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EFFECT OF ANTIOXIDANTS AND HEPATOPROTECTIVE ACTIVITIES OF METHANOL EXTRACT OF BEET ROOT (*BETA VULGARIS* L.) AGAINST CARBON TETRACHLORIDE INDUCED HEPATOTOXICITY IN RAT MODELS

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ABSTRACT: A study on the effect of methanol extract of red beetroot (***MEBV***) against CCl_4 induced hepatotoxicity in male albino rats of Wistar strain (150 to 180g) was done. Five groups with six rats each were used as positive control, negative control and experimental with an oral dose of CCl_4 through intra-peritoneal (IP) route and ***MEBV*** at 100µgm/ml, 200µgm/ml & 300µgm/ml *per os,* respectively, for 14 days. After 14 days, animals were sacrificed; blood samples were obtained and analyzed. The results showed that CCl_4 administration was associated with triple fold increases in the activities of alanine amino transferase (ALT), aspartate amino transferase (AST) and Bilirubin ($P<0.05$) compared with the respective mean control values. The enzymatic and non-enzymatic antioxidants such as, serum catalase, GSH, Vit. E, Vit.C, & SOD, were lower than the respective mean values of the normal control. The blood parameters have got reduced. The hapatochords and hepatocytes architecture was also observed to be disturbed. However, treatment with ***MEBV*** restored the enzyme activities of the liver ALT, AST, Bilirubin, WBC, RBC, PCV and hepatic lobule architecture, to a very near normal level. This work showed that methanol extract of *Beta vulgaris* was successful at 300µgm/ml, in counteracting CCl_4 induced hepatotoxicity and amelioration of the same (hepatic remediation) to near normalcy level in rat liver functions and amelioration may be due to the total phenolics, flavonoids and other antioxidants composition in the plant extract.

INTRODUCTION: The largest exocrine gland of our body, the liver, plays vital functions in association with homeostasis of the body.

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Anabolic and catabolic pathways of nutrients that we consume and de-toxification of ingested food based chemicals are taken care by our liver. The variety of ingested chemicals induces liver injury by mostly causing oxidative stress in hepatic tissue and accounts for numerous diseases, including cancer.

Considering the fact of widespread and casual abuse of liver, like environmental toxins, prescription and over-the-counter drug use, which

lead to hepatitis, cirrhosis and liver disease, more research light is thrown in the use of antioxidants for prevention and/or amelioration of hepatic injury [1]. This amelioration process is often referred to as "The chemoprevention", and a large body of evidence from various experiments has supported its efficiency [2].

Carbon tetrachloride (CCl_4) is one of the most potent hepatotoxins and is widely used in scientific research to evaluate hepatoprotective agents [3]. The hepatotoxic effect of CCl_4 is largely due to its active metabolite, trichloromethyl free radical (CCl_3 – and / or CCl_3OO^-) by chronic or acute vehicles [4]. The regular exposure to hepatotoxic substances causes hepatocellular lipid accumulation (steatosis), hepato-cellular necrosis or hepatobiliary dysfunction[5]. Cirrhotic or neoplastic changes are usually considered as a result of chronic exposures. Peroxidation was first proposed as mechanism of carbon tetrachloride induced liver injury.

The CCl_4 is shown to induce hepatocellular carcinomas in rodents by oral, inhalation, and parenteral exposure [3]. The hepatotoxic effect of CCl_4 is a result of its reductive dehalogenation by CYP2E1 enzyme into the highly reactive trichloromethyl and trichloromethyl peroxy free radicals**.** Both radicals form adducts with cellular macromolecules and abstract hydrogen from different molecules, thus initiating oxidation of lipids, proteins and DNA. These observations are consistent with the hypothesis that toxicants-induced hepatocellular injury results in the influx of Ca^{2+} (calcium ion) into the cell, initiating a series of cyto-toxic events common to various hepato-toxicants and resulting in cell death.

Several drugs and chemicals inhibit hepatocyte antioxidant defines against lipids peroxidation, thus increasing the severity and duration of peroxide damage [6].

Enzyme substances of liver like ALT (SGPT), AST (SGOT), and Bilirubin are also present in blood serum in very low concentration. These enzymes provide information about hepatic health [7]. Liver diseases were among the first disorders to which serum test were applied and have proved to be useful in diagnostic purposes.

The red beetroot, (*Beta vulgaris* L.), a flowering plant of Amaranthaceae family, is widely available vegetable and is commonly consumed. It is reported to have water-soluble, non-phenolics antioxidants, the betalins, red betacyanins (principally betanin) and yellow betaxanthines, in its consumable leaves and roots [8]. The antioxidant effects of betalains have been demonstrated mainly in various in vitro experiments. As very few investigations on beetroot activity have been performed in vivo, the present study is aimed at an evaluation of methanolic extract of *Beta vulgaris* ***(MEBV)*** and its anti-oxidants in ameliorating the induced hepatotoxicity to CCl_4 exposure and circulating liver marker enzyme levels and proteins using Wistar albino rat models.

MATERIALS AND METHODS:

Chemicals and reagents: CCl_4 , 3-(4,5-dimethyl thiazol-2yl)-2,5-diphenyl tetrazolium bromide (MTT), 2,2-diphenyl-1picrylhydrazyl (DPPH), Anthrone Reagent, Folin-Ciocalteau Reagent, Ninhydrin, thiobarbituric acid (TBA) and 5,5'-dithiobis-2-nitrobenzoic acid (DTNB), Methanol, 2,4- dinitrophenyl hydrazine-thiourea-CuSO4 reagent, and isobutyl alcohol were procured from Sigma-Aldrich Chemicals (Bangalore). All other chemicals and estimation kits used were of analytical grade and purchased from commercial sources.

Plant Material: Beetroot (*Beta vulgaris* L.) were purchased from a local market, Vellore, Tamil Nadu, India and the plants were authenticated by the Botany Department, Govt. Arts and Science College, Thiruvannamalai (Thiruvalluvar University), Tamil Nadu, India. The plant materials were washed in tap water, shade dried and powdered.

Extraction and fractionation: The powdered root material (800 g) was successively extracted with 95% methanol using Soxhlet extractor. The marc left after the methanol extraction was macerated with distilled water for 24 h. The solvents were distilled off under reduced pressure below 45ºC to afford *Beta vulgaris* methanol extract (***MEBV***, 12.7% w/w).

Preliminary phytochemicals screening: Preliminary phytochemicals analysis was performed to identify the nature of phyto-constituents in the methanol extract of beetroot (*Beta vulgaris* L.) [9].

1. **Antioxidant enzymes assay (DPPH scavenging assay):** The free radical scavenging activity of methanol extract *of Beta vulgaris (MEBV)* was measured ***in vitro*** by 2, 2-diphenyl-1-picrylhydrazyl (DPPH) method [10]. The extract of the plant was prepared at various concentrations (0.8, 4, 20 and 100 µg/ml) and added to a solution of 1.5 x 10^{-4}m DPPH (sigma, Bangalore) in methanol and the reaction mixture was shaken vigorously. The amount of DPPH remaining was determined at 520nm and the radical scavenging activity was obtained from the equation. The absorbance (Abs) was taken at 520 nm. Quercetin, a known antioxidant was used as positive control. The amount of DPPH remaining was determined at 520nm and the radical scavenging activity was obtained from the equation.

Radical Scavenging activity (%)

$$= \frac{\text{OD control} - \text{OD sample}}{\text{OD control}} \times 100$$

2. **Determination of total phenolics and flavonoids content:** Total phenolics assay by Folin-Ciocalteu reagent method is routinely employed in studying phenolics antioxidants [11]. The total phenolics and flavonoids content of plant extracts were determined as per Folin-Ciocalteau assay method [12]. The total phenolics content was expressed as milligrams of gallic acid equivalents/g extract (mg GAE/g of dry mass) and the total flavonoids content was expressed in milligrams of quercetin equivalents/g of extract (mg QE/g of dry mass).

Experimental animals: The experimental animals used for the study were male Wistar rats *Rattus norvegicus*, weighing (160–180 g) and procured from Kings Institute of Preventive Medicine, Guindy, Chennai, and fed with standard rat chow (Amrut Laboratory Animal Feed, Bangalore, India) and water *ad libitum*. Forty eight animals were used for the experiment and maintained as per the Committee for the purpose of control and supervision of Experiments on Animals (CPCSEA) guidelines.

Animals were acclimatized for one week prior to experiment. The animals were maintained under standard laboratory conditions of temperature (25 ± 2%) and humidity (55 ± 5%) with 12 h light- dark cycle.

1. **Determination of LD_{50} of CCl_4 for Male Wistar rats:** The acute oral toxicity studies of *Beta vulgaris* extract was carried out as per OECD guidelines. The rats were orally fed with different doses of CCl_4 and the LD_{50} value was calculated as per the method of OECD – 423 guidelines and was found to be 2400ml/kg body weight for a period of 14 days (OECD – 423, 2001). The rats were treated with 1.9ml/kg of CCl_4 daily intra-peritoneal (IP) to induce hepatotoxicity [13].

In vivo **hepatoprotective activity:** The rats were randomly divided into five groups, each consisting of five rats and treated as follows:

- Group I fed with normal diet and distilled water (served as positive control).
- Group II received CCl_4 100mM (IP), (served as negative control)
- Group III received CCl_4 100mM (IP) and 100µgm/ml (***MEBV***) *p.o.,*
- Group IV received CCl_4 100mM (IP) and 200µgm/ml (***MEBV***) *p.o.,*
- Group V received CCl_4 100mM (IP) and 300µgm/ml (***MEBV***) *p.o.,*

The rats were maintained in the above condition for 14 days and on the 15th day, the rats were anesthetized and sacrificed. Then the blood and liver samples were collected, processed, analyzed for various biochemical factors and antioxidant enzymes in the blood samples and they were duly reported.

Liver marker enzymes and Blood sampling assay: Activities of Alanine transaminase (ALT) or Serum Glutamic Pyruvic Transaminase (SGPT) and Aspartate aminotrasferase (AST) or Serum Glutamic Oxaloacetic Transaminase (SGOT) and Bilirubin levels were determined in serum proteins for the study [7].

However, the level of tissue toxicity is tested as Level of lipid peroxidation (LPO) and expressed in terms of GPx (Glutathione peroxidase), Glutathione (GSH) Superoxide dismutase (SOD) and catalase (CAT) [14]. They were determined by the standard methods to assess oxidative stress, for the *'in vivo'* subjects.

The blood samples were also analyzed for the red blood cells count, white blood cells count, packed cell volume (PCV %) also known Hematocrit (HCT) as per standard methods [14]. The whole blood samples were collected into heparinized capillary tubes, filled up to 2/3 the length, sealed and centrifuged at 3,000rpm in a haematocrit centrifuge for 10 minutes. Packed cell volume was determined using a haematocrit reader and PCV was expressed as percentage erythrocytes blood contained and the haemoglobin concentration was estimated as per using the formula.

Hb concentration = ⅓ x PCV concentration

Histopathological Examination: Following standard procedure, the histopathological studies were done [15]. For the observations at the light microscopic level, fresh tissue pieces of liver were fixed in Bouin's fluid. Following two days of fixation, the specimens were washed and dehydrated through an ascending series of ethanol (70 – 100%).

Then, they were cleaned with xylene and embedded in paraffin wax, then sectioned at 5µm thickness using a rotary microtome. Sections were rehydrated in distilled water and stained with Hematoxylin-Eosin (H&E) and then examined under light microscopy.

Statistical analysis: The results were expressed as mean ± SE (n=6) and analyzed by one-way analysis variance (ANOVA) [16]. The significant was considered to be as, $p < 0.05$ vs control.

RESULTS:

Preliminary phytochemicals screening: Screening plant extracts for their antioxidant potency in order to identify their ability in scavenging free radicals (ROS) and binding with metal ions of oxidative reactions is considered as an important step prior to the isolation of antioxidant phytochemicals [17].

In the preliminary phytochemicals analysis, methanol extract of *Beta vulgaris* showed the presence of various phytoconstituents **(Table 1 & Fig. 1)**. It was observed that, carbohydrate, protein, fat, total amino acid content and proline content are moderately high. The carbohydrates were 41.3g/ 100g, protein was 14.3g/100g, fat was 1.79g/100g and total amino acid content was (15.9 g/100g), indicating the phytoconstituents were moderately available in beetroot.

TABLE 1: BIOCHEMICAL FACTORS OF *BETA VULGARIS* (g/100g)

Factors	*Beta vulgaris*
Carbohydrates	41.3 (g/100g)
Protein	14.3 (g/100g)
Fat	1.79 (g/100g)
Total amino acid	15.9 (g/100g)

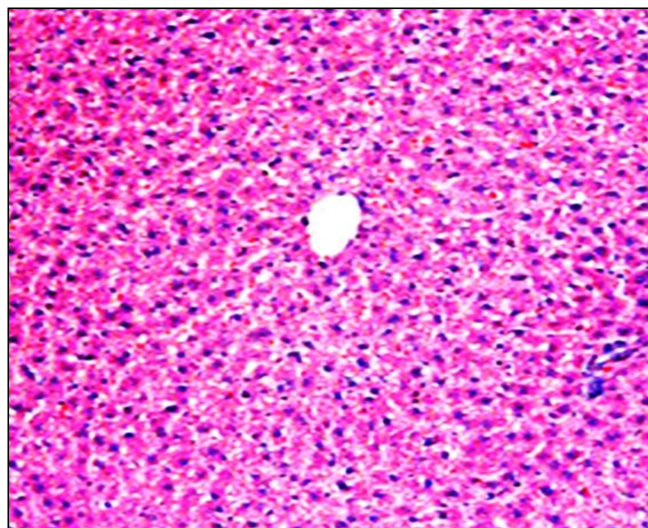

FIG. 1: LIGHT MICROGRAPH OF A HE STAINED LIVER OF A POSITIVE CONTROL RAT ×300

Table 2 represents the Vitamins and Minerals Factors of the leafy parts of the plant *Beta vulgaris*. All the non – enzymatic antioxidants such as Total phenolics, β- Carotene, Ascorbic acid, Thiamine and Flavonoids were found to increase in high content.

Vit. C is measured to be at 441mg/100g, sodium at 454mg/100gm, calcium at 385mg/100g, Iron 11.6mg/100g, total ash at 4.8g/100g, β- Carotene and Thiamine at 32.6 and 0.28 μg/100g, respectively. The metal elements and vitamins have impact on various physiological routes involving non-enzymatic antioxidants [18].

Effect of *MEBV* on Antioxidant Activity (DPPH assay): The DPPH assay is the widely used screening method for antioxidant activity of plant extracts [19]. DPPH is relatively stable, nitrogen-centered free radical which produces violet color in ethanol solution. It will be reduced to a yellow colored product, diphenylpicryl hydrazine, with the addition of the plant extracts in a concentration dependent manner. The reduction in the number of DPPH molecules can be correlated with the number of available hydroxyl groups. The Detoxification of excess Reactive Oxygen Species (ROS) produced during stress is important as it may induce membrane lipid peroxidation, enzyme inhibition and nucleic acid damage [20].

TABLE 2: VITAMINS AND MINERALS FACTORS OF *BETA VULGARIS*

Factors	*Beta vulgaris*
beta Carotene	32.6 (μg/100g)
Ascorbic acid	*441 (mg/100g)*
Thiamine	0.28 (μg/100g)
Total Ash	4.8 (g/100g)
K	*524 (mg/100g)*
Na	*454 (mg/100g)*
Fe	11.6 (mg/100g)
Ca	*385 (mg/100g)*

Table 3 shows the antioxidant properties which include the (1, 1-diphenyl-2- picrylhydrazyl) DPPH assay, the antioxidant enzymes such as catalase and superoxide dismutase (SOD) and the non-enzymatic antioxidant glutathione. The DPPH assay shows that ***MEBV*** has a 67% antioxidant activity. Similarly the other antioxidant enzymes such as SOD, GSH and Catalase showed decent levels of content [***MEBV*** = SOD - 214μgm/ minute/ mg protein; the catalase activity was 56.9μmol/min/g; and *Glutathione **of MEBV*** was 56 mg/g;] the extract showed significant inhibition percentage (stronger hydrogen donating ability) and may be positively correlated with total phenolics content (TPC) [10].

TABLE 3: ANTIOXIDANTS ACTIVITY, TOTAL PHENOLICS AND FLAVONOIDS CONTENTS OF METHANOL EXTRACT OF *B. VULGARIS*

Factors	*MEBV*
AOA	67 (%)
SOD	214± 1.01 (mgm / minute / mg protein)
Catalase	56.9± 1.01 (μ/mol/min/g)
GSH	56± 1.01 (mg/g)
Total phenolics Content	26.47 ± 1.05 (mg GAE/g dry extract)
Total flavonoids content	15.7 ± 0.01 (mg QE/g dry extract)

GAE = Gallic acid equivalent; QE = Quercetin equivalent

Effect of *MEBV* on total phenolics (TPC) and flavonoids contents (TFC): The phytochemicals analysis showed the presence of total phenolics content (TPC) as 26.47 mg GAE/g and total flavonoids content (TFC) as 15.7 QE/g in ***MEBV***. Flavonoids are natural polyphenolic molecules common to most flowering plants. They include flavonols, flavones, flavanones, isoflavones, catechins, anthocyanidins and chalcones. Although not considered vitamins and flavonoids have a number of nutritional functions have been described as biological response modifiers. Most of them act as antioxidants and some have anti-inflammatory properties [21].

(Table 3) Antioxidants activity, total phenolics and flavonoids contents of methanol extract of *B. vulgaris*

Effect of *MEBV* on the liver function analysis: It was observed that, the level of ALT (SGPT) by CCl_4 was at 101 ±1.11 IU/L is considerably reduced to 41 ±1.02 IU/L at 300μgm/ml ***MEBV***, which is close to control value of 39.5 ±1.11 IU/L. (**Table 4**). Similarly the elevated level of AST (SGOT) by CCl_4 (1288±1.02 IU/L) is effectively reduced to 409 ±1.02 IU/L by ***MEBV***, at 300μgm/ml, thus reducing the liver marker enzymes near normal to the control 359.5 ±1.01 IU/L.

The elevated bilirubin level of 0.94mg/dL due to CCl_4 toxicity is reportedly reduced by ***MEBV*** at 300μgm/ml, to 0.58mg/dL which is almost close to the normal control value of 0.48mg/dL.

TABLE 4: EFFECT OF METHANOL EXTRACT OF *B. VULGARIS* (L.) ROOT (*MEBV*) ON ALT, GOT AND BILIRUBIN IN CCl_4-INDUCED HEPATOTOXICITY IN RATS

Groups	ALT (SGPT)	AST (SGOT)	Bilirubin
Group-I	39.5 ±1.11 (IU/L)	359 ±1.01 (IU/L)	0.48 ±1.01 (mg/dL)
Group-II	101 ±1.11 (IU/L)	1288 ±1.02 (IU/L)	0.94 ±1.01 (mg/dL)
Group-III	81 ±1.02 (IU/L)	855 ±1.02 (IU/L)	0.87 ±1.02 (mg/dL)
Group-IV	51 ±1.02 (IU/L)	521 ±1.02 (IU/L)	0.64 ±1.02 (mg/dL)
Group-V	41±1.02 (IU/L)	409±1.02 (IU/L)	0.58±1.02 (mg/dL)

Each value represents the mean ± SE (n = 6); Significance p≤0.05 vs. Control

Effect of *MEBV* on the level of non-enzymatic antioxidants: The level of non-enzymatic antioxidants (SOD, CAT and GPx, GSH, respectively) as observed in CCl_4 control group rats **(Table 5),** were significantly ($p<0.05$) low, indicating the rise of ROS group and significant damage to the antioxidant system. However, the ***MEBV*** treated groups, recorded with rising values close to the normal control levels, indicating remarkable antioxidant effect. Inactivation, detoxification, removal of ROS and other free radicals depend on enzymatic and non-enzymatic antioxidants. The important enzymatic antioxidants in the tissues were SOD, CAT and glutathione peroxidase (GPx). These antioxidants together with GSH act to prevent the formation of free radicals and thereby prevent oxidative stress [22].

TABLE 5: EFFECT OF METHANOL EXTRACT OF *B. VULGARIS* (L.) ROOT (*MEBV*) ON ENZYMATIC AND NON-ENZYMATIC ANTIOXIDANTS IN CCl_4-INDUCED HEPATOTOXICITY IN RATS

Groups	GPx	GSH	SOD	CAT
Group-I	5.01±1.02 (µgm/min/mg)	4.55±0.11 (U/min/mg)	7.88±1.05 (U/min/mg)	90.5±1.05 (U/min/mg)
Group-II	2.08±1.02 (µgm/min/mg)	1.42±1.05 (U/min/mg)	4.1±1.01 (U/min/mg)	42±1.04 (U/min/mg)
Group-III	3.6±1.02 (µgm/min/mg)	2.9±0.11 (U/min/mg)	6.4±1.01 (U/min/mg)	59±1.02 (U/min/mg)
Group-IV	4.45±1.11 (µgm/min/mg)	3.55±1.05 (U/min/mg)	6.9±1.02 (U/min/mg)	79.02±1.06 (U/min/mg)
Group-V	5.32±1.11 (µgm/min/mg)	4.7±1.05 (U/min/mg)	7.47±1.04 (U/min/mg)	84.72±1.08 (U/min/mg)

Each value represents the mean ± SE (n = 6); Significance p≤0.05 vs. Control

Reduction in the enzyme activities towards the respective normal values by ***MEBV*** at different dose levels (100, 200 and 300 mg/kg) is an indication of stabilization of plasma membranes and repair of liver tissue damage. The first line of defense against ROS is, displayed by the antioxidative enzymes, such as SOD and CAT. The GSH, a non-enzymatic antioxidant, plays a pivotal role in protecting against organ toxicity. These antioxidants mainly convert active oxygen molecules into non-toxic compounds. It was observed that, Glutathione peroxidase (Gpx), Glutathione (GSH), Superoxide dismutase (SOD) and Catalase (CAT) levels of liver protein, which was low in the CCl_4 diseased rats. And it may be due to the accumulation of superoxide radicals and hydrogen peroxide [23]. Upon exposure to methanol extract of *Beta vulgaris* ***(MEBV)***, in this study, they have got improved to near normal levels as shown by the control. (The values at 300µgm/ml are given as per below;) the GPx of ***MEBV*** was 5.32±1.11, against CCl_4 impacted value of 2.08±1.02µgm/ml; and the values were close to the control value of 5.01±1.02 µgm/ml. The GSH of ***MEBV*** = 4.7±1.05mg/mg, against CCl_4 =1.42±1.05mg/mg; and the value was near the normal value of 4.55±0.11mg/mg; The value for SOD of ***MEBV*** was 7.47±1.04u/min/mg, as against CCl_4 4.1±1.01u/min/mg; whereas the value of control was 7.88±1.02µgm/ml; and the Catalase (CAT) levels of ***MEBV*** at 84.72±1.08 micromol/mg, as against CCl_4 exposed rats level of 42±1.04 micromol /mg liver protein and the value of the control group was 90.5±1.05 micromol/mg.

Glutathione is a tripeptide composed of three different amino acids, glutamate, cysteine and glycine that has numerous important functions within cells. Glutathione contains an unusual peptide linkage between the amine group of cysteine and the carboxyl group of the glutamate side chain. Glutathione is involved in various liver detoxification processes. Glutathione serves as a reductant is conjugated to drugs to make them more

water soluble is involved in amino acid transport across cell membranes (the g-glutamyl cycle) is a part of the peptidoleukotrienes, serves as a cofactor for some enzymatic reactions and as an aid in the rearrangement of protein disulfide bonds [24].

Effect of *MEBV* on the blood parameters: As summarized in **table 6**, injection of CCl_4 in rats with significant ($P \leq 0.05$) reduction of WBC, RBCs counts, Hb content as compared with control. Oral administration of ***MEBV*** for 14 days along with CCl_4 ameliorated the suppressive effect of CCl_4 in group III, IV and V at 300µgm/ml when compared with 100µgm/ml and 200µgm/ml. The amelioration by ***MEBV*** is reported to the near normal values that were exhibited in control group. The WBC count of 12.2 thousand in a unit blood was raised to the level of 21.1 thousands which was very close to the level of control (group-I) at 22.1 thousands per unit. Similarly the RBC count is raised from 5.7 million to 8.3 million which is close to the control values of 8.7 million per unit blood. The haemoglobin content in group-V was raised to 13.1 g/dL from 8.2 g/dL of CCl_4 group-II which was close to 14.2 g/dL the value of the control. The packed cell volume too have bettered in group-V (43.1%) when compared with group-II (30%) which is near normal value of 47% in group-I.

TABLE 6: EFFECT OF METHANOL EXTRACT OF *B. VULGARIS* L ROOT (*MEBV*) ON THE BLOOD PARAMETERS OF THE CCl_4 INDUCED HEPATOTOXIC LIVER OF THE RAT

Parameters	WBC	RBC	Hb	HCT (PCV)
Group I	22.1±1.02 (*103 µL)	8.7±1.01 (*106 µL)	14.2±0.22 (g/dL)	47±1.12 (%)
Group II	12.2±1.02 (*103 µL)	5.7±1.01 (*106 µL)	8.2±0.22 (g/dL)	30±1.01 (%)
Group III	15.9±1.02 (*103 µL)	7.2±1.01 (*106 µL)	10.6±0.21 (g/dL)	37.5±1.02 (%)
Group IV	17.2±1.02 (*103 µL)	7.8±1.02 (*106 µL)	12.0±1.21 (g/dL)	39.7±1.02 (%)
Group V	21.1±1.12 (*103 µL)	8.3±1.02 (*106 µL)	13.1±1.21 (g/dL)	43.1±1.11 (%)

Each value represents the mean ± SE (n = 6); Significance $p \leq 0.05$ vs. Control

Histopathological findings: Oral adminstration of methanol extract of *Beta vulgaris* for 14 days in addition to 1.9ml/kg b.w of CCl_4 intraperitoneal route did offer remarkable hepatic remediation at 300µgm/ml level and restored the hepatic architecture close to the positive control group of rats [14]. However, the CCl_4 injection to negative control group revealed that, big areas of hepatic necrosis more or less in centro-lobular position and showed inflammation towards peripheral area (**Fig. 2**).

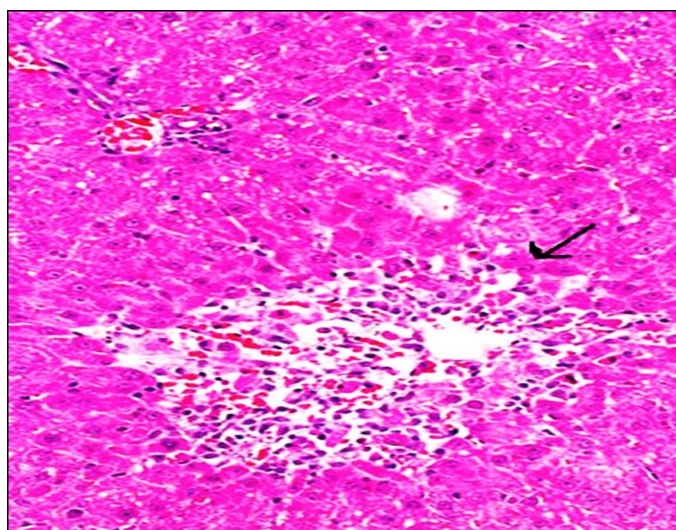

FIG. 2: LIGHT MICROGRAPH OF A HE STAINED LIVER OF A POSITIVE CONTROL RAT × 100

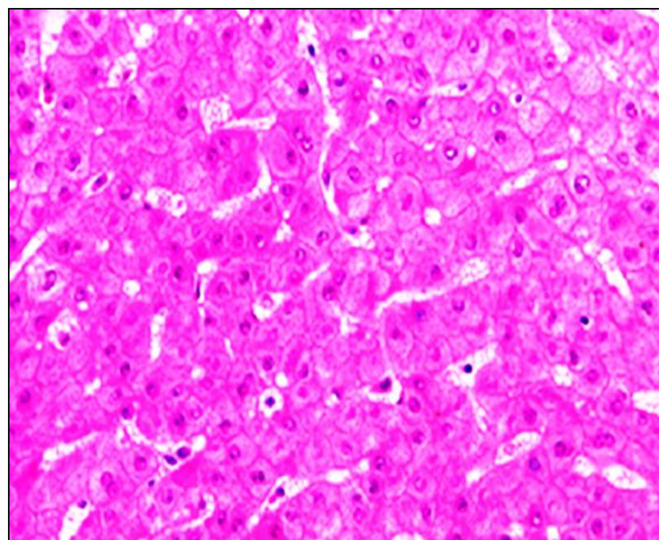

FIG. 3: LIGHT MICROGRAPH OF A HE STAINED LIVER OF CCL_4 INTOXICATED RAT (NEGATIVE CONTROL)

Fig. 2 shows hexagonal or pentagonal lobules with central veins and peripheral hepatic triads or tetrads embedded in connective tissue. Hepatocytes are regular and contain a large spheroidal nucleus with a distinctly marked nucleolus and peripheral chromatin distribution. **Fig. 3** show most hepatic lobules, the trabecular structure is lightly blurred and, in the remaining lobules, distinctly blurred. The cytoplasm of some cells shows rare empty vacuole-type spaces. ×300.

Also there was dilation of blood sinusoids, single cell necrosis which affected most of the cell cord.

The above results support the view on stabilization of plasma membranes of hepatocytes and thus reduced ROS levels by the activity of ***MEBV***. Previous phytochemicals investigations on *Beta vulgaris* reported the presence of flavonoids and phenolics[18, 25]. The antioxidant activity, flavonoids and phenolics may be responsible for hepatoprotective activity [26].

DISCUSSION: The CCl_4, a well-known hepato-toxin, has been widely used to screen new hepatoprotective agents. At cellular metabolism, this xenobiotic chemical is rapidly transformed by cytochrome P450 2E1 into a trichloromethyl radical which is converted into a peroxyl radical (ROO*) in the presence of oxygen. Similarly, superoxide radical (SO') and hydroxyl radicals (OH") are also produced as a part of normal metabolic processes. These radicals may interact with cellular macromolecules and initiate the peroxidative degradation of lipid membranes. Enhanced production of reactive oxygen species (ROS) and pro-inflammatory cytokines by activated Kupffer cells is also involved in liver damage initiated by CCl_4-derived radicals. As CCl_4 intoxication is known for the release of ROS and thus oxidative stress it leads to diverse pathological issues [27].

Generally the oxidative stress and cell damages are correlated with increased level of thiobarbituric reactive substances (TBA) and decrease in packed cell volume (PCV) due to destruction of erythrocytes (RBCs) [14]. The packed cell volume (PCV) and haemoglobin concentration of CCl_4 control group was reportedly decreased significantly ($p<0.05$), indicating that there might have been possible destruction of erythrocytes by CCl_4 [28]. This observation agrees with the report that CCl_4 toxicity can lead to destruction of hepatocytes and erythrocytes [14].

The elevated levels of ALT (SGPT), AST (SGOT) and Bilirubin due to CCl_4 intoxication were moderately ($p<0.05$) reduced with ***MEBV*** treatment when compared with CCl_4 control rats. Maximum activity was found with 300µgm/ml dose of ***MEBV***.

Aminotransferases (AST or SGOT) are concentrated mainly in the liver, and their leaching into the circulation implicate that the integrity of the hepatocytes is disturbed. In this study, there is significant increase in the levels of ALT (SGPT), AST (SGOT) and bilirubin in the CCl_4 treated group. Generally, bilirubin conjugates with glucoronic acid in the liver and is later excreted through bile. The presence of higher proportion of bilirubin in serum indicates that the process of conjugation is hampered, reflecting loss of hepatic function [29].

However treatments with ***MEBV*** has alleviated this situation and restored RBCs, WBCs levels to near normal values thus suggested ***MEBV*** has protective effect. The histopathological studies too support the hepatoprotective role of ***MEBV*** and its ability to suppress the oxidative stress caused by CCl_4. This may be due to improved levels of SOD, GSH, Catalase enzymes and phytochemicals that are present already in the plant part as per preliminary screening and these may offer antioxidant activity such as phenolics, flavonoids, Vit. E, Vit. C, β-Carotene, Thiamine etc.

Thus, enzymatic scavenging of ROS could be efficiently achieved through the complex but elaborate coordination among the enzymes involved. The Higher levels of antioxidant system enable the plant extract to act as a very good free radical scavenger and help to prevent diseases caused by oxidative damage [30].

The phenolics are ubiquitous secondary metabolites having wide therapeutic values like antioxidant, anti-carcinogenic and free radical scavenging activities. The scavenging ability of the phenolics is mainly due to the presence of hydroxyl groups. Flavonoids are a group of polyphenolic compounds that exhibit anti-hepatotoxic, anti-inflammatory activities. They inhibit enzymes such as aldose reductase and xanthine oxidase is capable of scavenging the ROS due to their phenolics hydroxyl groups and potent antioxidants. The presence of Flavonoids and Phenolics that are reported in the ***MEBV*** may reveal a positive correlation between phenolics content and antioxidant activity, suggesting phenolics and flavonoids might be the active phytochemicals in *Beta vulgaris.*

It may be recalled that the phenolics and flavonoids contents were found moderately in methanol extract of *Beta vulgaris* (***MEBV***).

CONCLUSION: In this study, male wistar rats were exposed to CCl_4 and methanol extract of *Beta vulgaris,* at 100, 200 and 300μgm/ml respectively. The rats, during the *in vivo* study, developed a significant hepatic damage and oxidative stress, as evidenced by the massive elevation in the liver marker enzyme activities of ALT (SGPT), AST (SGOT) and bilirubin as compared with normal control rats. The blood parameters and hepatic cellular architecture were also under disturbance. This may be an indication of cellular leakage and loss of functional integrity of hepatocytes cell membrane. The phytochemicals analysis revealed the high content of phenolics and flavonoids in *Beta vulgaris; u*pon exposure to ***MEBV*** it was observed and recorded that the reversal of hepatotoxic effects and hepato-remediation do occur.

Hence based on the preliminary findings on antioxidant and hepatoprotective activities here reported, it may be concluded that, methanol extract of *Beta vulgaris* (***MEBV)*** possess significant protection against CCl_4-induced hepatotoxicity in the *in vivo* studies. This study also delivers the scope to continue study, identify and characterize the active principle(s) and the mechanism involved in the field of Hepatoprotection and Chemo-prevention of Hepatotoxicity offered by the antioxidants fraternity of *Beta vulgaris.*

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