protective to assess its efficacy in the treatment and yet, its suspected toxicity. In assessing the therapeutic effect of the extract, *Beta vulgaris* aqueous root extract decreased the levels of total bilirubin, greatly evident in the indirect (unconjugated) bilirubin levels, suggesting that the hepatoprotective effect it has on the liver act not on the liver's ability to conjugate bilirubin, but on its ability to assemble bilirubin. The liver however would have shown defectiveness in conjugating bilirubin under intense damage of hepatocytes exhibited by extensive periportal fibrosis, central fibrosis, necrosis, inflammation and cytoplasmic vacuolation/fatty degeneration; which was not exhibited by the liver injury induced in the present study. The estimated levels of the serum biochemical parameters that are diagnostic markers of extent (Ansari *et al.,* 1991) and position of the liver injury also offered complementary explanation to the hepatoprotective ability of *Beta vulgaris root*. The extract lowered significantly the level of alanine aminotransferase (ALT), which is the most precise determinant for assessing hepatoprotectivity in biochemical analysis, even at a dose of 300 mg/kg body weight. This effect suggests that the liver' ability is further enhanced at this dose level. Histopathological examination of cells revealed that though its hepatoprotective effect did not show in bilirubin levels at dose 300 mg/kg, on cellular levels, the effect is comparable to the control. In prophylactic studies, *Beta vulgaris* root extract showed a significant reduction in aspartate aminotransferase, AST levels at a dose of 300 mg/kg body weight.



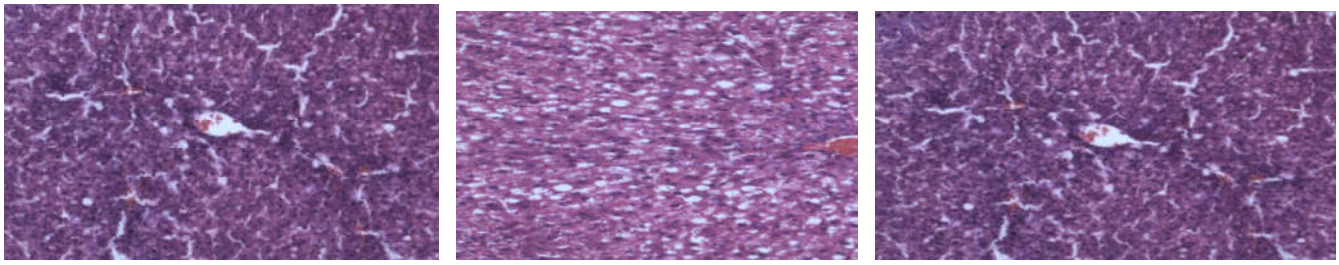
(A) (B) (C)

Plate 1 (A –C): Photomicrographs, X 100 of cells of rats of the negative control group that received only drug vehicle (A), rats that were treated with 300 mg/kg *Beta vulgaris root* (B) and 1000 mg/kg *Beta vulgaris root* (C); and induced with carbon tetrachloride



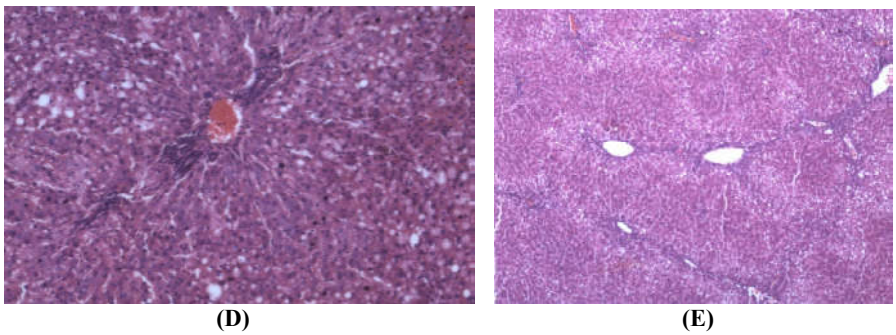
(D) (E)

Plate 2 (D –E). High power micrographs, X100 of cells of rats that were treated with 1500 mg/kg *Betavulgaris root*(D) and Silymarin (reference drug at 25 mg/kg) (E); and induced with carbon tetrachloride



(A) (B) (C)

Plate 3 (A – C). High power micrographs, X100 of cells of rats of the positive control group that were administered with carbon tetrachloride only (A), rats that were treated with 300 mg/kg *Beta vulgaris root*(B) and 1000 mg/kg *Beta vulgaris root* (C); and induced with carbon tetrachloride



(D) (E)

Plate 4 (D – E). High power micrographs, X100 of cells of rats that were treated with 1500 mg/kg *Beta vulgaris root*(D) and Silymarin (reference drug at 25 mg/kg) (E); and induced with carbon tetrachloride

The extract also significantly lowered the levels of alanine aminotransferase, ALT, dose-dependently. These reductions were comparable to the control and the reference drug. The level of bilirubin also lowered at a dose of 1000 mg/kg body weight. Examination of histopathological slides of liver of rats in various groups confirms its hepatoprotective ability. Presence of mild necrosis and fibrosis in liver cells of rats treated with doses is not comparable to the control, though slides of the CCl_4 only treated rats shown more extensive cell damage compared to control.

This extent of damage was also seen in slides of rats treated with the reference drug. These suggest that *Beta vulgaris* root can prevent liver injury by CCl_4, when administered for a longer period. When given concomitantly with carbon tetrachloride, *Beta vulgaris* root reduced the levels of aspartate aminotransferase and alanine aminotransferase at 300 mg/kg. These reductions were however not significant by Tukey's test. Histopathological examination confirms a reduction in cellular features, showing rectification. These changes were prominent at 1000 mg/kg, and were comparable to the control.

These suggest that the extract was able to reverse the injurious effects of CCl_4, or did not enable CCl_4 to cause pronounced injury in the cells, also evident in serum aminotransferase levels. If administered for a longer period, the extract could prevent completely, liver injury from CCl_4. When assessed the possible acute toxicity to mice, there was no lethality observed at 5000 mg/kg body weight, no physical changes were also observed. In sub-acute studies, daily administration of different doses of *Beta vulgaris* root extract for 14 days in rats had minimal effect. There was no significant change in the levels of the serum biochemical parameters AST, ALT, GGT, and bilirubin compared to the control group, though there was slight decrease in GGT levels (with the exception of 1500 mg/kg b. wt), synthesis of albumin was not least compromised, confirming no effect of extract on the metabolic roles of the liver. Histopathological analysis of liver cells confirms minimal effect of extract on liver and kidney at all doses. The slight decrease in GGT would therefore postulate an enhanced biliary tract. The extract therefore has a high safety on the target organs, liver and kidney. Evaluation of haematological indices also revealed minimal effect by the extract, except for white blood cell (WBC) count which showed a dose-dependent (300 - 1500 mg/kg) significant increment towards the control. At a low dose of 300 mg/kg body weight of the *Beta vulgaris root*, the extract showed a reduced count of WBC compared to the control. This is further increased at 1000 mg/kg and 1500 mg/kg body weights of the *Betavulgaris* root extract. This suggests that the extract has a positive effect on WBC count at high doses. WBCs are immunological cells that help fight the invasion of foreign bodies and disease infections in the system. The extract increasing the WBC count at high doses suggests that *Beta vulgaris* root extract may enhance the immune system by elevation of levels of WBCs. Although there were slight decreases in the relative weights of the liver, kidney, stomach and spleen, to the body in the various doses, compared to the control, the decreases were however only significant at 300 mg/kg body weight of extract in liver, kidney and stomach. Despite the decrease in the relative weight of liver, the levels of the serum alanine aminotransferase and aspartate aminotransferase were normal in all groups compared to the control. Bilirubin levels were also found to be normal in all groups compared to the control. This shows that the liver's ability to produce ALT and synthesise bilirubin were not compromised, indicating that the liver functioned adequately, and the extract had minor effect on the liver at all doses (from 300 mg/kg through 1000 mg/kg to 1500 mg/kg body weights). When extract administered concurrently with CCl_4, as seen in experiments to assess the concomitant and prophylactic effect of the extract with CCl_4, the hepatoprotective effect is minimal, and highly dose-specific. At the minimum dose, caution must therefore be taken in the administration of safe doses. Alkaline Phosphatase (ALP) and Gamma Glutamic Transpeptidase (GGT) shown an elevation in concomitant assessment at 1500 mg/kg, and GGT also elevated in the prophylactic assessment at a high dose of 1500 mg/kg, without elevations in the aminotransferases. This suggests a problem with the biliary tract at very high dose when treatment is being administered with CCl_4. Administering 1000 mg/kg body weight of *Beta vulgaris* aqueous root could be most effective in all cases of hepatoprotectivity.

Conclusion

The study conducted in this article reveals that: *Beta vulgaris* aqueous root extract has profound therapeutic ability on liver injury induced by carbon tetrachloride. *Beta vulgaris* aqueous root extract possesses adequate prophylactic ability against carbon tetrachloride-induced liver injury. *Beta vulgaris* aqueous root extract has slight hepatoprotective ability when administered concomitantly with carbon tetrachloride. The hepatoprotective ability of the extract, assessed at the three levels of therapeutic, prophylactic and concomitant are individually dose – specific. Administering 1000 mg/kg body weight of *Beta vulgaris* aqueous root extract could be most effective in all cases of hepatoprotectivity. Sub-acute toxicological studies also showed that: *Beta vulgaris* aqueous root extract caused an increase in food intake and body weights. Significant reduction in white blood cell count at a dose of 300mg/kg suggests that treatment with very low doses, though may be effective in protecting the liver, should be recommended with caution. In acute toxicity studies, the LD_{50} was found to be well beyond 5000mg/kg in mice and rats. Thus, *Beta vulgaris*is hepatoprotective, and It is non-toxic,Sub-acute toxicological studies also showed that: *Beta vulgaris* caused an increase in food intake and body weights. Significant reduction in white blood cell count at a dose of 300mg/kg suggests that treatment with very low doses, though may be effective in protecting the liver, should be recommended with caution. In acute toxicity studies, the LD50 was found to be well beyond 5000mg/kg in mice and rats. Thus, *Beta vulgaris* is hepatoprotective, and its non-toxic.

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