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Curative effect of *Beta vulgaris* (Beet root) on carbon tetrachloride CCl_4) induced cardiotoxicity in adult albino wistar rat

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Abstract
The heart is responsible for pumping oxygenated blood, hormones and other vital substances to different parts of the body, so it's usually exposed to toxic substances. The aim of this study was to evaluate the curative effect of *beta vulgaris* on the heart following CCl_4 injection. Twenty five adult albino wistar weighing between 150g and 200g were used in this study. The animals were divided into 5 groups (A, B, C, D, and E). Groups A and B served as control. Group A was fed with feed and water and injected with paraffin oil for 2 days. Group B were treated *Beta vulgaris extract* alone. Groups C and D were injected with CCl_4 (1.0ml/kg b.w i.p) for two consecutive days and group D fed with BE throughout the experimental days. Group E were fed with BVE throughout the experiment and injected CCl_4 on the last 2days of the experimental period (28 days). At the end of the experimental period animals were weighed and sacrificed using chloroform. The heart were harvested and trimmed for histological analysis. The result showed there were decrease in mean body weight of 5% in group C animals. The levels of aspartate aminotransferase (AST), alanine aminotransferase (ALT) and alkaline phosphatase (ALP) in group C were significantly higher (p<0.05) when compared with the control group while groups B, D and E remained fairly normal. The histological examination of the heart in group C animals showed massive distortion of the cytoarchitecture of the heart, while group A and B were fairly normal. Group D and E showed a reversal in the distortions of cardiac structures. The result of this study showed that *Beta Vulgaris extract* has the ability to prevent and also restore histological and biochemical changes in the heart following intoxication.

Keywords: beet root, carbon tetrachloride, albino wistar rats, aspartate aminotransferase (AST), alanine aminotransferase (ALT) and alkaline phosphatase (ALP)

Introduction
The beetroot is the taproot portion of the beet plant, usually known in North America as the beet, also table beet, garden beet, or red or golden beet. It is several of the cultivated varieties of *Beta vulgaris* grown for their edible taproots and their leaves (called beet greens).
The usually deep purple roots of beetroot are eaten boiled, roasted or raw. It is also eaten in combination with other vegetables as salad. Yellow-colored beetroots are grown on a very small scale for home consumption (Grubben, *et al*, 2004) [1].
In preliminary research, beetroot juice reduced blood pressure in hypertensive individuals (Lundberg, *et al*, 2011) and so may have an effect on mechanisms of cardiovascular disease (Hobbs, *et al*, 2012; Siervo, *et al*, 2013) [2, 3].
Beets contain betaines which may function to reduce the concentration of homocysteine, (Pajares, and Pérez-Sala, 2006) [4] a homolog of the naturally occurring amino acid cysteine. High circulating levels of homocysteine may be harmful to blood vessels and thus contribute to the development of cardiovascular disease. This hypothesis is controversial as it has not yet been established whether homocysteine itself is harmful or is just an indicator of increased risk for cardiovascular disease (Potter, *et al*, 2008) [5].

Aim of the Study
The aim of this study was to investigate the curative ability of *Beta vulgaris* on carbon tetrachloride induced toxicity of the heart.

Scope of Study
This research work is limited to determining the effect of *beta vulgaris* juice extract carbon tetrachloride induced cardiotoxicity on the physical and behavioural changes of experimental

animals, histological changes in the heart, and blood enzyme reactivity level of AST, ALT and ALP.

Materials and Methods

Experimental Animals Model

25 five adult wistar rats weighing between 150-200g purchased from the animal house of the Faculty of Basic Medical Sciences, Abia State University were used for this experiment. The animals were housed in wooden cages at animal house and fed with feed and water for a period of two weeks to acclimatize. The animals were handled in accordance with the guidelines for animal care and research as detailed in NIHG 2011 publication.

Plant Extract

The beetroot purchase from a fruit garden at shoprite supermarket, Enugu State. The beetroot were extracted using standard histochemistry proceedures at histochemistry laboratory of Human Anatomy Department, Abia state university, Nigeria. Beta Vulgaris Extract (BE) were refrigerated at a temperature of about 4^{o}C to maintain its constituents. Phytonutrient analysis were done from the plant extracts.

Experimental groups

Twenty five adult wistar rats weighing 150g to 200g were divided into five groups of four animals each (A, B, C, D and E). Group A served as control group and was fed with standard diet and water only. Group B were fed with standard diet and beet juice extract, while group C administered CCl_4 for 48 hours and were fed with water and animal feed throughout the experimental period of 28 days. Group D were administered CCl_4 on the first two days of the experiment and treated with Beta vulgaris extract for the remaining duration of the experiment. Group E were fed with beet root extract and feed throughout the experiment and administered CCl_4 48 hours before animal sacrifice.

Induction cardiotoxicity

Cardiac injury were induced by the intraperitoneal injection of CCl_4 (1 ml kg^{-1} b.wt.), diluted in paraffin oil in the ratio of 1;1, for 2 days of the experiment as stated by Ihentuge, *et al*, (2015). Group C received 1ml kg-^{1}b.wt of CCl_4 injections on the first two successive days of the 28 days and was given water and standard rat feed for the duration of the experiment. Group D rats received 1ml kg-^{1}b.wt of CCl_4 injections on first 2 days of the experiment and were fed with beet juice extract for the remaining days of the experiment (600ml/day/cage). Group E rats were fed with beta vulgaris extract for the duration of the experiment and administered Carbon tetrachloride on the last 2 days, to evaluate the resistance of this group to intoxication. Group A was administered paraffin oil only. Group B were administered beet juice (600ml/24/cage) for the duration of the experiment.

Samples and organ collection

24 hours after the last administration, the animals were anaesthetized with chloroform. Blood samples were collected by cardiac puncture using sterile syringes with needles. Blood for serum preparation was collected into sterile plain tubes. Serum samples were separated from clots by centrifugation at 3000g for five 10 minutes. The serum samples was separated into sterile plain tubes and stored in the refrigerator for analysis.

Histopathological and serum biochemistry Studies

Following a careful and neat dissection of the animals, the organ of study (the liver and heart) was harvested, with scalpel, blade and forceps and trimmed down to size of 3mm x 3mm thick. The cut tissue slides were prepared in the Histology preparation room of the Department of Human Anatomy, Faculty of Basic Medical Sciences, Abia state university, Nigeria. Biochemical study for AST, ALT and ALP serum activities were carried out to the determine enzyme activities.

Tissue Processing

Fixed tissue was processed using a standard routing histological processing (H&E method). Paraffin blocks of tissue were obtained and sections of 7 µm sliced for staining procedure and microscopic analysis.

Data analysis

Results collected were expressed in mean±standard deviation using IBM SPSS verson 16. The significance of the differences at $p<0.05$ significance level was calculated using student t-test.

Lethal dose of carbon tetrachloride

LD 50 of CCl_4 has been reported to be 1.7ml/kg (Klingensmith *et al*, 1982) on intraperiotoneal injection. But in our experiment the lethal dose at which most of these animals died was 1.5ml/kg intraperitoneal injection.

Physical And Behavioural Changes

On administration of carbon tetrachloride (CCl_4) to groups C, D and E, the following observations were made:

1. Staggering and loss of balance
2. Withdrawal from feeding
3. Reduce activity and sluggishness
4. Laboured breathing

These observations were not made of group A and B that was giving paraffin oil and beetroot juice.

Body Weights Analysis of

Table 1: comparison of mean initial weight, final weight and weight difference (g). Data expressed in mean ± SEM.

Body Weight	Grp A	Grp B	Grp C	Grp D	Grp E
Initial Weight (G)	153.80±4.97	151.40±2.61	157.80±6.46	167.20±16.18	174.40±35.59
Final Weight(G)	167.20±12.46	155.60±15.49	151.00±14.30	181.00±20.81	187.00±34.20
Weight Difference (G)	13.40±7.49	3.80±12.88	-5.20±7.84[a]	13.80±4.63	12.60±1.39

(a is significant at $p<0.05$)

Table 1.0: The table showed Groups A,B,D, and E recorded a significant increase in body weight at the end of the experiment period except for group C. Group C which, recorded a significance decrease in body weight ($p<0.05$) when compared with the control group.

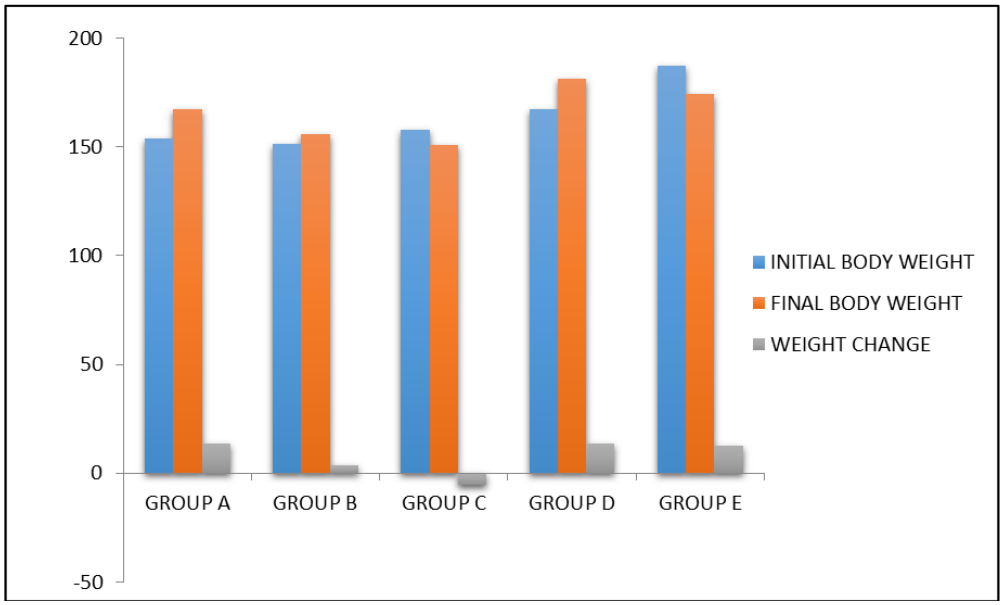

Fig 1: The bar chat below represents the mean initial body weight, final body weight and weight difference across the groups. The chat shows that during the experimental period, animals in group A,B,D and E recorded a statistical increase, while animals in group C show a decrease in body weight compared to initial weight before administration of carbon tetrachloride.

Table 1: Showing Activity of ASP, ALT and ALP in mU/L

In u/L	Grp A	Grp B	Grp C	Grp D	Grp E
AST	46.30±3.37	43.52±5.87	154.14±26.89[a]	50.57±2.40	65.07±5.12
ALT	91.44±29.83	75.17±12.11	144.73±20.89 [b]	67.13±6.14	83.79±2.65
ALP	134.93±9.44	127.33±9.61	359.22±105.33[c]	170.36±10.16	236.5±32.10[a]

Alkaline Phosphatase (ALP), Aspartate Aminotransferase (AST), and Alanine Aminotransferase (ALT) Activities

Table 1.1, a significant increase in the activities of AST, ALP and ALT in group C administered ccl4, when compared with groups D and E treated with beta vulgaris extract. Groups A and B showed a fairly comfortable AST, ALP and ALT levels.

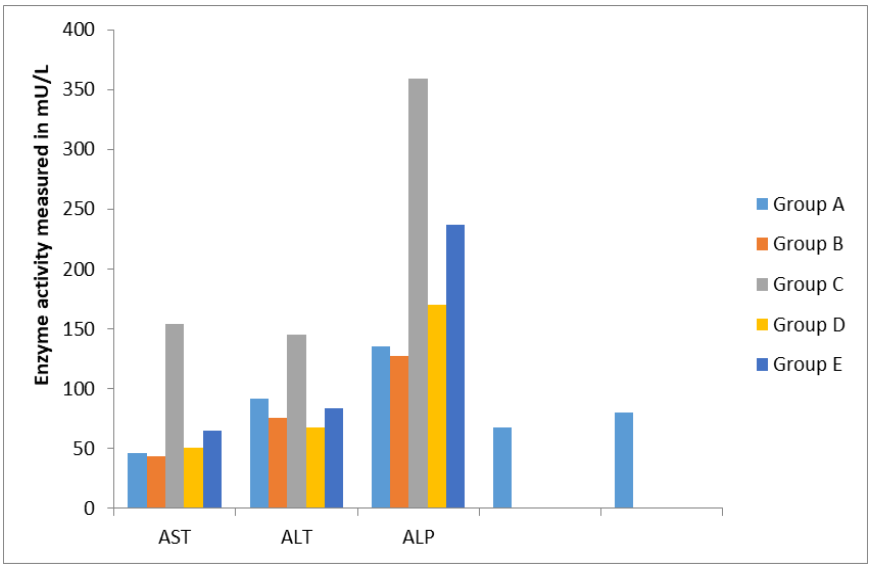

Fig 2: showing the three liver enzymes activities for different groups.

The graph above illustrates the levels of different enzymes activities for different groups. The levels of the three liver enzymes was significantly higher in group C administered carbon tetrachloride compared to the control groups treated with Beta vulgaris extract.

Histological Findings

Cytoarchitecture of the heart stained with hematoxylin and eosin (H&E), were evaluated using standard techniques to observe the extent of carbon tetrachloride induced toxicity and the regenerative or protective properties of *Beta vulgaris* (beetroot).

Heart Slides

Photomicrograph 1, is a light micrograph of control group (group A), Section shows normal cardiac muscles with arranged cardiac fiber.

Photomicrograph 2 is a light micrograph of group B animals which took *Beta vulgaris* (beetroot juice). The micrograph

shows normal cardiac tissues with no pathological changes or alteration.

Photomicrograph 3 is a light micrograph of group C animals which was intoxicated with CCl_4 alone. The micrograph shows extensively affected heart tissue with sever loss of myocardial tissue (SLMT), sever intra myocardial hemorrhage (SIMH) and moderate inflammation of cardiac cells (MICC).

Photomicrograph 4 is a light micrograph of group D which was intoxicated with ccl_4 for 2days and was treated with *beta vulgaris* during the period of the experiment. The micrograph showed moderate healing of heart tissue with moderate loss of myocardial tissue (MLMT), moderate intra myocardial hemorrhage (MIMH) and moderate inflammation of cardiac cells (MICC).

Photomicrograph 5 is a photomicrograph of group E fed with beta vulgaris for the duration of the experiment (21 days) and administered CCl_4 on the last 2days (48 hours) to check the protective effect of beta vulgaris on the heart showed normal cardiac fiber but mild intra-myocardial hemorrhage

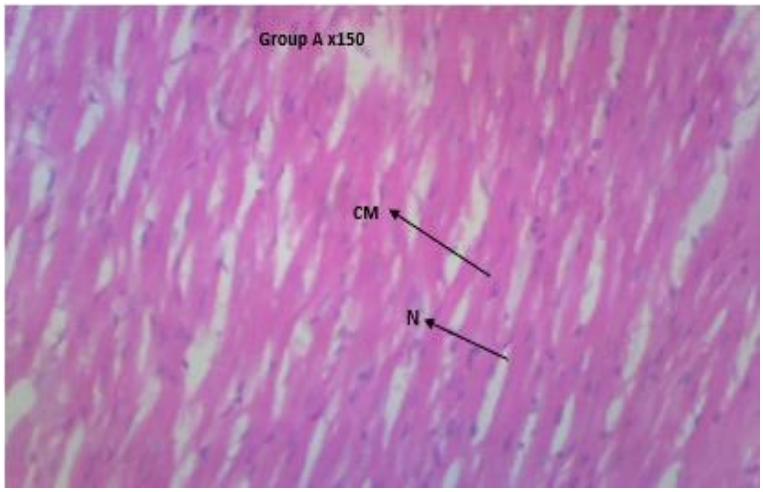

Fig 1: A: x150 Micrograph showed normal cardiac muscles with arranged cardiac fiber peripheral nucleus.

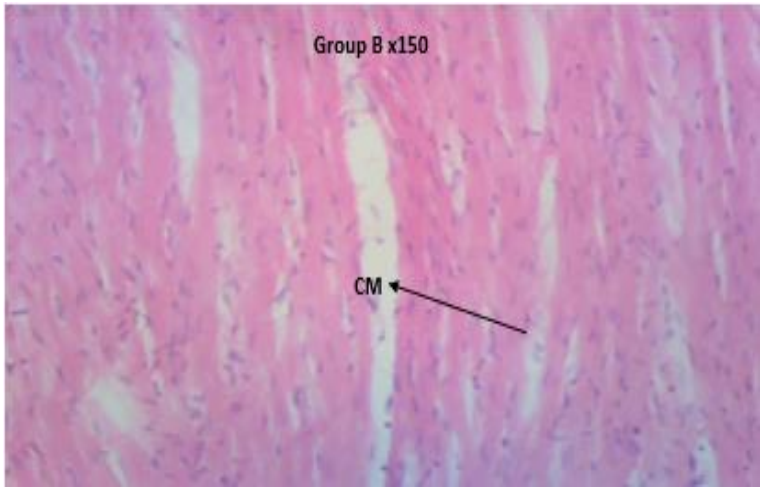

Fig 2: B: x150 Micrograph showed normal cardiac muscle (CM) and cardiac fibers (CF).

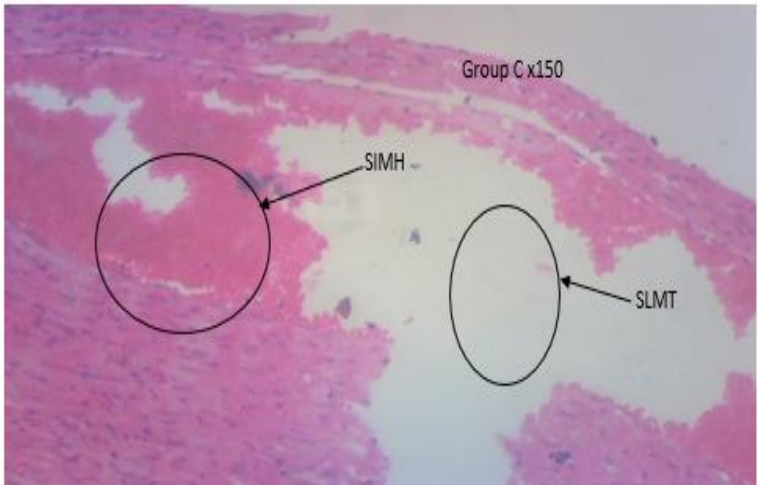

Fig 3: C: x150 Section shows extensively affected heart tissue with sever loss of myocardial tissue (SLMT), sever intra myocardial hemorrhage (SIMH) and moderate inflammation of cardiac cells (MICC).

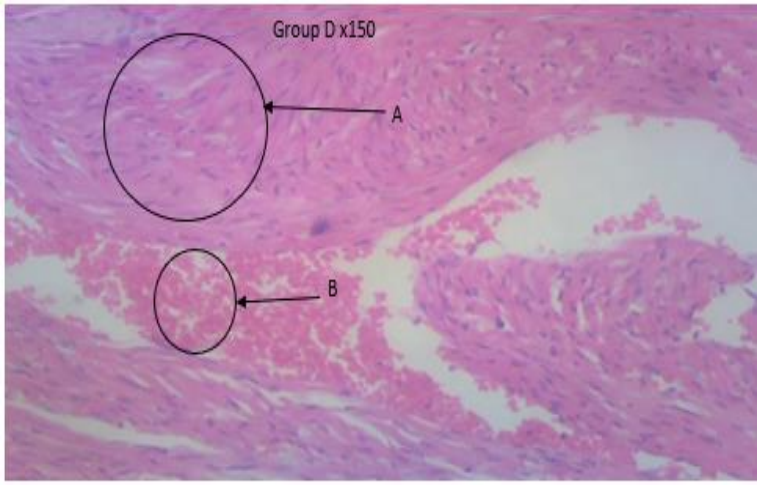

Fig 4: D: x150 Group D shows A: regeneration of cardiac tissues and B: showing reversal of hemorrhagic condition seen in group C animals.

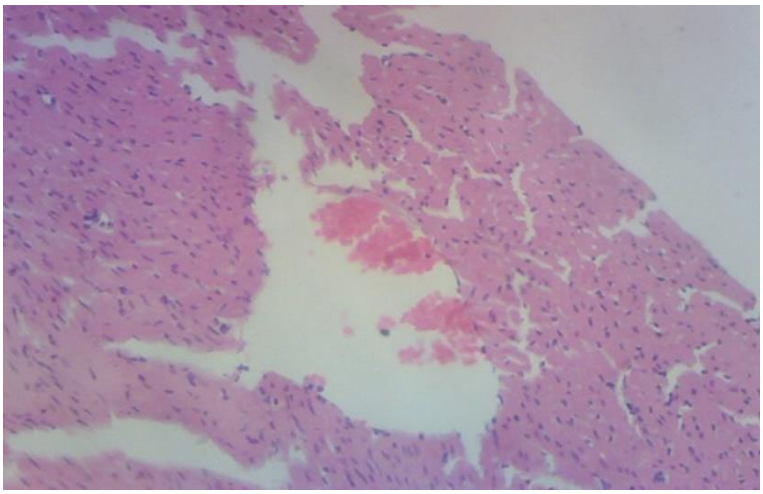

Fig 5: E: x150 Photomicrograph showing sagittal section of group E. There is regeneration of the cardiac tissues, their little or no hemorrhagic conditions but mild fatty change.

Discussion

The heart also as a muscular pump is responsible for cardiovascular supplies to the body. For the heart to meet with the demand of cardiovascular supply to the body its musculature must remain in tone for optimum function. The heart is also faced with deformation due to toxic substance that comes in contact with. Animals in group C, D and E administered CCl_4 on observation witnessed loss of balance and staggering, labored breathing and withdrawal from feeding just as reported by Ihentuge *et al* (2014). This as a result of reactions of free radicals in the CCl_4 in the liver which caused reduced metabolic activities.

The result of this study showed significance weight loss in animals administered carbon tetrachloride only (Group C), when compared with groups A and B (control group) and groups D and E which was treated with *beta vulgaris* before and after carbon tetrachloride intoxication. This result also shows a normal weight of the liver and heart of the control groups (A and B) and experimental groups of D and E which was treated with *beta vulgaris* before and after intoxication with ccl_4. There were also a significant increase in the weight of the heart in group C administered ccl_4 alone. The decrease in body weight maybe as a result of decreased food and water intake due to anorexia caused by the toxic effect of carbon tetrachloride while the increase liver weight and heart weight was as a result of fatty changes and inflammations induced by ccl_4 as reported by Ihentuge *et al* (2015).

In this study, animals in group C which were intoxicated with carbon tetrachloride only, showed noticeable fatty changes, massive infiltration of inflammatory cells, degeneration of the hepatocytes and centrilobular necrosis as histopathological changes of the liver (plate 1a and b). There was also massive deformation of cardiac tissues and occurrence of hemorrhage in the histology of group C animals (micrograph 3). This was also evident in the research by Sener *et al.* (2002) that reported increases lipid peroxidation in the Aorta and heart tissues. This change was not noticed in group B animals (plate 2a, 2b and 7) which was fed with *beta vulgaris* alone. In group D (plate 4a, 4b and 9) that was administered carbon tetrachloride and treated with beet juice, there was noticeable healing of the liver cells and cardiac cell regeneration. This is an indication that *beta vulgaris* has regenerative potentials, stopping further damage caused by carbon tetrachloride as well as repairing the damaged cells as reported in Ranju *et al* (2010) research. The group pretreated with *beta vulgaris* before injection of carbon tetrachloride (Group E) showed near normal histology of the liver and reversal of fatty and haemorrhagic conditions in the cardiac cells of the heart (plate 5a, 5b and 10). This showed that *beta vulgaris* prevented carbon tetrachloride from causing liver damage Ihentuge *et al* (2015).

The histological finding is supported by biochemical results which showed a significant increase in the serum levels of ALT, AST, and ALP in group C when compared with the control group ($p<0.05$). The level of AST in group C was

significantly higher when compared to AST levels observed in the control groups A and B. The levels of AST in group D and E fall within normal range (table 1.1). Group C animals also showed a remarkable increase in the activities of ALT and ALP levels when compared to animals in groups A and B. Groups D and E showed a remarkable reduction ALT and ALP levels as shown in table 1.1.

The findings from this study has proved that treatment with *beta vulgaris* has the ability reverse and suppress damages done to the heart by free radicals and lipid peroxidation (Thnaian, 2012).

The findings in our study could as result of the phytonutrients and antioxidant properties of *beta vulgaris*. The reversal of the tissue degeneration in groups administered ccl_4 and treated with *beta vulgaris estract* maybe as a result of it phytonutrients such as betanin, thiamine, saponines and other vitamins (A, B6 and B12) which have been reported to increase pack cell volume and haemoglobin in the body (Indhumathi and Kannikaparmeswari, 2012).

Conclusion

From this research *beta vulgaris* has proven to reverse biochemical changes in blood caused by toxic effect of ccl_4.

Beta vulgaris has proven to be a potent agent in the reversal of distortions in cytoarchitecture of the heart caused by lipid peroxidation of ccl_4.

This work has also shown that this curative effect that beta vulgaris has on the heart is as a result of its high phytonutrient content of phenolic compounds, thiamine, and carotene.

Abbreviations

BVEE	-	Beta vulgaris ethanolic extract
ALT	-	Alanine aminotransferase
ALP	-	Alkaline phosphatase
AST	-	Aspartate aminotransferase
BVE	-	Beta vulgaris extract
CCL_4	-	Carbon Tetrachloride
H&E	-	Hematoxylene and Eosin
MEBV	-	Methanolic extract of beta vulgaris

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