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INTERNATIONAL JOURNAL OF ADVANCED RESEARCH (IJAR)

Article DOI:10.21474/IJAR01/10285
DOI URL: http://dx.doi.org/10.21474/IJAR01/10285

RESEARCH ARTICLE

PROTECTIVE EFFECT OF BETA VULGARIS ON CARBON TETRACHLORIDE INDUCED HEPATOTOXICITY IN ADULT WISTAR RAT

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Manuscript Info

Manuscript History
Received: 23 November 2019
Final Accepted: 25 December 2019
Published: January 2020

Key words:-
Beta Vulgaris, Carbon Tetrachloride, Hepatotoxixty, Wistar Rats, Aspartate Aminotransferase (AST), Alanine Aminotransferase (ALT) and Alkaline Phosphatase (Alp)

Abstract

Beta vulgaris also known as beet root has been used to treat a wide range of symptoms and diseases, such as fever, constipation, cancer, diabetes, liver and heart diseases. The aim of this study was to investigate the protective ability of beta vulgaris on the liver following Carbon tetrachloride (CCl_4) intoxication. Twenty five adult albino wistar weighing between 150g and 200g were used in this study. The animals were divided into five groups A, B, C, D, and E. Groups A and B served as control were injected with paraffin oil for two consecutive days and group B fed with 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 was fed with Beta vulgaris extract throughout the experiment and injected CCl_4 on the last two days of the experimental period (28 days). At the end of the experimental period animals were reweighed and sacrificed using chloroform vapour. Blood was collected for serum enzyme analysis and liver was harvested and trimmed for histological studies. The result showed that, there was a decrease in mean body weight of 5% in group C animals while there was an increase in other groups. The liver weight were significantly higher in group C ($p<0.05$) when compared with control groups and other experimental groups. 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 were normal. Histological examination of liver (H&E) showed numerous fatty changes, distortion of hepatocytes, focal necrosis, loss of nuclei and cytoplasmic shrinkage in group C animals, groups A, B and E appear normal, while group D showed mild focal inflammation and areas of regeneration of tissues. These results shows that Beta vulgaris extract has the ability to prevent and also restore histological and biochemical changes in the liver following CCl_4 injection.

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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). These varieties have been classified as B. vulgaris subsp. vulgaris Conditiva Group (University of Melbourne achieve, 2013).

Beta vulgaris, popularly known as Beet root, is mostly cultivated in the coasts of Mediterranean, it is also extensively cultivated in Europe, America and many parts of Asia. It belongs to the family amaranthaceae (Kujala et'al., 2000).

The usually deep purple roots of beetroot are eaten boiled, roasted or raw. It is eaten alone or combined with any other salad vegetable. A large proportion of the commercial production is processed into boiled and sterilised beets or into pickles. In Eastern Europe, beet soup, such as borsch, is a popular dish. In Indian cuisine, chopped, cooked, spiced beet is a common side dish.

Medicinally, the roots and leaves of the beet have been employed as a folk remedy to treat a wide variety of ailments including, hepatotoxicity and nephrotoxicity, constipation, and diabetes (Kujala et'al, 2000). It is also employed as a special diet in the treatment of cancer (Chevallier, 1996).

Beetroot was also in the middle ages used as treatment for a variety of conditions, especially illnesses relating to digestion and the blood. Bartolomeo Platina recommended taking beetroot with garlic to nullify the effects of garlic-breath.

Carbon tetrachloride, also known by many other names (the most notable being tetrachloromethane, also recognized by the IUPAC), carbon tet in the cleaning industry, Halon-104 in firefighting and Refrigerant-10 in HVACR, is an organic compound with the chemical formula CCl_4. It was formerly widely used in fire extinguishers, as a precursor to refrigerants, and as a cleaning agent. It is a colourless liquid with a "sweet" smell that can be detected at low levels. It has practically no flammability at lower temperatures.

Carbon tetrachloride was widely used as a dry cleaning solvent, as a refrigerant, and in lava lamps (Doherty, 2000).

Carbon tetrachloride is one of the most potent hepatotoxins (toxic to the liver), so much so that is widely used in scientific research to evaluate hepatoprotective agents (Seifert et'al, 1994). Exposure to high concentrations of carbon tetrachloride (including vapor) can affect the central nervous system, degenerate the liver and kidneys and prolonged exposure may lead to coma or death. Chronic exposure to carbon tetrachloride can cause liver (Masuda, 2006; Recknagel, 1967) and kidney damage and could result in cancer (Rood et'al, 2001).

The liver is involved with almost all the biochemical pathways to growth, fight against disease, nutrient supply, energy provision and reproduction. Because of this multiple function of the liver it is always a first point of abuse from toxins. So, this study is aimed to investigate the hepatoprotective effect of beta vulgaris following carbon tetrachloride induced liver toxicity.

Materials and Methods:-

Experimental Animals:-

Twenty five adult albino wistar rats weighing between 150-200g were used for this study. The experimental animals were obtained from the animal holding of college of medicine and health sciences, Abia State University, Uturu, Nigeria. The animals were bred in wooden cages at normal room temperature ($27^oC – 30^oC$). Animals were divided into five groups containing five animals each.

The animals were fed ad libitum with water and standard guinea feed pellets obtained from the market. The animals received care in accordance with the guideline for care and use of laboratory animals, as published by the research and ethics committee of the faculty of basic medical science, Abia State University.

Preparation of Plant Extract:-
The beetroot was purchase from the vegetable section of shoprite supermarket, Polo park mall, Enugu. The beetroot juice was extracted at the histochemistry laboratory in Anatomy department, Abia state university uturu, Nigeria. Extract stored in the refrigerator at a temperature bellow 4^{o}C to maintain its freshness.

Experimental groups and protocol:-
Twenty five adult wistar rats weighing 150g to 200g were divided into five groups of four animals each. The groups was designated A, B, C, D and E. Group A served as control group and was fed with standard diet and tap water only, during the experimental period. Group B were fed with standard diet and beet juice (600ml/day), while group C was intoxicated with CCl_4 48 hours (first 2 days) consecutively and were fed with tap water and diet throughout the experimental period (21 days). Group D were intoxicated with CCl_4 on the first two days of the experimental days and then treated with beet juice for the remaining duration of the experiment. Group E were fed with beet juice and diet throughout the experiment duration and administered CCl_4 48 hours (last 2days) before sacrifice.

Induction of hepatotoxicity:-
Liver damage was induced by intraperitoneal injection of CCl_4 (1 ml kg-^{1} b.wt.), 1:1 diluted with paraffin oil, for two successive days of the experiment according to Ihentuge, et'al, 2015. Group C received 1ml kg-^{1}b.wt of CCl_4 injections on the first two days of the 21 days and was fed with tap 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 two days of the experiment and were administered beet juice (600ml/day/cage) for the remaining days of the experiment. Group E rats were administered beetroot juice (600ml/day/cage) for the duration of the experiment and injected with CCl_4 for the last two days of 21 days experimental period. Group A was injected with paraffin oil (1 ml kg-^{1} b.wt.) only and group B was administered beet juice (600ml/day/cage) for the duration of the experiment.

Samples and organ collection:-
Twenty four hours after the last administration, the animals were anaesthetized with diethyl ether. 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 minutes. The serum samples were separated into sterile plain tubes and stored in the refrigerator for analysis.

Histopathology and serum biochemical Studies:-
Following a careful and neat dissection of the animals, the organ of study (the liver) were 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 laboratory, Anatomy Department, college of Medicine and Health Sciences, Abia state university Uturu, Nigeria.

AST, ALT and ALP serum activities were measured to assess hepatotoxicity by CCl_4. The biochemical study was carried out in the Department of Anatomy, college of medicine and health sciences, Abia State University Uturu, Nigeria.

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 using mean±standard deviation using SPSS verson 15. The significance of the differences at $p<0.05$ significance level was calculated using student t-test.

Results:-

Activities of Serum Levels of Alkaline Phosphatise (Alp), Aspartate Aminotransferase (Ast), And Alanine Aminotransferase (Alt).

u/L	Group A	Group B	Group C	Group D	Group E
Aspartate Aminotransferase (Ast)	46.30±3.37	43.52±5.87	154.14±26.89[a]	50.57±2.40	65.07±5.12

Alanine Aminotransferase (Alt)	91.44±29.83	75.17±12.11	144.73±20.89 [b]	67.13±6.14	83.79±2.65
Alkaline phosphatise (alp)	134.93±9.44	127.33±9.61	359.22±105.33[c]	170.36±10.16	236.5±32.10[a]

Table 1.0:- showing serum level of activity of ASP, ALT and ALP in u/L.

From the results obtained above for the activity level of liver enzymes (AST, ALT and ALP), the levels of AST were not significantly different in Groups B and D when compared with the control group but there was a significant increase in group C (Group with carbon tetrachloride intoxication only). The levels ALT was significantly increased in Group C treated with carbon tetrachloride relative to control group and groups B, D and E. The ALP activity levels in Group C were significantly higher ($p<0.05$) than control (A) and groups D and E.

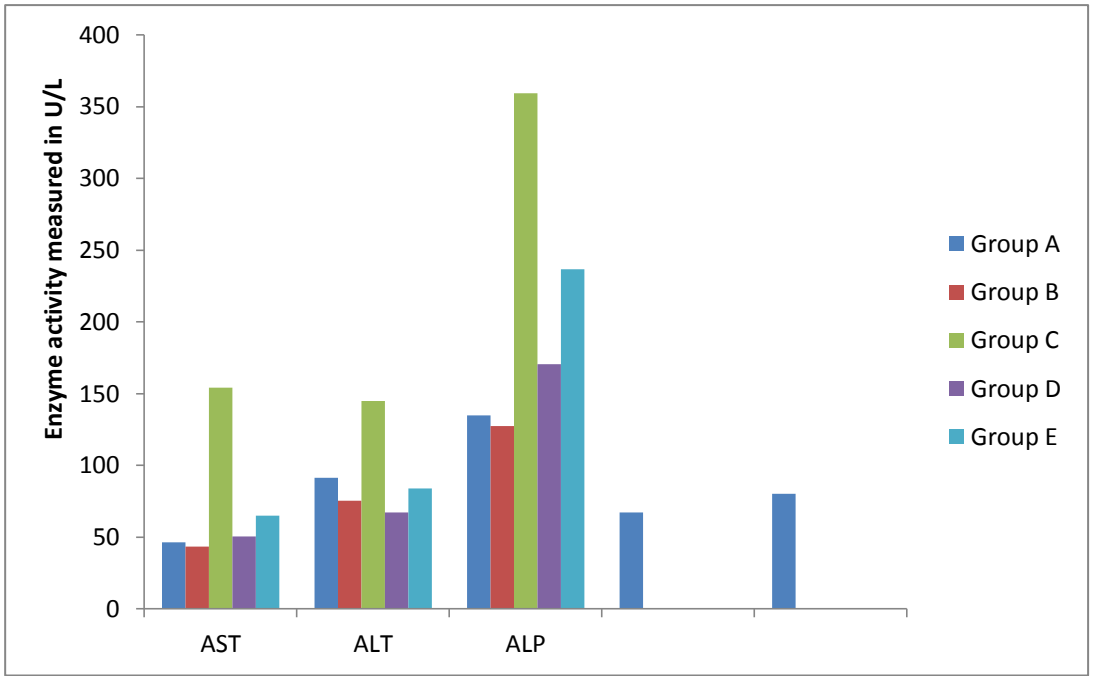


Figure 1.0:- showing the three liver enzymes activities for different groups.

The bar chat above illustrates the levels of different enzymes activities for different groups. The levels of three liver enzymes was significantly higher in group C (with carbon tetrachloride intoxication only) when compared with the control group and other groups.

Histological Findings:-

The morphology of the liver sections stained with hematoxylin and eosin (H&E). Standard techniques were evaluated to observe the extent of carbon tetrachloride induced toxicity and the regenerative or protective properties of beta vulgaris (beetroot) on the liver.

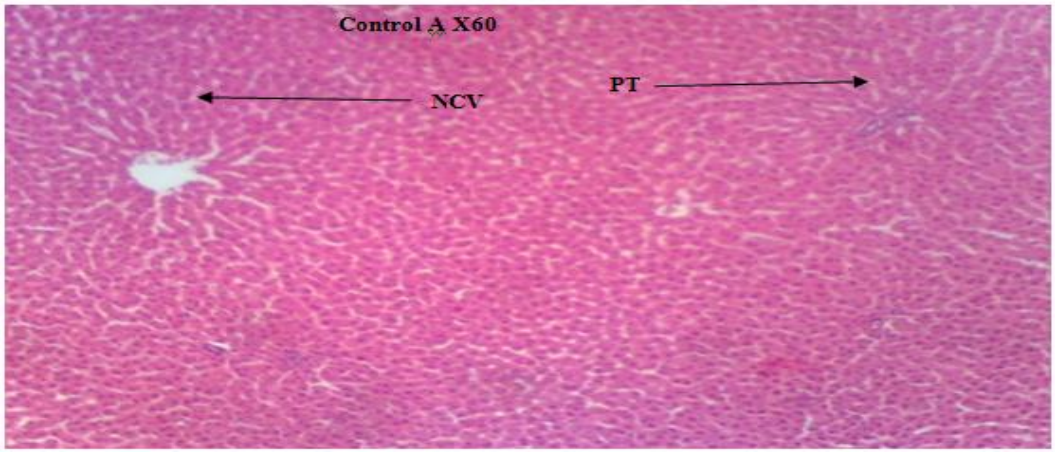
Photo plate 1a and 1b is a photomicrograph of the control group that showed normal histoarchitecture of the liver.

Photo plate 2a and 2b is a photomicrograph of the liver of the animals (Group B) that took only beta vulgaris (beetroot). This micrograph also shows healthy hepatocytes, normal central vein and portal triad.

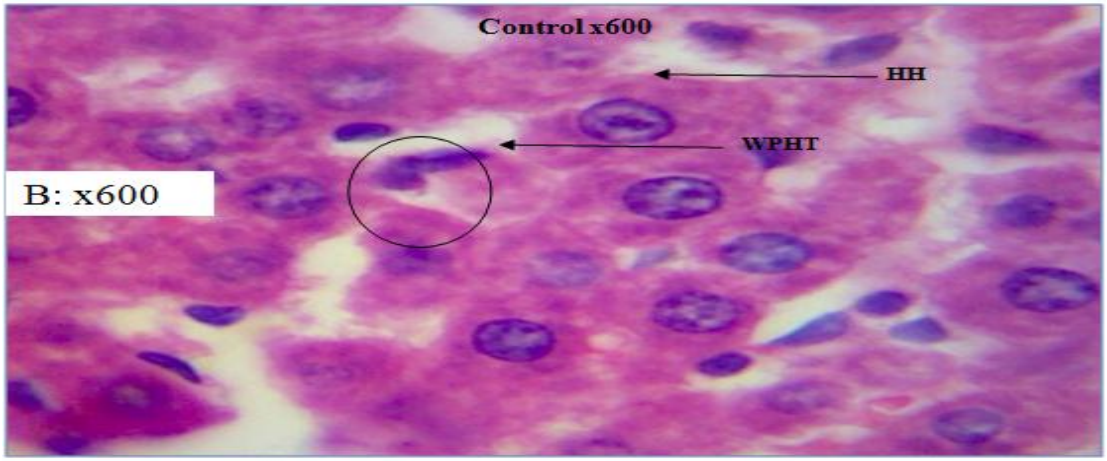
Photo plate 3a and 3b represents the animals in group C which was intoxicated with CCl_4. This group showed massive degenerative changes and tubular necrosis and degradation of hepatic tissues.

Photo plate 4a and 4b represents animals in group D with CCl_4 intoxicated rats and treated with beta vulgaris. The slide shows clear regeneration of hepatocytes and hepatic tissue appear more or less normal.

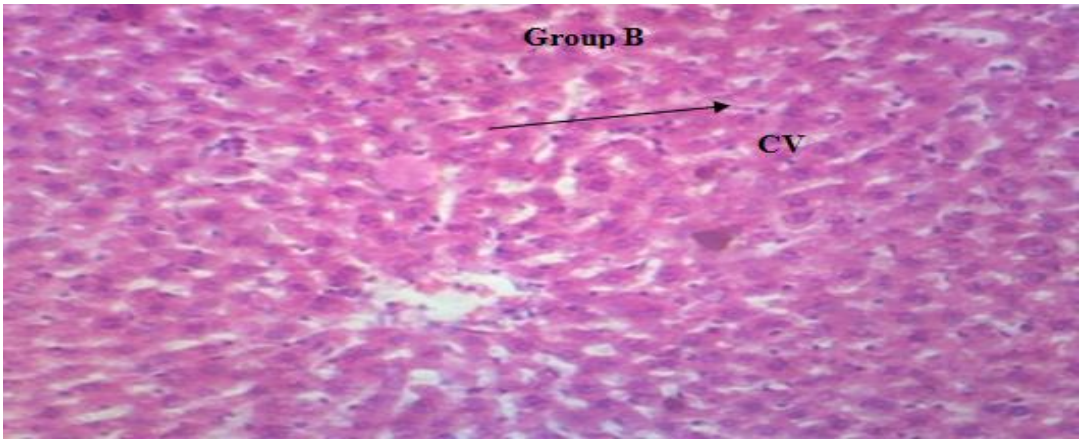
Photo plate 5a and 5b which represents group E animals that were giving beet juice for the duration of the experiment and intoxicated with CCl_4 on the last 2days shows normal hepatic tissues and portal vein and sinusoids appearing normal. However there are still areas of moderate focal area of fatty change (MFAFC) and focal aggregate of inflammatory cell (FAIC).



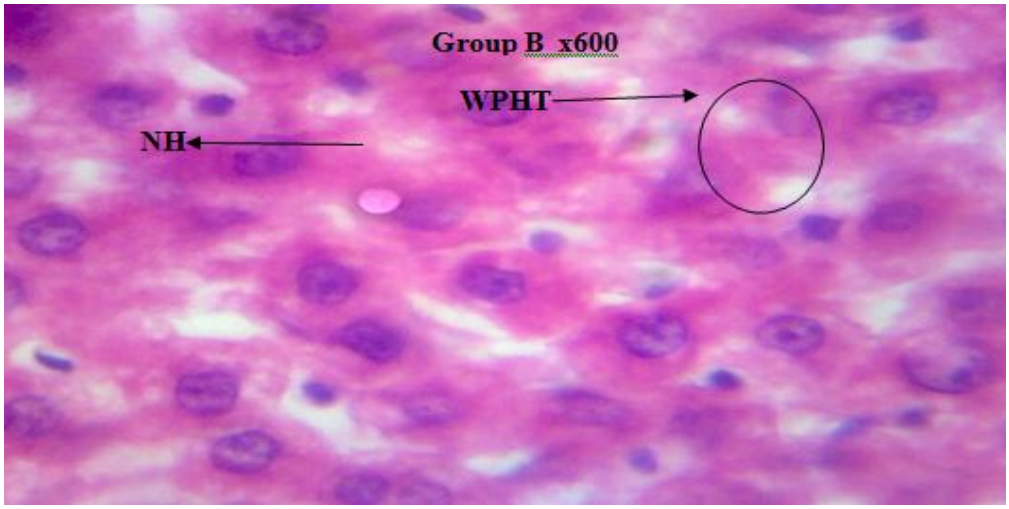
A: x60



Photomicrograph of group A control (X60 and X600) (H/E) showing well perfused normal hepatic tissue (WPHT) with normal central vain (NCV), Portal traid (PT) and healthy hepatocyte (HH).

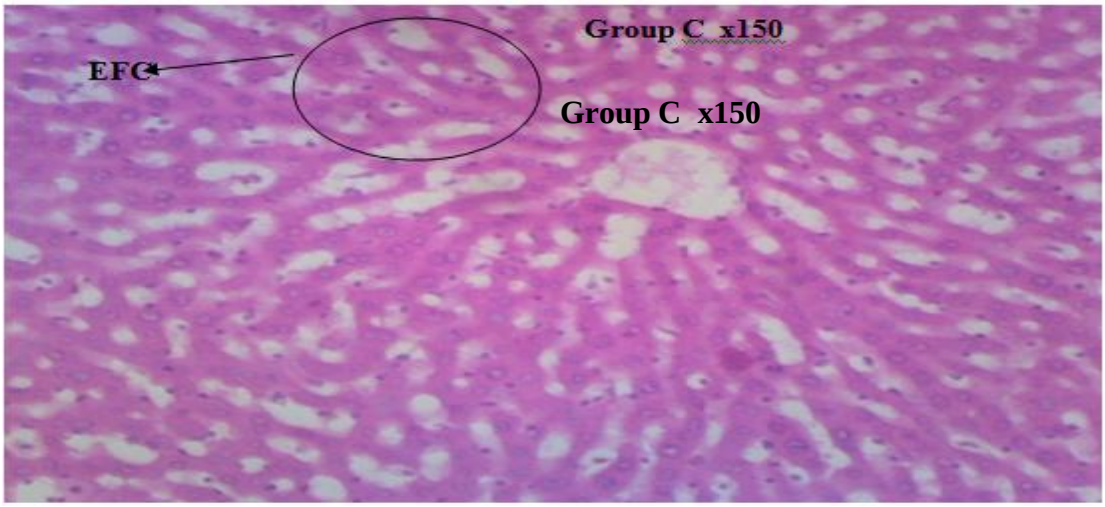


A: x60

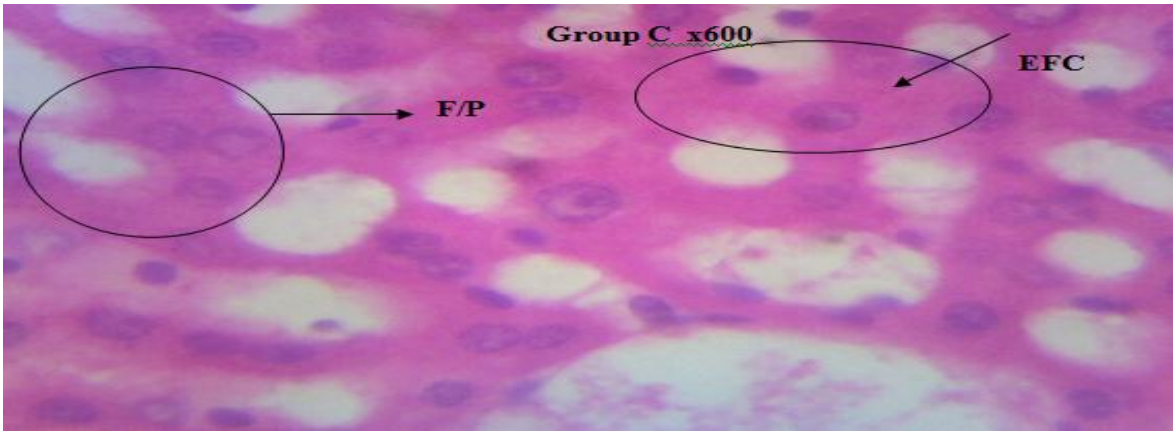


B: x600

Photomicrograph of group B (X60 and X600)(H/E) shows well perfused normal hepatic architecture with normal central vain (NCV), and normal hepatocyte (NH) as with control group A

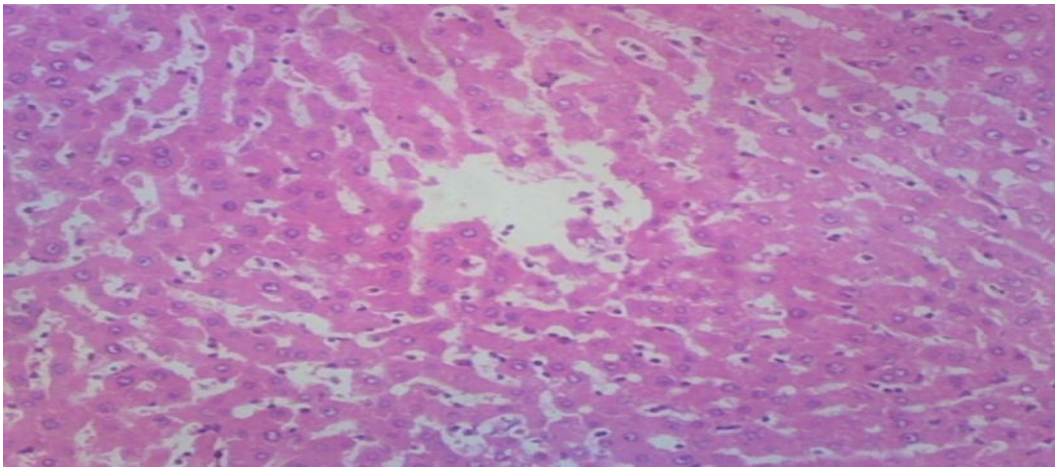


A: x60

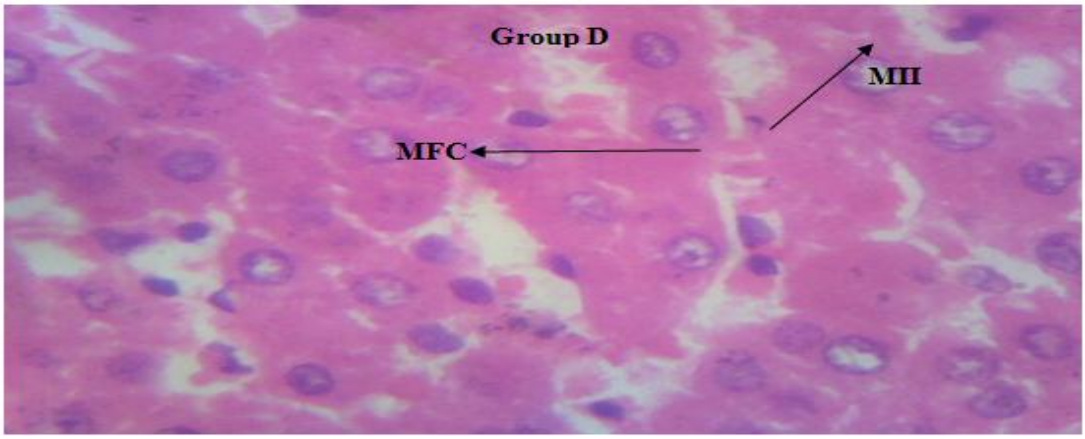


B: x600

Photomicrograph of group C (X150 and X600)(H/E) showing extensively affected hepatic tissue with extensive fatty changes (EFC), fainty and pyknotic appearance of the hepatocyte (F/P) and infilterate of inflammatory cell (IIC). There are cells at different stages of necrosis.

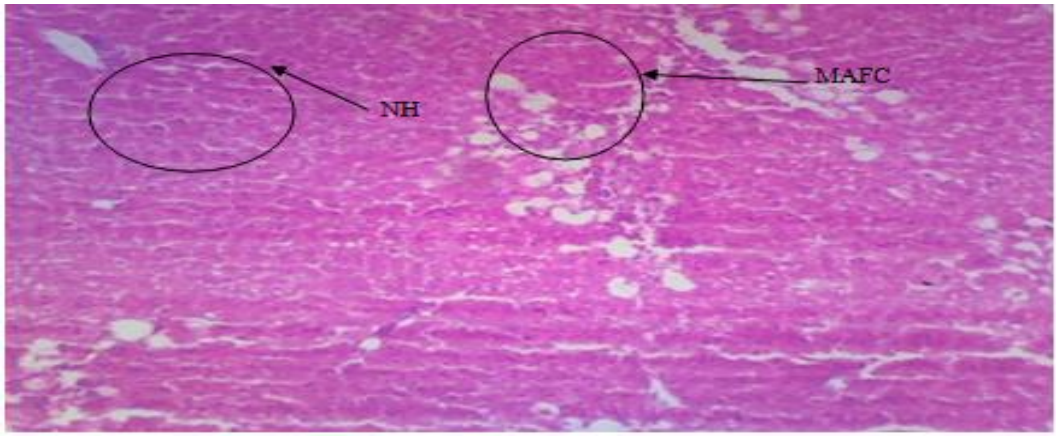


A: x150

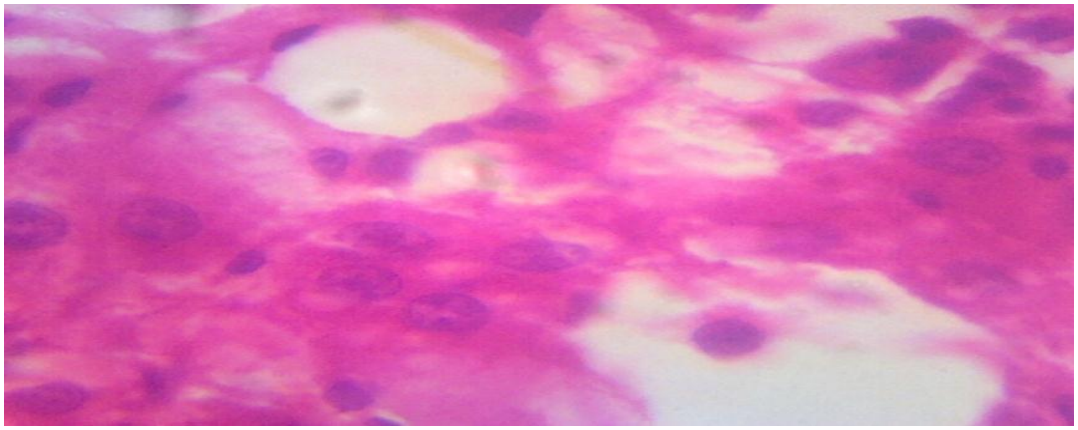


B: x600

Photomicrograph of group D (A:X150 and B:X600)(H/E) shows moderate regeneration and healing of hepatic tissue (MRHT). However there are still areas of moderate fatty change (MFC), moderate infiltrate of inflammatory cell (MIIC) this shows the healing effect of beta vulgaris.



A: x60



B: x600

Photomicrograph of group E (A: X60 and B:X600). The liver cells are well differentiated with little inflammation. There is no evidence of cellular necrosis as cells are essentially well arranged, normal hepatocytes and sinusoids are well dilated and oriented however there is a still area of moderate area of fatty change (MAFC)

Discussion:-

The hematologic testing showed significant increase in the levels of liver enzyme markers. The result as shown in table 1.0, showed a significant increase in the serum levels of ALT, AST, and ALP in group C when compared with the control group A ($p<0.05$), depicting a serious liver injury. The level of AST in group C was significantly high compared to AST levels observed in groups A and B. The levels of AST in group D and E fall within normal range (table 1.0). Group C also showed a remarkable increase in ALT and ALP serum levels compared to groups A and B. Groups D and E showed decrease ALT and ALP levels as shown in table 1.0. This shows that beta vulgaris has curative effect on the liver, combating toxins and free radicals that has deleterious effect on the liver. This study has proved that ccl_4 has the capabilities of elevating serum levels of AST, ALT and ALP. The report of hepatotoxic effect by ccl_4 are lipid peroxidation and are largely due to its active metabolite ccl_3 (that abstract hydrogen from fatty acids, initiating the lipid peroxidation), leading to cell injury (Sener et'al, 2002). Treatment with beta vulgaris has been found to significantly suppress the increase in serum AST and ALT activities induced by ccl_4 in rats (Thnaian, 2013). This study is in line with the above findings as shown in table 1.0, that there was no significance change in serum levels of ALT, AST and ALP in groups D and E which were treated with beta vulgaris when compared with the control group. This finding suggest that beta vulgaris has protective effect on the liver tissues.

The histological findings from this study, shows that 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 tissue (plate 1a and b). This change was not noticed in group B animals (plate 2a and 2b) which were beta vulgaris juice alone. In group D (plate 4a and 4b) that was administered carbon tetrachloride and treated with beet juice, there was noticeable regeneration of the liver cells. This is an indication that beta vulgaris has regenerative potentials, stopping further damage caused by carbon tetrachloride as well as repairing the damaged cells. The group pre-treated 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 liver cells (plate 5a and 5b). This showed that beta vulgaris prevented carbon tetrachloride from causing liver damage. This is in line with the findings of Ihentuge et'al (2015).

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 liver.

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