

## PHYTOCHEMICAL ANALYSIS AND *IN VIVO* HEPATOPROTECTIVE POTENTIAL OF *IPOMOEA ERIOCARPA* R.BR.

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### ABSTRACT

The present study investigate the phytochemicals of ethanolic extract of *Ipomoea eriocarpa* and hepatoprotective potential in vivo against carbon tetrachloride (CCl<sub>4</sub>)-induced hepatotoxicity in rats as animal model at the doses of 100 mg/kg and 200 mg/kg body weight. The phytochemical analysis revealed that flavonoids, tannins and terpenoids were strongly present in plant extract. An oral dose plant extract of 100mg/kg body weight was identified from toxicity test and administered to albino mice. The results showed that ethanolic extract of *I. eriocarpa* at the doses of 100 mg/kg and 200 mg/kg produced significant ( $p < 0.05$ ) decreases in paracetamol induced increased levels of liver serum enzymes such as ALT (alanine aminotransferase), AST (aspartate amino transferase), ALP (alkaline phosphatase), LDH (lactate dehydrogenase) and Total bilirubin and Total protein. The biochemical results of extract and toxic groups were also compared with standard drug silymarine. So, the present study showed that methanolic extract of *C. pendulus* possessed hepatoprotective activity and it is a good antioxidant source. It is concluded that ethanolic plant extract have strong potential for antioxidant and hepatotoxicity and also proved themselves as strong therapeutic and remedial agent against human and animal disease.

### INTRODUCTION

Medicinal plants have significant importance for the health care of local communities as a source of medicine. Since immemorial time plants have been playing an important part in human health care system. They are also used as significant source of food, dyes, cosmetics etc. (Amer *et al.*, 2013). The total 422,000 have been reported from the world about of which more than 50,000 are used for medicinal purpose for the majority of rural population (Hamilton, 2004). The 60% of

the world population and 80% population of developing countries depend upon the traditional medicines (Bhat *et al.*, 2014). More than 4.5 million Due to the high cost of allopathic drugs and their side effects, the popularity of medicinal plants in the rural area have been increased as the component of their healthcare (Marwat *et al.*, 2008). Formulations based on natural drugs derived from herbs were used significantly to relieve diseases in early twentieth century. By using synthetic drugs only one third of diseases can be cured therefore plant derived drugs are mostly used in the management of health care system (Ekor, 2014).

Liver plays different functions in body that important for life, such as bile secretion, detoxification and metabolization of harmful drugs, storage of glycogen, fats, minerals, iron and vitamins from diet (Waugh *et al.*, 2001). Liver disease is worldwide problem today. Many synthetic and chemotherapeutic drugs are used for the recovery from liver problems but these drugs are not useful and effective (Murugesan *et al.*, 2009). The hepatotoxic CCl<sub>4</sub> cause cellular necrosis of liver tissue and necrotic cell death by the peroxidation of the lipid membrane (Alirezaei *et al.*, 2012).

*Ipomoea eriocarpa* R.Br. belonging to Family Convolvulaceae are annual and perennial plants, also known as annual morning glories. It is distributed all over the South American, African and Asian tropical areas and Northern Australia. *I. eriocarpa* is a prostrate or twinning herb, have butterfly shaped cotyledons. First true leaves are large, heart shaped, with deep lobes at the base, alternate, ovate-cordate to linear-oblong, having almost 1-4 cm long petiole and about 1-2 m long stems. Inflorescence axillary, flowers are small, funnel-shaped, regular, penta-merous, bisexual pedicel about 3-5 mm long. Fruit are pods and seeds are released through slits. (Ghazanfar, 1992).

The plants are used in traditional medicines for treatment of illness, epilepsy, sores, rheumatism, fevers, Hansen's disease, seizures, headache, leprosy and ulcer (Kumar, 2013), leprosy, epilepsy and ulcers (Madhava, 2005). A broad literature review of *I. eriocarpa* have shown that the extract of this plant was screened for its anti-secretory, anti-nociceptive, antipyretic, cerebro-protective properties (Vijayakumar, 2012) antioxidant activities (Thamizhvanan, 2012) and toxicity studies (Harish, 2012).



**Fig. 1:** *Ipomoea. eriocarpa* R. Br. in Natural Habitat

## **MATERIALS AND METHODS**

### **Plant material**

The whole plant (Fig. 1) of study i.e. *I. eriocarpa* was collected from different regions of District Kotli Azad Kashmir. The collected plant material was identified, authenticated. (Herbarium Department of Botany, MUST, voucher number; MUST.BOT.5372)

### **Maceration of Plant Material**

The whole plant (500 g) were shade dried and macerated in ethanol solvent (1L). The resultant extracts were dried on rotary evaporator at 40°C to get concentrated extracts.

### **Phytochemical Analysis**

Qualitative tests for phytochemical screening of plant extracts were performed to evaluate the presence and absences of active secondary metabolites like alkaloids, flavonoids, cardiac glycosides, saponins tannins, terpenoids, reducing sugars, and protein test by using the method of Ayoola *et al.*, (2008).

### **Hepatoprotective activity**

The CCl<sub>4</sub> -induced hepatotoxicity study was carried out by following the method of Suja *et al.*, (2004) in which rats were divided into five groups containing four rats in each. The Group I is normal control, Group II as disease control in which CCl<sub>4</sub> is used to induce hepatotoxicity, Group III as standard control treated with silymarin while Group IV and V are plant extract treated group (100, 200 mg/kg/day) of plant extract respectively.

## Experimental Animals

The hepatoprotective activity was carried out by using adult albino wistar rats (100–150 g). The rats were kept in the animal house in cages of the Punjab University College of Pharmacy with standard conditions of temperature ( $25\pm 5$ ) and humidity ( $55\pm 5\%$ ) under 12 h light/dark cycle. The animals had free access of drinking water with provision of regular normal rat diet *ad libitum*. All the procedures performed by the permission of Animal Ethical Committee of Punjab University College of Pharmacy, Lahore Pakistan.

## Acute toxicity study

The doses of *I. eriocarpa* extract at 100, 200, and 1,000 mg/kg were given orally to the rats to observe the mortality in all rats during the duration of 24–72 hours. The rats were died at a dose of 1,000 mg/kg after 24 hours so only doses of 100 and 200 mg/kg were selected for this study which showed significant results.

## RESULTS AND DISCUSSION

### Phytochemical analysis

The phytochemical screening was carried out to observe the presence of phytochemical such as alkaloids, flavonoids, terpenoids, saponins, carbohydrates, proteins, phenols and phytosterols in ethanolic extract of *I. eriocarpa*. The concentration of phytochemicals varies in extracts. The ethanolic extracts showed the presence of terpenoids, flavonoids, phytosterols, and phenolic compounds (Table 1).

**Table 1: Phytochemical evaluations of extracts of *I. eriocarpa***

Phytochemical Constituents	<i>I. eriocarpa</i>
Alkaloids	-
Flavonoid	++
Carbohydrates	-
Cardiac glycosides	-
Reducing sugar	-
Protein test	-

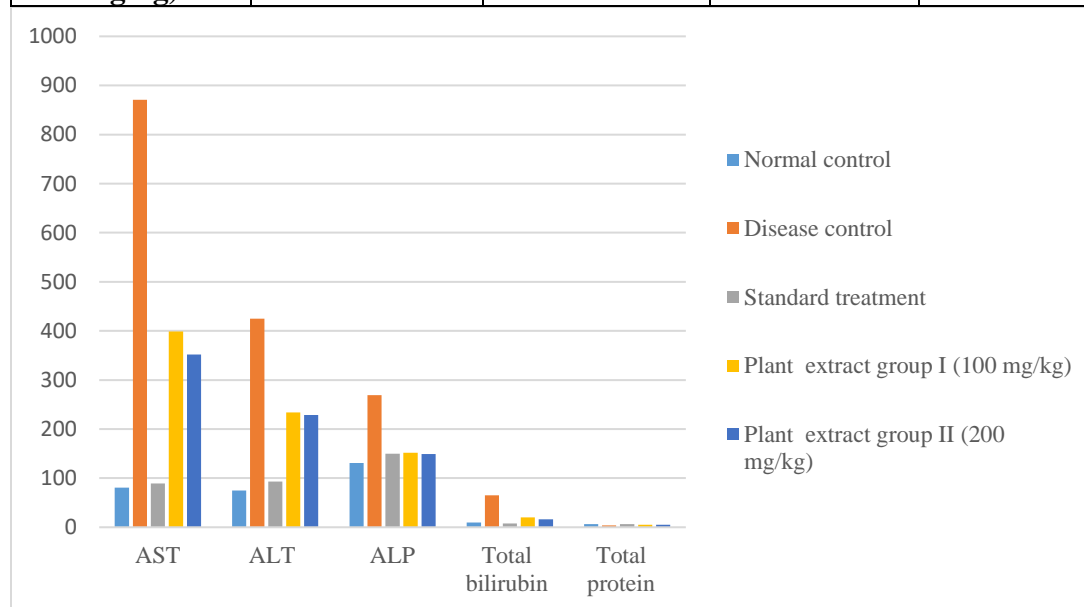
Phytosterols	-
Saponins	-
Steroids	-
Tannins	+++
Terpenoids	+++

### **Hepatoprotective potential of *I. eriocarpa***

The ethanolic extract of *I. eriocarpa* was used to investigate the hepatoprotective potential in CCl<sub>4</sub>-induced hepatotoxicity rats in which silymarin (25 mg/kg) was used as a standard drug. The selected doses of *I. eriocarpa* were dose dependent and significantly decrease ( $P < 0.05$ ) in the serum of AST, ALT, ALP, and bilirubin as compared with the CCl<sub>4</sub>-treated group (Table 2; Fig.2). For CCl<sub>4</sub> administrated hepatotoxicity study in rats the actions of serum hepatic marker enzymes including, alkaline phosphatase (ALP), alanine aminotransferase (ALT), aspartate aminotransferase (AST) and total bilirubin were carried out. Liver is the major source of ALP, which helps body for the breakdown of proteins. Its elevated level in blood stream signals the malfunctioning of liver. To access the liver functioning of experimental animals the ALP test was carried out. The silymarin drug reduced the alkaline phosphates level of blood in treatment groups. 200 mg/kg plant extract showed significant results as compare to 100mg/kg plant extract. The ethanolic extract of *I. eriocarpa* showed significant results of hepatoprotective effect when compared with silymarin by increasing the level of protein and albumin in serum and significant decrease in AST, ALT, ALP and bilirubin. The plant contains different phytochemical and the hepatoprotective effect is due to flavonoid contents.

**Table 2: Effect of *I. eriocarpa* extract (100 and 200 mg/kg) on CCl<sub>4</sub> -induced hepatotoxicity in rats.**

Groups	AST (U/ml)	ALT (U/ml)	ALP (U/ml)	Total bilirubin gm/dL	Total protein gm/Dl
Normal control	80.9 ± 1.63	75.6 ± 1.92	131.4 ± 4.13	10.3 ± 0.41	6.30 ± 0.05
Disease control	871.2 ± 3.37	425.8 ± 3.23	269.7 ± 5.24	65.40 ± 0.12	3.72 ± 0.19
Standard treatment	89.91 ± 3.20	93.1 ± 6.5	150.4 ± 3.63	8.41 ± 0.31	6.71 ± 0.3
Plant extract group I (100 mg/kg)	399.4 ± 3.4	234.23 ± 5.0	152.3 ± 4.92	20.79 ± 0.09	5.21 ± 0.61
Plant extract group II (200 mg/kg)	352.9 ± 4.99	229.6 ± 4.1	149.21 ± 4.6	16.56 ± 0.4	5.41 ± 0.21



**Fig. 2: Effect of *I. eriocarpa* on CCl<sub>4</sub> -induced hepatotoxicity in rats.**

## DISCUSSION

It is well known fact that phyto-chemical properties of plants are due to presence of important biochemical constituents in plants. Phyto-chemistry assessment of plant is quite necessary before determining biological properties of any plant (Rauf and Muhammad, 2013). It is revealed during the phyto-chemical screening of *I. eriocarpa* that the ethanolic extracts showed the presence of terpenoids, flavonoids, phytosterols, and phenolic compounds. The results revealed that the qualitative phytochemical study of plant extract contain the presence secondary metabolites which are similar to the results of Rabari *et al.* (2010).

To evaluate the hepatoprotective efficacy of *I. eriocarpa*, CCl<sub>4</sub> was used to induce hepatotoxicity in rabbits. CCl<sub>4</sub> effects hepatic cells and disturbing its metabolic activities. It mounts up in parenchyma cells of liver and to induce lipid peroxidation trichloromethyl (CCl<sub>3</sub>) free radical attached with cell proteins and lipids in aerobic environment (McGregor and Lang, 1996). CCl<sub>3</sub> toxicity resulted in variations in structure of endoplasmic reticulum and other membranes of hepatic cells. Serum marker enzymes including AST, ALT, ALP, serum bilirubin level increases significantly and protein synthesis rate reduces due to loss of metabolic enzyme activation it causes increased lipid peroxidation rate and obliteration of Ca<sup>2+</sup> homeostasis Enzymes outflow from hepatic cells due to changed impairment functions of membrane the AST, ALT and ALP and bilirubin level in the hepatic cells decreased and elevated in serum (Shilpi *et al.*, 2006). For CCl<sub>4</sub> liver damage evaluation the AST and ALT enzyme levels were considered greatly. To determine function of hepatic cells the level of serum ALP and bilirubin calculated. Bilirubin is important clinical evidence to determine necrosis in hepatic cells. Total serum protein level reduces in control group which showed that the protein synthesis ability of liver cells decreased due to imperfect activity of liver (Giannini *et al.*, 1999).

The effect of ethanolic extract of *Ipomoea eriocarpa* against CCl<sub>4</sub> Induced hepatotoxicity were evaluated by measuring AST, ALT, ALP, total bilirubin and total protein levels. The AST level of blood sample in *I. eriocarpa* extracts treated groups greatly drops. The maximum results were showed by plant extracts (352.9 ± 4.99) at highest dose. For the reduction in the ALT level of blood in treatment groups the highest activity was revealed by plant extract (229.6 ± 5.0) at highest dose as compared to low dose. The results of the study approximately comparable to the

hepatoprotective functioning recorded by Anupam *et al.*, (2013) while estimating hepatoprotective and nephroprotective action of hydroalcoholic extract of leaves of *I. staphylina*.

In carbon tetrachloride caused liver damage in rabbits, the present results recommend that the treatment with *I. eriocarpa* extracts expressively reduced the greater level of serum marker enzymes including ALT, AST, ALP and bilirubin by defending and sustaining the biological role of liver cells. The elevation in protein levels revealed the effective activity of membranes of hepatic cells (Wahid *et al.*, 2009). This hepatoprotective property of the *I. eriocarpa* extracts may be due to presence of antioxidants such as phenolic and flavonoids compounds. The results of this research exhibit the hepatoprotective significance of ethanolic extract of *I. eriocarpa*. The similar results were showed by *Convolvulus arvensis* Ali *et al.* (2013) and *Malva praviflora* Mallhi *et al.*, (2014).

## **CONCLUSION**

The findings obtained after the phytochemical screening, and hepatoprotective potential of *I. eriocarpa* demonstrated that of the chemical constituents responsible for these activities. It is needed to be done the experiments to evaluate biological significance of plants. The main phytoconstituents from the plant are presently being isolated. The phytochemicals present in *I. eriocarpa* are alkaloids, flavonoids, terpenoids, saponins, carbohydrates, proteins, phenols and phytosterols. The extract of whole plant used for treatment of hepatotoxicity, its valuable effects can be very helpful for prevention of complications related to liver cells. *I. eriocarpa* can control improve liver enzyme markers by expressively reduced the greater level of serum enzymes including ALT, ALP bilirubin and AST by defending and sustaining the biological role of liver cells. The elevation in protein levels revealed the effective activity of membranes of hepatic cell.

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