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## **A study on antioxidant potential of *Glycyrrhiza glabra* linn. in 1,4-dichlorobenzene induced liver carcinogenesis**

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### **ABSTRACT**

*1,4 Dichlorobenzene (1,4 DCB) has been used as a space deodorant and moth repellent as well as an intermediate in the chemical industry. Given its broad applications and high volatility, considerable concern exists regarding the adverse health effects of 1,4 Dichlorobenzene in the home and the workplace. In this study changes in lipid peroxidation, antioxidants and liver marker enzyme in the serum of 1,4 DCB treated rats were investigated to determine their roles in toxicity. The rats were given 300mg/kg of DCB then treated with Glycyrrhiza glabra Linn., leaf extract. The level of malinaldehyde (MDA), an end product of lipid peroxidation, markedly increased in the 1,4 DCB treated rats, after treating with Glycyrrhiza glabra Linn., extract its level returned to its original level. Thus G.glabra exhibits its best antioxidant potential and liver protective effects like strand drug – silymarin.*

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### **INTRODUCTION**

*Glycyrrhiza glabra* Linn., also known as licorice and sweetwood, is native to the Mediterranean and certain areas of Asia. In modern medicine, Licorice extracts are often used as a flavoring agent to mask bitter taste in preparations and as an expectorant in cough and cold preparations (Olukoga and Donaldson, 1998).

A number of components have been isolated from licorice including water soluble, biologically active complex. This complex is composed of triterpene saponins, flavonoids, polysaccharides, pectins, simple sugars, amino acids, mineral salts and various other substances (Obolentseva et al., 1999). Glycyrrhizin, a triterpenoid compound, accounts for the sweet taste of licorice root.

The yellow colour of licorice is due to the flavanoid content of the plant, which induces liquidity, isoliquiritin (a chalcone) and other compounds (Yamamura et al., 1992). The isoflavone glabridin and hispa glabridins A and B have significant antioxidant activity (Vaya et al., 1997). In view of this, the present study was aimed at evaluating the hepatoprotective activity of leaf extract of *Glycyrrhiza glabra* Linn., against 1,4 Dichlorobenzene induced hepatotoxicity in albino rats.

## EXPERIMENTAL SECTION

### Preparation of plant extract:

Weighed amount of dried powder of *Glycyrrhiza glabra* Linn., leaves taken and added 50ml of 99.9% hot ethanol mixture; evaporated at 55<sup>o</sup>c by using hot air oven, the collected extract used for hepatotoxicity studies.

1,4 DCB is used to induce liver toxicity in albino rats. The rats were assorted into the following group.

**Group I:** (n=6) – Negative control rats. Diet and water were available adlibitum.

**Group II:** (n=6) – 1,4 DCB induced rats. 300mg of 1,4 DCB dissolved in 1ml of corn oil & given per day for a period of 45 days.

**Group III:** (n=6) – *Glycyrrhiza glabra* Linn., leaf extract treated rats. 100mg of *Glycyrrhiza glabra* Linn., leaf extract/ kg body weight given after treating with 1,4 DCB.

### Group IV:

100mg/kg body weight Silymarin – [standard drug] was given after treating with 1,4 DCB.

### Collection of blood:

The blood was collected by sino – orbital puncture and allowed to clot for few minutes, the clotted blood was transformed to centrifuge tube. The blood was centrifuged at 3000rpm for 5min. The serum was used for estimation of biochemical parameters.

### Analysis Biochemical Parameters :

Lipid profile [LDL, VLDL & HDL Cholesterol] and enzymatic antioxidants [SOD, CAT, GPX, GSH & GST] were analysed in plasma and liver tissues of 1, 4 DCB intoxicated and plant extract treated rats.

## RESULTS AND DISCUSSION

Liver toxicity was assessed by the increased level of plasma lipids such as LDL, VLDL and Cholesterol with concomitant decrease in enzymatic antioxidants, SOD, CAT, GPX and GSH. Table 1 shows 1,4 DCB induced changes in the level of lipid profile [LDL, VLDL & HDL] and restoring capacity of *Glycyrrhiza glabra* Linn., leaf extract in those toxic rats.

Table 1. Effect of Glycyrrhiza Glabra Leaf Extract on Lipid Profile in 1, 4 Dichloro benzene induced rats

Groups	Dosage mg/ kg of body wt	VLDL (mg//dL)	LDL (mg//dL)	HDL (mg//dL)	Total Cholesterol		Triglycerides	
					Liver (mg/100g tissues)	Plasma (mg/dl)	Liver (mg/100g tissues)	Plasma (mg/dl)
Negative Control	-	14.17± 1.36 <sup>b</sup>	29.17± 2.80 <sup>bc</sup>	49.98± 4.81 <sup>a</sup>	333.94±32.14 <sup>b</sup>	87.72±8.44 <sup>b</sup>	398.61±38.37 <sup>a</sup>	61.71±.5.94 <sup>b</sup>
1.4 Dichloro benzene induced	300 mg / kg of body wt	30.09± 2.89 <sup>a</sup>	76.29± 7.34 <sup>a</sup>	28.56± 2.74 <sup>b</sup>	505.00±48.61 <sup>a</sup>	128.01±12.32 <sup>a</sup>	520.30±50.08 <sup>c</sup>	137.49±13.23 <sup>a</sup>
Glycyrrhiza glabra	100 mg/kg of body wt	15.81± 1.52 <sup>b</sup>	33.15± 3.19 <sup>c</sup>	47.94± 4.61 <sup>b</sup>	387.90±37.33 <sup>c</sup>	91.12±8.77 <sup>b</sup>	418.81±40.31 <sup>bc</sup>	64.66±6.22 <sup>b</sup>
Silymarin [Standard drug]	100 mg / kg of body wt	14.68± 1.41 <sup>b</sup>	27.54± 2.65 <sup>b</sup>	48.96± 4.71 <sup>a</sup>	367.91±35.41 <sup>bc</sup>	85.27±8.20 <sup>b</sup>	413.20±39.77 <sup>b</sup>	59.46±5.72 <sup>b</sup>

Values are means ±S.D. of eight rats in each group.

Values not sharing a common superscript letter differ significantly at  $p < 0.05$  (Duncan's multiple range test)

Table 2: Effect of Glycyrrhiza Glabra Leaf Extract on Enzymatic antioxidants in 1, 4 Dichloro benzene induced rats

Groups	Dosage mg/kg of body wt	SOD		CAT		GPX		GST		GSH	
		Liver (50% inhibition of NBT reduction/min/mg protein)	Lysate (enzyme required for 50% inhibition of NBT reduction/min/mg haemoglobin)	Liver (µmoles H <sub>2</sub> O <sub>2</sub> utilized/min/mg protein)	Lysate (µmoles of H <sub>2</sub> O <sub>2</sub> utilized/min/mg haemoglobin).	Liver (µg of GSH utilized/min/mg protein)	Lysate (µmoles of GSH utilized/min/mg haemoglobin)	Liver (µmoles CDNB-GSH conjugate formed /min/mg protein)	Lysate (µmoles CDNB-GSH conjugate formed/min/mg haemoglobin)	Liver (mmoles/mg tissue)	Plasma (mmol/mg protein)
Negative Control	-	9.89±0.95 <sup>a</sup>	12.67±1.22 <sup>a</sup>	83.74±8.06 <sup>a</sup>	49.17±4.73 <sup>a</sup>	18.66±1.79 <sup>a</sup>	17.85±1.71 <sup>a</sup>	11.73±1.12 <sup>a</sup>	17.91±1.72 <sup>a</sup>	22.12±2.12 <sup>ad</sup>	35.95±3.46 <sup>a</sup>
1.4 Dichloro benzene induced	300 mg / kg of body wt	5.61±0.54 <sup>b</sup>	8.50±0.81 <sup>b</sup>	54.26±5.22 <sup>b</sup>	28.05±2.70 <sup>b</sup>	10.09±0.97 <sup>b</sup>	11.04±1.06 <sup>b</sup>	7.75±0.74 <sup>B</sup>	12.64±1.21 <sup>b</sup>	13.18±1.26 <sup>b</sup>	20.86±2.00 <sup>b</sup>
Glycyrrhiza glabra	100 mg/kg of body wt	9.58±0.92 <sup>a</sup>	11.62±1.11 <sup>c</sup>	78.74±7.57 <sup>a</sup>	47.60±4.58 <sup>a</sup>	16.72±1.61 <sup>d</sup>	16.60±1.59 <sup>a,c</sup>	10.60±1.02 <sup>a</sup>	18.54±1.78 <sup>a</sup>	20.47±1.97 <sup>d</sup>	31.47±3.02 <sup>d</sup>
Silymarin [Standard drug]	100 mg / kg of body wt	10.09±0.97 <sup>a</sup>	12.77±1.22 <sup>a</sup>	85.47±8.22 <sup>a</sup>	48.74±4.69 <sup>a</sup>	18.87±1.81 <sup>a</sup>	17.96±1.72 <sup>a</sup>	11.52±1.10 <sup>a</sup>	18.31±1.76 <sup>a</sup>	23.06±2.21 <sup>a</sup>	36.55±3.51 <sup>a</sup>

Values are means ± S.D. of eight rats in each group.

Values not sharing a common superscript letter differ significantly at  $p < 0.05$  (Duncan's multiple range test).

The antioxidant effects of *Glycyrrhiza glabra* Linn., were examined in hepatotoxic male albino rats. Table – 2 shows one week administration of GC powder to toxic rats resulted in significant reduction in plasma and hepatic total lipids, triglycerides LDL, VLDL & cholesterol accompanied by significant increase in HDL cholesterol level. The leaf powder administration to hepato toxic rats also decreased hepatocytes lipid peroxidation with a concomitant increase in SOD, CAT, GPX, GSH & GST activities.

Thus the hypocholesteramic and antioxidant effects of *Glycyrrhiza glabra* Linn., leaf powder appeared to be mediated via (i) accelerated cholesterol elimination through faecal matter. (ii) improving the activities of hepatic SOD & Catalase. The antioxidant status of these animals also was improved upon treatment (Visavadiya NP, Narasimhacharya AV. 2006).

Certain *Glycyrrhiza glabra* Linn., constituents possess significant antioxidant & hepatoprotective properties. Glycyrrhizin & glabridin inhibit the generation of Reactive oxygen species (ROS) by neutrophils at the site of inflammation (Akamatsu et al., 1991). *Glycyrrhiza glabra* Linn., leaf powder inhibits Fe<sup>3+</sup> induced mitochondria lipid peroxidation in rat liver cells (Hiraguchi et al., 2000). Other research indicates *G. glabra* lowers lipid peroxide values in animal models of liver injury caused by ischaemia reperfusion (Nagai et al., 1991). *Glycyrrhiza glabra* Linn., constituents also exhibit hepatoprotective activity by lowering serum liver enzyme levels and improving tissue pathology in hepatitis patients ( Van Rossum et al., 2001).

### CONCLUSION

*Glycyrrhiza glabra* Linn., is a commonly available herbaceous medicinal plant belonging to pea family for the present investigation.

Ethanollic extracts of *Glycyrrhiza glabra* Linn., were evaluated according to Indian pharmacopeia by soxhlet method. The antioxidant potential of ethanolic extract of *Glycyrrhiza glabra* Linn., was analysed by 1,4 Dichlorobenzene induced hepatotoxicity. Antioxidant potential activity and hepatoprotective function of *Glycyrrhiza glabra* Linn., may be due to the presence of active chemical constituents like glycyrrhizin and glycyrrhithic acid.

It may be concluded that the herbal drug appears to fulfil the necessary therapeutic criteria expected of conservative remedy for hepatoprotective activity. Furthest investigations are needed to substantiate this effect which may throw more light on its mechanism and verification in a larger series for definite conclusion.

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