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Hepatoprotective and Antioxidant effect of *Polygala rosmarinifolia* Wight & Arn against CCl₄ induced hepatotoxicity in rats

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Carbon tetrachloride (CCl₄) intoxicated rats showed significant elevation in serum enzymes, bilirubin and lipid peroxidation of the liver tissues and reduction in serum total protein, superoxide dismutase, catalase, reduced glutathione peroxide activity. Treatment with ethanol extract of *Polygala rosmarinifolia* whole plant altered the above parameters to the levels of near normal. All the above results were comparable with the standard drug silymarin (100mg/kg) treated group. Thus the present study ascertains that the ethanol extract of *Polygala rosmarinifolia* whole plant possesses significant hepatoprotective activity.

Keyword: *Polygala rosmarinifolia*, Hepatoprotective, ALP, Bilirubin.

1. Introduction

Liver regulates various important metabolic functions. Hepatic damage is associated with distortion of these metabolic functions^[1]. Liver disease is still a worldwide health problem. Unfortunately, conventional or synthetic drugs used in the treatment of liver diseases are inadequate and sometimes can have serious side effects. This is one of the reasons for many people in the world over including those in developed countries turning complementary and alternative medicine (CAM)^[2]. Many traditional remedies employ herbal drugs for the treatment of liver ailments^[3-6].

Carbon tetrachloride (CCl₄) is one of the most commonly used hepatotoxins in the experimental study of liver diseases. The hepatoprotective effect of CCl₄ is largely due to its active metabolite, trichloromethyl radical. The administration of CCl₄ in rats enhances hepatic

protein oxidation and results in the accumulation of CCl₄ oxidized proteins in the liver^[7]. The present study was conducted to evaluate the hepatoprotective effect of the extracts of whole plant of *Polygala rosmarinifolia* on carbon tetrachloride induced liver damage in experimental rats.

Polygala is a branching type native to the Central and Western United states. There are several varieties of *Polygala*. It earned its nick name "Seneca Snake Wood" in 1700's when Seneca Indians used to treat snakebites in American settlers. *Polygala* was traditionally used by Americans to treat snake bite^[8] and as an expectorant to treat cough and bronchitis. In traditional Chinese medicine; *Polygala* is used for variety of purposes including the promotion to sleep and calming the spirit. *Polygala* is considered as a powerful tonic herb^[9] that can

help to develop the mind and aid in creative thinking. Taking into consideration of the medicinal importance of *Polygala rosmarinifolia*, the ethanol extract of the whole plant *Polygala*

rosmarinifolia were analyzed for their hepatoprotective activity against CCl₄ induced hepatotoxicity in rats.

Table 1: Effect of whole plant extracts of *Polygala rosmarinifolia* on the protein, albumin, globulin concentration and enzyme activity of serum GOT, GPT, and ALP in the normal, liver damaged and drug treated rats.

Groups	Parameters						
	T.Protein (mg/dl)	Albumin (g/dl)	Globulin (g/dl)	A/G Ratio	SGOT (U/L)	SGPT (U/L)	ALP (U/L)
I	7.98±0.81	4.88±0.34	3.1±0.11	1.5:1	19.56±1.36	26.16±0.93	143.29±5.32
II	6.18±0.34*	3.28±0.11*	2.9±0.23	1.1:1	40.11±1.21*	43.19±1.08*	196.11±6.84*
III	7.08±0.15	4.11±0.17	2.97±0.18	1.3:1	24.11±1.16	28.51±1.22	156.22±6.94
IV	7.76±0.13*	4.46±0.12*	3.3±0.12	1.3:1	20.96±1.13*a	24.11±1.73*a	151.03±8.28*a
V	7.48±0.11*	4.51±0.31*	2.97±0.16	1.5:1	21.33±1.19*a	27.06±1.33*a	146.55±6.94*a

Each Value is SEM ± 5 individual observations * P < 0.05 ; ** P < 0.01 Compared normal control vs liver injured rats. A P < 0.05 ; aa P < 0.01 Compared liver injured rats vs drug treated

Biochemical active such as anticancer, antidiabetic, anti-inflammatory, anti-fertility and antioxidant activity were reported^[10,11,12,13,14] However, potential of literature reveals that, hepatoprotective activity of *Polygala rosmarinifolia* is totally lacking and hence the present investigation was undertaken.

2. Materials and Methods

2.1 Plant material

The whole plants of *Polygala rosmarinifolia* Wight & Arn were collected in the month of February and March, 2012, from the Vadavalli, Coimbatore district, TamilNadu. The plant specimen were identified with the help of local flora and authenticated in Botanical survey of India, Southern Circle, Coimbatore, Tamil Nadu, India. A voucher specimen was deposited in Ethnopharmacology Unit, Research Department of Botany, V.O. Chidambaram College, Tuticorin, Tamil Nadu.

2.2 Preparation of plant extracts for phytochemical Screening and Hepatoprotective Studies

The whole plant of the *Polygala rosmarinifolia* plant was dried under shade and then powdered with a mechanical grinder to obtain a coarse powder, which was then subjected to extraction in

a Soxhlet apparatus using ethanol. The extract was subjected to qualitative test for the identification of various phytochemical constituents as per standard procedures^[15-17]. The ethanol extracts were concentrated in a rotary evaporator. The concentrated ethanol extract were used for hepatoprotective studies.

2.3 Animals

Normal healthy male Wistar albino rats (180-240g) were used for the present investigation. Animals were housed under standard environmental conditions at room temperature (25±2°C) and light and dark (12:12h). Rats were fed with standard pellet diet (Goldmohur brand, MS Hindustan lever Ltd., Mumbai, India) and water *ad libitum*.

2.4 Acute Toxicity Studies

Acute oral toxicity study was performed as per OECD-423 guidelines (acute toxic class method), albino rats (n=6) of either sex selected by random sampling were used for acute toxicity study^[18]. The animals were kept fasting for overnight and provided only with water, after which the extracts were administered orally at 5mg/kg body weight by gastric intubations and observed for 14 days. If mortality was observed in two out of three animals, then the dose administered was assigned

as toxic dose. If mortality was observed in one animal, then the same dose was repeated again to confirm the toxic dose. If mortality was not observed, the procedure was repeated for higher doses such as 50, 100 and 2000 mg/kg body weight.

Table 2: Effect of whole plant extracts of *Polygala rosmarinifolia* on the serum Total, conjugated and unconjugated bilirubin levels in the normal control, liver injured and drug treated rats.

Groups	Parameters		
	Total Bilirubin ($\mu\text{mol/L}$)	Conjugated ($\mu\text{mol/L}$)	Unconjugated ($\mu\text{mol/L}$)
I	0.68 \pm 0.03	0.24 \pm 0.01	0.44 \pm 0.02
II	3.69 \pm 0.43**	1.49 \pm 0.03*	2.20 \pm 0.06**
III	1.69 \pm 0.01*	0.34 \pm 0.03*	1.35 \pm 0.11*
IV	1.34 \pm 0.01*a	0.24 \pm 0.02a	1.10 \pm 0.01*a
V	0.88 \pm 0.01**a	0.20 \pm 0.01*a	0.68 \pm 0.3*aa

Each Value is SEM \pm 5 individual observations * P < 0.05 ; ** P<0.01 Compared normal control vs liver injured rats. A- P < 0.05 ; aa - P<0.01 Compared liver injured rats vs drug treated

2.5 Experimental Design

In the investigation, a total of 30 rats (25 CCl₄ hepatic toxicity induced rats and 5 normal rats) were taken and divided into five groups of 5 rats each.

- Group I: Rats received normal saline was served as a normal control.
- Group II: CCl₄ hepatic toxicity induced control: Rats received 2.5ml/kg body weight of CCl₄ for 14 days.
- Group III: Liver injured rats received ethanol extract of whole plant of *Polygala rosmarinifolia* at the dose of 100mg/kg body weight for 14 days.
- Group IV: Liver injured rats received ethanol extract of whole plant of *Polygala rosmarinifolia* at the dose of 200mg/kg body weight for 14 days.
- Group V: Group VI: Liver injured rats received standard drug silymarin at the dose of 100mg/kg body weight for 14 days.

2.6 Biochemical Analysis

The animals were sacrificed at the end of experimental period of 14 days by decapitation. Blood was collected, sera separated by centrifugation at 3000g for 10 minutes. Serum protein^[19] and serum albumins was determined quantitatively by colorimetric method using bromocresol green. The total protein minus the albumin gives the globulin. Serum glutamate pyruvate transaminase (SGPT) and serum glutamate oxaloacetate transaminase (SGOT) was measured spectrophotometrically by using the method of Reitman and Frankel^[20]. Serum alkaline phosphatase (ALP) was measured by the method of King and Armstrong^[21]. Total bilirubin and conjugated bilirubin were determined as described by Balistrei and Shaw^[22].

The unconjugated bilirubin concentrations were calculated as the difference between total and conjugated bilirubin concentrations. Gamma-glutamyltransferase (GGT) was estimated by the method of Szasz^[23]. Liver homogenates (10%W/V) were prepared in ice cold 10mM tris buffer (pH7.4). Quantitative estimation of MDA formation was done by determining the concentration of thiobarbituric acid reactive substances (TBARS) in 10% liver homogenates by the method of Okhawa^[24]. Enzymatic antioxidants, superoxide dismutase (SOD)^[25] Catalase^[26,27] and non-enzymatic antioxidant glutathione peroxidase (GPx)^[28] glutathione reductase (GRD)^[29] and reduced glutathione (GSH)^[30] were also assayed in liver homogenates.

2.7 Statistical Analysis

The data were expressed as the mean \pm S.E.M. The difference among the means has been analyzed by one-way ANOVA. p<0.001, p<0.01 and p<0.05 were considered as statistical significance using SPSS Software.

Table 3: Effect of whole plant extracts of *Polygala rosmarinifolia* on liver LPO,GPX,GRD,SOD and CAT in the normal control, liver injured and drug treated rats.

Groups	Parameters				
	LPO (n mole of MDA/mg protien)	GPX (u/mg Protien)	GRD (u/mg)	SOD (u/mg)	CAT (u/mg)
I	0.82±0.03	13.54±1.23	8.56±0.59	10.45±0.19	9.56±0.82
II	3.16±0.54**	4.22±0.56**	3.05±0.28*	4.22±0.28**	2.87±0.70**
III	2.24±0.85*	7.34±0.37*	4.78±0.45 ^{ns}	4.88±0.41*	4.67±0.27*
IV	1.92±0.52*	9.12±0.28 ^{ns}	6.99±0.67 ^l	5.97±0.26*	5.98±0.48*
V	0.89±0.11 ^{aa}	12.56±0.97 ^{aa}	8.67±0.11 ^{aa}	11.71±0.12 ^{aa}	13.56±0.55 ^{aa}

Each Value is SEM ± 5 individual observations * P < 0.05; ** P<0.01 Compared normal control vs liver injured rats. A P < 0.05; aa P<0.01 Compared liver injured rats vs drug treated

3. Result

The ethanol extract of whole plant *Polygala rosmarinifolia* subjected for phytochemical study showed the presence of alkaloids, coumarin, glycosides, flavonoides, saponins, steroids, phenols, tannins, and xanthoproteins. The ethanol extract did not show any sign and symptoms of toxicity and mortality upto 2000mg/kg dose. The effect of ethanol extract of *Polygala rosmarinifolia* on serum total protein, albumin, A/G ratio, serum transaminases, alkaline phosphates in CCl₄ intoxicated rats are summarized in table 1.

There was a significant ($p < 0.01$) increase in serum GOT, GPT and ALP levels in CCl₄ intoxicated group (Group II) compared to the normal control group (Group I). The total protein and albumin levels were significantly ($p < 0.001$) decreased 6.18g/dl and 3.28g/dl in CCl₄ intoxicated rats from the levels of 7.98g/dl and 4.88g/dl respectively in normal group. Ethanol extract of *Polygala rosmarinifolia* at the dose of 100 and 200mg/Kg orally significantly decreased the elevated serum marker enzymes and reversed the altered total protein and albumin to almost normal level.

The effect of ethanol extract of *Polygala rosmarinifolia* on total, conjugated and unconjugated bilirubin is shown in Table 2. A significant elevation of total, conjugated, unconjugated bilirubin in the serum of CCl₄ intoxicated group (Group II) when compared to normal control (Group I). The ethanol extract of *Polygala rosmarinifolia* at the dose 100 and 200mg/kg reduced the levels of total, conjugated

and unconjugated bilirubin (Group III and Group IV). The decreases in the concentration of total bilirubin, conjugated bilirubin, unconjugated bilirubin were found to be greater in standard silymarin (Group V) followed by Group IV and Group III (Table 2).

The effects of ethanol extract of *Polygala rosmarinifolia* on lipid peroxidation (LPO), Glutathione peroxidase (GPx), glutathione reductase (GRD), superoxide dismutase (SOD) catalase (CAT) and reduced glutathione (GSH) activity is shown in Table 3. Lipid peroxidation level was significantly ($p < 0.01$) increased and glutathione peroxidase, glutathione reductase, superoxide dismutase and catalase activity were significantly ($p < 0.01$) decreased in CCl₄ intoxicated rats when compared with those of the animals in normal control group. Rats treated with ethanol extract of *Polygala rosmarinifolia* at the doses of 100 and 200mg/kg significantly decreased the elevated lipid peroxidation levels and restored the altered glutathione peroxidase, glutathione reductase, superoxide dismutase, catalase and reduced glutathione levels towards the normal levels in a dose dependent manner. The results are well comparable with silymarin (standard drug) treated group.

4. Discussion

It is well established that CCl₄ induces hepatotoxicity by metabolic activation; therefore it selectively causes toxicity in liver cells maintaining semi-normal metabolic function. CCl₄ is bio-transformed by the cytochrome P₄₅₀ in the endoplasmic reticulum system to produce

trichloromethyl free radical (CCl_3). Trichloromethyl free radical then combined with cellular lipids and proteins in the presence of oxygen to form a trichloromethyl peroxy radical, which may attack lipids on the membrane of endoplasmic reticulum faster than trichloromethyl free radical. Thus, trichloromethyl peroxy free radical leads to elicit lipid peroxidation, the destruction of Ca^{2+} homeostasis, and finally, results in cell death^[31,32]. These results in changes of structures of the endoplasmic reticulum and other membrane, loss of enzyme metabolic enzyme activation, reduction of protein synthesis and loss of glucose - 6- phosphate activation, leading to liver damage^[33,34]. Hepatotoxic compounds like CCl_4 are known to cause marked elevation in serum enzymatic activities. In the present study, treatment with *Polygala rosmarinifolia* whole plant extract attenuated the increases in the activities of SGOT, SGPT and ALP produced by CCl_4 indicating that *Polygala rosmarinifolia* whole plant extract protects liver injury induced by CCl_4 towards normalization.

Alkaline phosphatase concentration is related to the functioning of hepatocytes, high level of alkaline phosphatase in the blood serum is related to the increased synthesis of its by cells lining bile canaliculi usually in response to cholestasis and increased biliary pressure^[35]. Increased level was obtained after CCl_4 administration and it was brought to near normal level *Polygala rosmarinifolia* by treatment.

Protein metabolism is a major project of liver and a healthy functioning liver is required for the synthesis of the serum proteins. Hypoproteinemia is a feature of liver damage due to significant fall in protein synthesis. Albumin is decreased in chronic liver disease. Hypoproteinemic was observed after CCl_4 ingestion but the trend turns towards normal after *Polygala rosmarinifolia* treatment.

Bilirubin is a yellow pigment produced when heme is catabolised. Hepatocytes render bilirubin water soluble and therefore easily excretable by conjugating it with glucuronic acid prior to secreting it into bile by active transport. Hyperbilirubinemia may result from the production of more bilirubin than the liver can

process, damage to the liver impairing its ability to excrete normal amount of bilirubin or obstruction of excretory ducts of the liver^[36]. Serum bilirubin is considered as one of the true test of liver functions since it reflects the ability of the liver to take up and process bilirubin into bile. Elevated levels may indicate several illnesses. High levels of total bilirubin in CCl_4 treated rats may be due to CCl_4 toxicity. This may have resulted in hyperbilirubinemia. The significant reduction in the level of total bilirubin in the serum of whole plant extract treated rats suggested the hepatoprotective potential of whole plant extract against *Polygala rosmarinifolia* CCl_4 intoxication.

Lipid peroxidation has been postulated to the destructive process of liver injury due to CCl_4 administration. In the present study, the elevations in the levels of end products of lipid peroxidation in the liver of rat treated with CCl_4 were observed. The increase in malondialdehyde (MDA) levels in liver suggests enhanced lipid peroxidation leading to tissue damage and failure of antioxidant defense mechanisms to prevent formation of excessive free radicals. Treatment with ethanol extract of *Polygala rosmarinifolia* significantly reversed these changes. Hence, it may be possible that the mechanism of hepatoprotection by ethanol extract of *Polygala rosmarinifolia* is due to its antioxidant effect.

In the present investigations, CCl_4 intoxicated rats decreased the content of GPx and GRD in liver, whereas, treatment with ethanol extract of *Polygala rosmarinifolia* (100 and 200mg/kg) able to reverse such effects. Superoxide dismutase (SOD), a metallo protein is the most sensitive enzyme index in liver injury and one of the most important enzyme in the enzymatic antioxidant defense system. It scavengers the superoxide anion to form hydrogen peroxide and oxygen, hence diminishing the toxic effect caused by this radical^[37]. In the present study, it was observed that the ethanol extract of *Polygala rosmarinifolia* whole plant significantly increased the SOD activity in CCl_4 intoxicated rats there by diminished CCl_4 induced oxidative damage.

Catalase (CAT) is an enzymatic antioxidant widely distributed in all tissues and the highest

activity is found in red cells and liver. Catalase is a heme protein, localized in the peroxisomes or the microperoxisomes. This enzyme catalyses the decomposition of H_2O_2 to water and oxygen and thus protecting the cell from oxidative damage by H_2O_2 and OH. Therefore, the reduction in the activity of catalase may result in a number of deleterious effects due to accumulation of hydrogen peroxide³⁸. In the present study, treatment with ethanol extract of *Polygala rosmarinifolia* whole plant increased the level of catalase significantly in dose dependent manner and protected the liver from CCl_4 intoxication.

In conclusion, the results of this study demonstrate that the ethanol extract of *Polygala rosmarinifolia* whole plant has a potent hepatoprotective action against CCl_4 induced hepatic damage in rats. It's mode in affording the hepatoprotective activity against CCl_4 induced liver damage may be due to cell membrane stabilization, hepatic cells regeneration and enhancement of antioxidant enzymes such as catalase, superoxide dismutase and glutathione peroxidase production. The hepatoprotective and antioxidant potential of whole plant extract could have been brought about by various phytochemical principles i.e. flavonoids, alkaloids, phenolics and tannins present in *Polygala rosmarinifolia* whole plant. So results of this study demonstrated that the *Polygala rosmarinifolia* has significantly protection on CCl_4 induced hepatotoxicity.

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