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Research Article

HEPATOPROTECTIVE & ANTIOXIDANT EFFECT OF WHOLE PLANT EXTRACT OF *POLYGONUMGLABRUM* WILLD. ON CCL_4 INDUCED HEPATIC DAMAGE IN RATS

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ABSTRACT

The present work has been designed to evaluate hepatoprotective&in vivo antioxidant effect of Methanol-Aqueous Extract of whole plant *Polygonumglabrum*willd. (MAEP) against carbon tetrachloride(CCl_4)- induced hepatotoxicity in experimental rats. The levels of serum glutamate oxaloacetate transaminase (SGOT), serum glutamate pyruvate transaminase (SGPT), serum alkaline phosphatase (SALP), uric acid, total protein and total bilirubin were determined. Antioxidant status in liver was determined by measuring the activity of malonaldehyde (MDA), glutathione (GSH), total thiol and catalase (CAT). Total wet weight and histopathological study of isolated liver specimen was also carried out. . The oral pre-treatment with MAEP (200 and 400 mg/kg) showed significant hepatoprotective activity against CCl_4 induced hepatotoxicity by decreasing the activities of serum marker enzymes, bilirubin and lipid peroxidation, and significant increase in the levels of uric acid, GSH, Total thiol, Catalase and protein in a dose dependent manner, which was confirmed by the decrease in the total weight of the liver and histopathological examinations. Data also showed that MAEP possessed strong antioxidant activity, which may probably leads to the promising hepatoprotective activities of Methanol-Aqueous Extract of whole plant *Polygonum glabrum willd. (MAEP)*. These findings, therefore supported the traditional believes on hepatoprotective effect of the whole plant *Polygonum glabrum*willd.(MAEP).

Keywords: *Polygonum glabrum*willd.*(MAEP)*, Hepatoprotective activity, Carbon tetrachloride, Antioxidants, malonaldehyde (MDA), glutathione (GSH), Total thiol and catalase (CAT).

INTRODUCTION

The liver is the key organ regulating homeostasis in the body. The liver is expected not only to perform physiological functions but also to protect against the hazards of chemicals and harmful drugs[1]. Any injury to liver or impairment of its function may lead to many implications on one's health[2]. There are numerous plants and traditional formulations available for the treatment of liver diseases[3,4]. In spite of tremendous advances made in allopathic medicine, no effective hepatoprotective medicine is available[5]. Use of the herbal medicine in jaundice, presumably viral hepatitis, has been known in India since the Vedic times. About 170 phyto-constituents isolated from 110 plants belonging to 55 families have been reported so far to possess liver protective activities. It is estimated that about 6000 commercial herbal formulations are sold worldwide as hepatoprotective drugs, of them about 40 patent polyherbal formulations representing a variety of combinations of 93 Indian herbs from 44 families are available in the Indian market[6]. However, only a small proportion of hepatoprotective plants as well as formulations used in traditional medicine are pharmacologically evaluated for their safety and efficacy[7]. Some herbal preparations exist as standardized extracts with major known ingredients or even pure compounds which are being evaluated[4].

Hepatotoxicity from drugs and chemicals is the commonest form of iatrogenic disease. Some of theinorganic compounds producing hepatotoxicity are arsenic, phosphorus, copper and iron. Antioxidants are believed to play a very important role in the body defense system against reactive oxygen species (ROS).

It involves a variety of components, both endogenous and exogenous in origin, that function interactively and synergistically to neutralize free radicals[9].

Oxygen is essential for the survival of all on this earth. During the process of oxygen utilization in normal physiological and metabolic processes, approximately 5% of oxygen gets univalently reduced to oxygen derived free radicals like superoxide anion (O_2^-), hydrogen peroxide (H_2O_2) radical, nitric oxide (NO^-) radical, hydroxyl radical (OH^-), NOO, etc. These radicals known as reactive oxygen species (ROS) exert oxidative stress on the cells of human body rendering each cell to face about 25000 oxidative hits per second. The ROS have also been implicated in the pathogenesis of diabetes, liver damage, nephrotoxicity, inflammation, cancer, cardiovascular disorders, neurological disorders and in the process of aging[10].

There is a need to strengthen the antioxidant system or exogenous administration of antioxidants to overcome such challenges as a prophylactic or as curatives[10]. Conventional or synthetic drugs used in the treatment of liver diseases are sometimes inadequate and can have serious adverse effects. So there is a worldwide trend to go back to traditional medicinal plants.[11] Many classes of substituents from natural sources are reported to possess antioxidant property. Many plants often contain substantial amount of antioxidants including vitamin C and E, carotinoids, flavonoids, tannins etc., and can be utilized to scavenge the excess of free radicals from human body[10].

Administration of antioxidants may be useful in protecting organs against pro-oxidative challenges. There are reports that antioxidants of natural origin obtained from various herbs are highly effective not only in quenching the free radicals but also in protecting various organs and organ systems[10].

There is phenomenonal increase in the search for the herbal antioxidants throughout the world. Keeping the tone of the trends in view, we in our laboratory have undertaken research for herbal antioxidants and hepatoprotective that are available at a hand's stretch. As the part of our work, we normally undertake field survey and contact with the native practitioners. We found a plant by name *Polygonum glabrum* Willd.

The literature survey of this plant revealed that traditionally it has been used for the treatment of colic, febrifuge, piles, debility[12] and jaundice[13] it also possesses anthelmintic[14] and anti-inflammatory[15] activities. In modern literature, the reports indicate that plant possesses flavonoids, tannins, carotinoids etc., and these constituents are known to be antioxidants and hepatoprotective. In the present study the plants of *Polygonum glabrum* Willd. was screened for the antioxidant and hepatoprotective properties against experimentally induced liver damage.

MATERIALS AND METHODS

Plant material and preparation of the extract:

The whole plant of *Polygonum glabrum* Willd. was collected in the month of April from the surrounding fields of Dharwad & Bhadravati and authenticated by HOD, Dr. Hebbar, and Professor Dr. Gurumurthy Department of Botany, Karnataka University, Dharwad. The whole plant was shade dried at room temperature. The dried whole plant was powdered and extracted with methanol aqueous using soxhlet extractor, the concentrate was evaporated to dryness under reduced pressure and low temperature (40^o C) on a rotary evaporator. The extract was concentrated and stored in desiccators, until further use for biological investigation and *in-vivo* antioxidant studies, after subjecting it to preliminary qualitative phytochemical evaluation.

Chemicals

All the chemicals and solvents were of analytical grade and were procured from Ranbaxy Fine Chemicals Ltd., Mumbai, India. Carbon tetrachloride (CCl_4) were procured from S.D. Fine Chemicals – Mumbai (India) Ltd, Mumbai. Standard drug Silymarin was obtained as gift sample from Microlab – Bangalore. Dithiobisnitrobenzoate (DTNB) was obtained from Sigma co. Standard kits for SGOT, SGPT, SALP, uric acid and bilirubin were obtained from Erba Diagnostics Ltd, India. UV-Visible spectrophotometer; Shimadzu Pharmaspec 1700 was used to measure the absorbance.

Experimental animals

The female albino rats (180- 220 g) were used for the experimentation. The inbreed colonies of rats were collected from Venkateshwara Enterprises Bangalore, Karnataka. They were maintained in the animal house of SET's College of Pharmacy, Dharwad for experimental purpose. After randomization into various groups, animals were acclimatized for period of 7 days under standard husbandry conditions. Room temperature (23 ±2°C), relative humidity (50±5%), 12 hrs light/dark cycle. All the animals were fed with rodent pellet diet (Amrut feeds, Venkateshwara Enterprises, Bangalore) and water allowed *ad-libitum* under strict hygienic condition. Ethical clearance for performing experiments on animals was obtained from Institutional Animal Ethics Committee (IAEC) prior to the initiation of the experiment and the care of the laboratory animals was taken as per the CPCSEA regulations.

Determination of acute toxicity (LD50)

The acute toxicity of methanol-aqueous extract of whole plant *Polygonumglabrum* Willd. (MAEP) was determined in female albino rats weighing 150-200 g, maintained under standard husbandry conditions. The animals were fasted overnight prior to the experiment. Fixed dose (OCED Guideline No. 420) method of CPCSEA was adopted for toxicity studies by up and down/staircase method as per OECD guidelines. The methanol-aqueous extract of whole plant *Polygonum glabrum* Willd. was orally administered to different groups of rats at the doses of 50, 300, 1000, 2000 and 4000 mg /kg body weight respectively. Animals were observed for 48 h to study the general behavior of animals, sign of discomfort and nervous manifestation. The *Polygonum glabrum* Willd. was found devoid of mortality of animals at the dose of 4000 mg /kg body weight. Hence the 1/10th (100 mg/kg, p o.) and 1/20th (200 mg/kg, p.o.) of the dose selected for the screening of hepatoprotective and antioxidants activity of *Polygonum glabrum* Willd.(MAEP) on CCl_4 induced hepatic damage in rats[1].

Phytochemical screening

Freshly prepared methanol-aqueous extract of whole plant *Polygonum glabrum* Willd. (MAEP) was subjected to

preliminary phytochemical screening for detection of major chemical constituents[16].

Evaluation of hepatoprotective activity in CCl_4 induced hepatotoxicity.

Albino rat of wistar strain weighing 150 – 200 g were selected and divided into five groups of each containing six animals.

Group I: Normal control rats – (Olive oil 0.5ml/kg body wt given i.p)

Group II: CCl_4 in olive oil 0.5ml/kg body wt.

Group III: Methanol-aqueous extract of PG(lower dose) +CCl_4

Group IV: Methanol-aqueous extract of PG(Higher dose)+ CCl_4

Group V: *Silymarin*(25 mg/ kg p.o). + CCl_4

Animals were treated as shown above for a period of 21 days. Every 7th, 14th and 21st day CCl_4 in Olive oil (0.5ml/kg i.p) was administered to all groups except group I. Group V received standard drug, Silymarin 25mg/kg p.o. once in a day and CCl4 as mentioned above. Whereas group III and IV were treated with MAEP test extract dose of (200 and 400 mg/kg p.o.) respectively. During this period of treatment the rats were maintained under normal diet and water. All the animals were sacrificed 24 hrs after the last injection of CCl_4 i.e. on 21st day. Blood was collected by retro orbital bleeding under mild ether anaesthesia and was allowed to clot at room temperature for 30 min, subjected to centrifugation (3000 rpm for 15 min.) and stored serum at 4° C until used for estimation of biochemical parameters[2]. The serum was used to estimate serum glutamate oxaloacetate transaminase (SGOT), serum glutamate pyruvate transaminase (SGPT), serum alkaline phosphatase (SALP), uric acid, total protein and total bilirubin content[4,5,6,7,8].

Liver was dissected out and subjected for morphological study such as wet liver weight of each animal. Further the liver was placed in 10% formalin solution dehydrated in graded alcohol and then embedded in paraffin. Microtome sections (5 µm thick) were prepared from each liver samples and stained with haematoxylin-eosin (H&E) dye for histopathological study[3].

Lipid peroxidation

Thiobarbituric acid reactive substances (TBARS) in the liver homogenate were estimated by using standard protocol. Briefly, homogenate was incubated with 15% TCA, 0.375% TBA and 5N HCl at 95°C for 15 min, the mixture was cooled, centrifuged & absorbance of supernatant measured at 532 nm against appropriate blank. The amount of lipid peroxidation was determined by using the formula = 1.56 x 10^5 M^{-1} cm^{-1} and expressed as TBARS (n moles) per g of tissue[9].

Catalase

Catalase activity was determined spectrophotometrically according to previously published method. Briefly, to 1.95 ml of 10 mM H_2O_2 in 60 mM phosphate buffer (pH=7.0), 0.05 ml of the Heart & Kidney homogenate was added and rate of degradation of H_2O_2 was followed at 240 nm/ min. Catalase content in terms of U/mg of protein was estimated from the rate of decomposition of H_2O_2 using the formula k=2.303/ Δt x log (A_1/A_2) s^{-1} (A unit of catalase is defined as the quantity which decomposes 1.0 µmole of H_2O_2 per min at pH=7.0 at 25°C, while H_2O_2 concentration falls from 10.3 to 9.2 mM)[10].

Total thiols

The assay is based on the formation of a relatively stable yellow product when sulphydryl groups react with DTNB. Briefly, 0.2 ml of liver homogenate was mixed with phosphate buffer (pH=8), 40 µl of 10mM DTNB and 3.16 ml of methanol. This mixture was incubated for 10 min and the absorbance was measured at 412 nm against appropriate blank. The total thiol content was calculated by using the formula ε=13.6 x 10^3 cm^{-1} M^{-1}. [11]

Glutathione (GSH)

The assay is based on the formation of a relatively stable yellow product when sulphydryl groups react with DTNB. Briefly, proteins were precipitated using 10% TCA, centrifuged and 0.5 ml of the supernatant was mixed with 0.2M phosphate buffer (pH 8.0) and 10 mM DTNB. This mixture was incubated for 10 min and the absorbance was measured at 412 nm against appropriate blank. The glutathione content was calculated by using the standard plot under same experimental conditions. Plotting the absorbance against GSH concentration (0.005-0.042 µmoles) gave the standard curve. A linear correlation coefficient (r^2 = 0.999) was obtained[12].

RESULTS AND DISCUSSION

The qualitative chemical investigation of Methanol-Aqueous extract of whole plant *Polygonum glabrum* Willd. (MAEP) was carried out to check the presence of various phytoconstituents in extract and the results are given in Table No 1.

It is observed from the phytochemical study that flavonoids, glycosides, tannins, protein, terpenes, volatile oils and saponins are present in the extracts.

Acute toxicity (LD50) studies

Acute toxicity studies were carried out according to OECD guidelines (Up and Down method). No mortality was observed at 4000mg/kg body weight. Therefore 1/10th and 1/20th doses were taken as low and high effective dose for all further *in-vivo* studies.

Effect of MAEP on serum marker enzymelevels

Rats subjected to the CCl_4 challenge alone (positive control group) developed significant liver injury as evident from a significant elevation in the biochemical markers like SGPT, SGOT, ALP, TP, TB & DB when compared with normal control group (Table No. 2a). Oral administration of the test extract exhibited dose dependent significant reduction in the CCl_4 induced increase in the biochemical levels. Treatment with the reference standard Silymarin (25mg/kg p.o.) also reversed the hepatotoxicity significantly. Hepatoprotective potency of the test extract at the dose 400mg/kg was found closer to that of standard.

There was a marked increase in AST(SGOT) (IU/L) levels observed in CCl_4 treated group (279.3±13.06) as compared to normal (182±19.8 as show in Table 2a). However, the AST levels were reversed to near normal levels with the treatment of 200 mg/kg (223±6.50) and 400 mg/kg (209.7±2.34) of methanol-aqueous extract of *Polygonumglabrum*Willd. In addition, the standard Silymarin has restored the AST levels towards normal (203.9±7.46). Serum ALT(SGPT) levels have been also elevated in the CCl_4 treated group (192.3±8.17).

Phytochemical investigation of Methanol-Aqueous Extract of *Polygonum glabrum* Willd.MAEP:

Extract	% Yeild (gms)	Colour	Odour	Consistency
Methanol-Aqueous	5.6	Dark grayish	Characteristic	Sticky mass

Table 1: Results of the preliminary phytochemical investigation on EEET

Types of Phytochemical constituents	Methanol-aqueous extract
Alkaloids	–
Carbohydrates	+
Flavonoids	+++
Glycosides	+
Tannins	++
Protein	++
Terpenes	++
Volatile oils	++

- Absent, + indicates presence, ++ more clarity, +++better response

Treatment with standard Silymarin has brought back the ALT levels to the near normal levels (113.6±7.99 as show in Table 2a). However treatment with the methanol-aqueous extract of *Polygonumglabrum*Willd. has restored the ALT levels in a dose dependent manner in both the doses 200 mg/kg and 400 mg/kg up to (144.9±13.09 and 144.8±9.35) respectively.

Rise in ALP (IU/L) serum levels were remarkable in CCl_4 treated group (56.33±2.09) and with the 200 mg/kg and 400 mg/kg doses of methanol-aqueous extract of *Polygonumglabrum*Willd. Reduced to (43.83±1.62 and 41.67±1.99as show in Table 2a) respectively, whereas standard Silymarin responded well and restored the ALP levels to 38.5±3.00.

In case of total and direct bilirubin there was a noticeable rise (1.962±0.008 and 1.073±0.033) in serum levels on CCl_4 treatment. Treatment with 200 mg/kg and 400mg/kg of extract has reversed the Total bilirubin serum levels to (1.517±0.040 and 1.458±0.037) respectively and also treatment with 200 mg/kg and 400mg/kg of extract has reversed the direct bilirubin serum levels to (0.79±0.033 and 0.735±0.025) which is statistically significant when compared with CCl_4 treated group. The reversal by standard Silymarin with (1.36±0.164 and 0.703±0.103) in case of total and direct bilirubin as show in Table 2arespectively.

There was a marked depletion in the Total protein level in CCl_4 treated group (5.99±0.085). The Slilymarin and methanol-aqueous extract 200 mg/kg and 400 mg/kg (7.78±0.209, 6.93±0.224 and 7.27±0.209) which were near to the normal value (9.56±0.131) respectively.

The results are summarized in Table 2a and graphically depicted (Fig.1a,1b,1c,1d,1e,1f,)

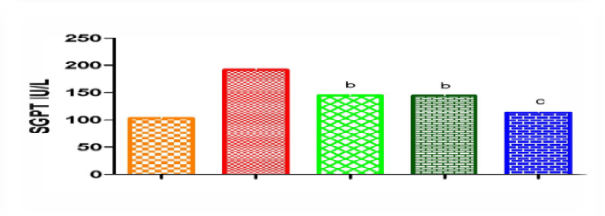

Fig 1a: SGOT. Each bar represent the Mean ± SEM (n = 5). [a] P < 0.05; [b] P < 0.01; [c] P < 0.001 compared with CCl_4 rats

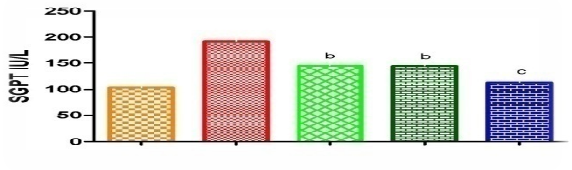

Fig 1b: SGPT. Each bar represent the Mean ± SEM (n = 5). [a] P < 0.05; [b] P < 0.01; [c] P < 0.001 compared with CCl_4 rats

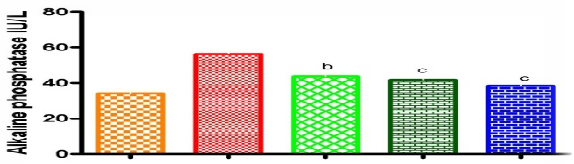

Fig 1c: SALP. Each bar represent the Mean ± SEM (n = 5). [a] P < 0.05; [b] P < 0.01; [c] P < 0.001 compared with CCl_4 rats

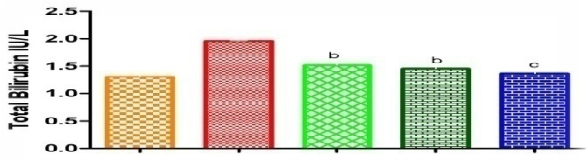

Fig 1d: Total Bilirubin. Each bar represent the Mean ± SEM (n = 5). [a] P < 0.05; [b] P < 0.01; [c] P < 0.001 compared with CCl_4 rats

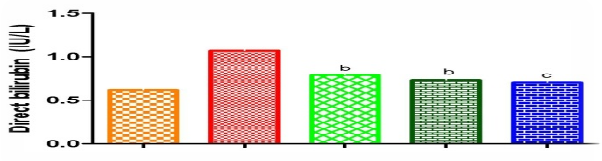

Fig 1e: Direct Bilirubin. Each bar represent the Mean ± SEM (n = 5). [a] P < 0.05; [b] P < 0.01; [c] P < 0.001 compared with CCl_4 rats

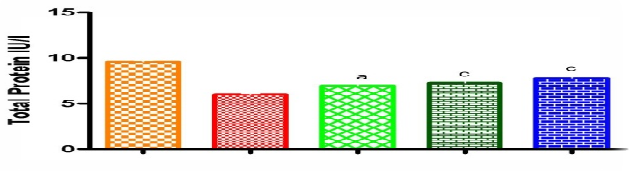

Fig 1f: TotalProteins. Each bar represent the Mean ± SEM (n = 5). [a] P < 0.05; [b] P < 0.01; [c] P < 0.001 compared with CCl_4 rats

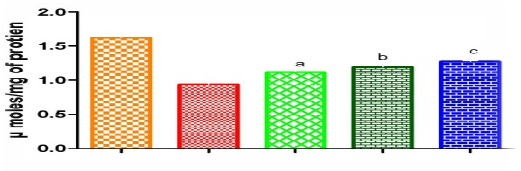

Fig 2b: Total Thiol. Each bar represent the Mean ± SEM (n = 5). a P < 0.05; b P < 0.01; c P < 0.001 compared with CCl_4 rats.

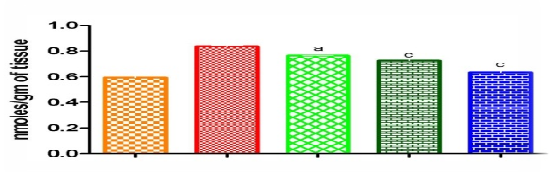

Fig 2c: Lipid Peroxidation. Each bar represent the Mean ± SEM (n = 5). a P < 0.05; b P < 0.01; c P < 0.001 compared with CCl_4 rats

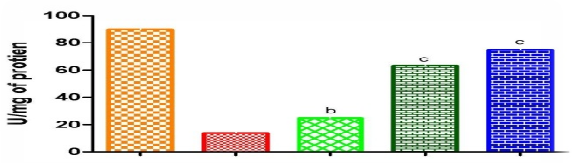

Fig 2d: Catalase. Each bar represent the Mean ± SEM (n = 5). a P < 0.05; b P < 0.01; c P < 0.001 compared with CCl_4 rats

Effect of MAEP on Liver Antioxidant Enzymes Reduced glutathione (GSH)

Normal control rats showed basal GSH levels of about (0.0618±0.0026 nMoles/mg of Protein). Hepatotoxicity rats showed significantly decreased levels of GSH (0.0074±0.001nmoles/mg of protein) in comparison to normal rats. Pre-treatment with different doses of MAEP (200 and 400 mg/kg) and Silymarin (25 mg/kg) showed significant improvement in GSH levels (Table 2b, Fig No: 2a).

Total thiols

Basal total thiol levels in normal rats were found to be (1.623±0.064μmoles/mg of protein). CCl_4-induced Hepatotoxicity rats exhibited significantly decreased levels of total thiols (0.939±0.02μmoles/mg of protein) in comparison to normal rats. Moreover, pretreatment with different doses of MAEP (200 and 400 mg/kg) and Silymarin (25 mg/kg) showed significantly increased levels (Table 2b ,Fig No: 2b)

Lipid peroxidation

Normal control rats showed basal TBARS levels of about (0.594±0.016nmoles/g of liver tissue homogenate) and Hepatotoxicity rats showed significantly increased in TBARS levels (0.8372±0.00689nmoles/g of tissue). Pre-treatment with different doses of the MAEP (200 and 400 mg/kg) and Silymarin (25 mg/kg) significantly abolished the increase in TBARS levels induced by CCl_4 (Table 2b ,Fig No: 2c)

Catalase

Normal basal level of catalase activity in normal control rats was found to be (89.77±2.69U/mg of protein). Hepatotoxicity rats showed significantly decreased levels of catalase by several folds (13.81±1.26U/mg of protein). Pre-treatment of doses of MAEP (200 and 400 mg/kg) and Silymarin (25 mg/kg) significantly increased the levels of catalase to the near normal values (Table 2b ,Fig No: 2d).

The results are summarized in Table 2b and graphically depicted (Fig.2a,21b,2c,2d,21e,2f,).

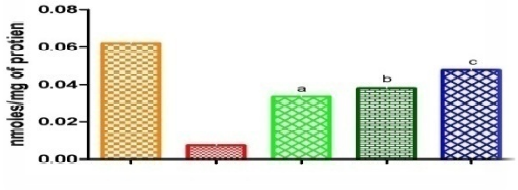

Fig 2a: GSH. Each bar represent the Mean ± SEM (n = 5). [a] P < 0.05; [b] P < 0.01; [c] P < 0.001 compared with CCl_4 rats

Effect of MAEP on Histopathological profile

Histopathological profile of liver from CCl_4 intoxicated rats reveals hepatic globular architecture disrupted, hepatic cells has shown various degree of fatty degeneration like ballooning of hepatocytes, fatty cyst, infiltration of lymphocytes and proliferation of kupffer cells. Congestion of liver sinusoids. Protective effect of test extract was confirmed by histopathological examination of liver section. Administration of test extract at the dose of 400mg/kg that is (Fig No-3d) showed a significant improvement of the hepatic architecture and areas of Kupffer cell proliferation and sinusoid appeared normal on contrary with 200mg/kg (Fig No- 3c).

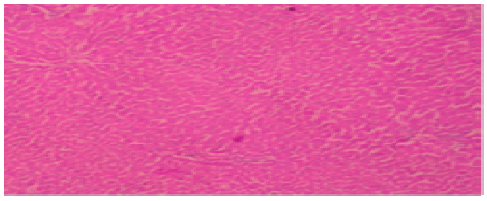

Fig-3a- Normal

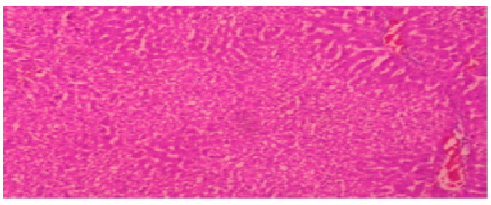

Fig-3b- CCl4 induced

Fig-3c- MAEP 200mg + CCl_4

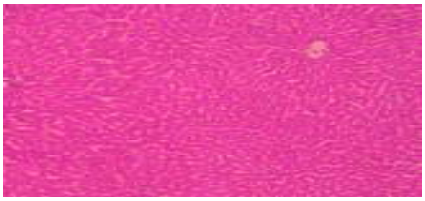

Fig-3d-MAEP 400mg + CCl_4

Fig-3e- Silymarin+ CCl_4

DISCUSSION

Human beings constantly struggle against the changing environmental condition to maintain optimum health and vigour throughout their life, during all the seasons. In addition they are also exposed to various xenobiotics including drugs, toxins, etc. and other stresses.

Physiological homeostasis is maintained in spite of exposure to several such external challenges and endogenous aberrations. However such challenges or aberrations over power the endogenous defence mechanisms, which leads to the disturbances in the physiological homeostasis. Further this causes various diseases. It is increasingly being realized now that a majority of the disease/disorders are mainly due to imbalance between pro-oxidant (free radicals) and anti-oxidant homeostatic phenomenon.[1]

Liver is such an organ that its physiological role and its self-protective mechanism are well developed. In spite of such balanced internal milieu, hepatic aberration, damage and necrosis commonly occurring due to over exposure to hepatotoxic causes to such an extent that it over powers the mechanism.

An attempt has been made in the present study to evaluate the hepatoprotective and antioxidant activity of methanol-aqueous extract of *Polygonumglabrum*Willd. against CCl_4 induced hepatotoxicity in rats on the basis of its traditional and folklore use in jaundice[13].

The Methanol-aqueous extract prepared was subjected to phytochemical tests and the outcome of these tests revealed the presence of carbohydrate, glycosides, flavonoids, tannins, protein, terpenes and volatile oils.

The Methanol-aqueous extract was subjected to acute toxicity studies as per OECD guidelines (Up and Down method). Since no death was observed at 4000 mg/kg, it was accepted as cut off dose 1/20th (200 mg/kg) and 1/10th (400 mg/kg) of doses were taken as effective lower and higher doses for screening hepatoprotective and antioxidant activities.

In this study, rats treated with CCl_4 developed a significant hepatic damage and oxidative stress, which was observed from a substantial increase in the levels of SGPT, SGOT, ALP and Bilirubin (Direct & total). This is indicative of cellular leakage and loss of functional integrity of cell membrane in liver[2].

Liver damage was assessed by biochemical studies and by histopathological examinations. Toxicity begins with the change in endoplasmic reticulum, which results in the loss of metabolic enzymes located in the intracellular structures. The toxic metabolite CCl_3 radical is produced which further reacts with oxygen to give trichloromethylperoxy radical. Cytochrome P_{450} 2E1 is the enzyme responsible for this conversion. This radical binds covalently to the macromolecule and causes peroxidative degradation of lipid membrane of the adipose tissue. In this view, the reduction in levels of SGPT, SGOT, ALP and Bilirubin (Direct & total) treated with methanol-aqueous extract of *Polygonumglabrum*Willd. exhibited stabilization of plasma membrane as well as repair of hepatic tissue damage caused by CCl_4. This effect was in agreement with the commonly accepted view that serum levels of transaminases return to normal with the healing of hepatic parenchyma and regeneration of hepatocytes[3].

Histopathological profile of liver from CCl_4 intoxicated rats reveals hepatic globular architecture disrupted, hepatic cells has shown various degree of fatty degeneration like ballooning of hepatocytes, fatty cyst, infiltration of lymphocytes, proliferation of kupffer cells and congestion of liver sinusoids.Similarly, a histopathological observation showed that hepatic globular architecture was normalized, fewer lymphatic infiltrations were seen and kupffer cells proliferation appear to be normal.

The observation suggests that the methanol-aqueous extract of *Polygonumglabrum*Willd. possessed hepatoprotective activity against CCl_4 induced hepatotoxicity. Since the preliminary phytochemical analysis of the extract showed the presence of flavonoids and tannins, which have been known for their antioxidant and hepatoprotective properties. Methanol-aqueous extract of *Polygonumglabrum*Willd. was subjected to *In-vivo* antioxidant activity. A major defence mechanism involves the antioxidant enzymes, including CAT, GSH, LPO, Total protein and Total Thiol which convert active oxygen molecules into non- toxic compounds when assayed by estimation

Thus it has been hypothesized that one of the principal causes of CCl_4 induced liver injury is formation of lipid peroxides by free radical derivatives of CCl_4 (CCl_3).The antioxidant activity or the inhibition of the generation of free radicals is important in the protection against CCl_4 induced hepatotoxicity. Reactive oxygen species (ROS) or oxygen containing free radicals that are generated endogenously during normal physiological functioning or exposure to stresses play vital role in causing oxidative stress and tissue damage. The most

important oxygen-containing free radicals in many disease states are superoxide anion (O2•), H_2O_2 radical, NO^- radical, hydroxyl ion radical (OH^-), NOO, etc. These are highly reactive species, capable to damage the biologically relevant molecules such as DNA, proteins, carbohydrates and lipids. Targets of free radicals include all kinds of molecules in the body[4,5]. Thus free radicals are involved in development of degenerative diseases. They have also been implicated in the pathogenesis liver damage[6].

An antioxidant is a molecule stable enough to donate an electron to a rampaging free radical and neutralize it, thus reducing its capacity to damage. These antioxidants delay or inhibit cellular damage mainly through their free radical scavenging property. These low molecular weight antioxidants can safely interact with free radicals and terminate the chain reaction before vital molecules are damaged.[4] Several endogenous antioxidants, that are playing vital role as organs protectants are GSH, catalase etc.

Naturally, there is a dynamic balance between the amount of free radicals generated in the body and antioxidants to quench them and protect the body against deleterious effects.[7] This is accomplished by a set of endogenous antioxidant enzymes such as CAT and GSH. These enzymes constitute a mutually supportive team of defence against ROS.In CCl_4 induced hepatotoxicity, the balance between ROS production and these antioxidant defense may be lost, oxidative stress results leading to hepatic necrosis. The reduced activities of CAT and GSH observed point out the hepatic damage in rats administered with CCl_4[8].

In the present study, a decrease in hepatic tissue GSH and CAT levels was observed in the CCl_4 treated groups. Pre-treatment with methanol-aqueous extract of *Polygonum glabrum* Willd.(MAEP) increased the depleted hepatic GSH and CAT levels in dose dependent manner. The elevation of lipid peroxidation in the liver of rat treated with CCl4 was observed. Hence also reduced by the pre-treatment of methanol-aqueous extract of *Polygonumglabrum* Willd.

Similarly, the liver weights of all animals were recorded and the slight increase in the liver weight was observed in the CCl_4 treated group. Hence, the groups treated with methanol-aqueous extract of *Polygonumglabrum*Willd. significantly reduced the liver weight when compared with CCl_4 control group.

It is well known that free radicals cause cell damage through mechanisms of covalent binding and lipid peroxidation with subsequent tissue. Antioxidants act as free radical scavengers that destroy single oxygen molecules (free radicals) in the body, thereby protecting against oxidative damage of cells. Catalase (CAT), GSH, etc. are the well-known enzymes present in plasma which act as antioxidants by transforming reactive oxygen species and reactive nitrogen species into the stable compounds and involved in a scavenging of the excessive free radicals. The treatment with methanol-aqueous extract of *Polygonum glabrum* Willd.(MAEP) has normalized the CCl_4 induced biochemical and tissue abrasion, therefore it may be suggested that hepatoprotective activity against CCl_4 challenge is probably due to its free radical scavenging activity and prevention of lipid peroxidation. The restoration of tissue GSH, CAT levels by the treatment with test extract is indicating that the inbuilt protective mechanism is being restored. This hepatoprotective activity may be attributed to the anti-oxidant activity of the plant.

CONCLUSION

Thus, it can be concluded that possible mechanism of hepatoprotective activity of *Polygonum glabrum* Willd.(MAEP) may be due to its antioxidant activity, which may be due to the presence of flavonoids and tannins in the extract. The hepatoprotective and antioxidant activity ofWhole plant extract of *Polygonum glabrum* Willd.(MAEP) was confirmed by biochemical and histopathological studies. There is room for further study to isolate, identify and characterize the active principle responsible for the hepatoprotective activity of the plant.

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Table 2a: Effect Methanol-aqueous extract of *Polygonum glabrum* Willd. (MAEP) on enzyme AST (SGOT), ALT (SGPT), ALP, Total Bilirubin , Direct bilirubin, and Total Protein levels in blood serum of CCl_4 induced hepatotoxicity

GROUPS	SGPT IU/l	SGOT IU/l	SALP IU/l	TB mg/dl	DB mg/dl	TP gms/dl
NORMAL	103±4.077	182±19.8	34±1.461	1.307±0.029	0.6167±0.015	9.563±0.131
CCl_4	192.3±8.175	279.3±13.06	56.33±2.092	1.962±0.008	1.073±0.0337	5.99±0.0850
MAE.P 200mg + CCl_4	144.9± 13.09**	223± 6.506*	43.83± 1.621**	1.517± 0.040**	0.79± 0.03337**	6.938± 0.224*
MAE.P 400mg + CCl_4	144.8± 9.357**	209.7± 2.348**	41.67± 1.994***	1.458± 0.037**	0.735± 0.0259**	7.275± 0.209***
STANDARD + CCl_4	113.6± 7.997***	203.9± 7.467***	38.5± 3.008***	1.368± 0.164***	0.7033± 0.103***	7.782± 0.209***

Table 2b: Effect Methanol-aqueous extract of *Polygonum glabrum* Willd. (MAEP) on enzyme AST (SGOT), ALT (SGPT), ALP, Total Bilirubin , Direct bilirubin, and Total Protein levels in blood serum of CCl_4 induced hepatotoxicity

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