# HEPATOPROTECTIVE ACTIVITY OF ETHANOLIC EXTRACT OF *OUGEINIA OOJEINENSIS* BARKS IN $CCL_4$ TREATED MALE RATS

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**Summary**

To examined the hepatoprotective effect of bark of ethanol extract of *Ougeinia oojeinensis* in male wistar albino rats treated with carbon tetrachloride. Liver damage was studied by assessing parameters such as serum glutamate oxaloacetate transaminase, glutamate pyruvate transaminase, alkaline phosphatase, acid phosphatase and bilirubin in serum. The effect of administration of ethanolic extract at dose of 100 mg/kg and 200 mg/kg on the above parameter was further investigated. Results of this study revealed that the suspension of ethanolic extract showed significant hepatoprotective activity ($P<0.05$) by reducing the levels of the biochemical parameters in experimental animals. The ethanolic extract of both doses afforded significant protection against $CCL_4$ induced hepatocellular injury. Histopathological studies too, are in conformity with findings.

**Key words** Carbon tetrachloride, marker enzymes, *Ougeinia oojeinensis*

## Introduction

Liver plays a major role in detoxification and excretion of many endogenous and exogenous compounds, any injury to it or impairment of its functions may lead to many implications on one's health. Management of liver disease is still a challenge to the modern medicine. The modern medicines have title to offer for alleviation of hepatic ailments instead of most important representatives are of phytoconstitutes[1]. *Ougeinia oojeinensis* (Roxb.) Hochr (Fabaceae) known in Hindi as Tinsa and in Sanskrit as Ratha is a deciduous trees, found in the outer Himalayas and sub-Himalayan tracts from Jammu to Bhutan up to an altitude of 1500m and extending through the whole of northern and central India into the greater part of Deccan peninsula[2-3]. The extract of the whole plant *O. oojeinensis* were scientifically evaluated for anti-inflammatory and analgesic activities in previous studies. The 50% of ethanolic extract of stem bark has been reported exhibit antispasmodic action [4].

Phytochemical investigated on *O. oojeinensis* have reported the presence of lupeol, hydroxlupeol, betulin and isoflavanones such as dalbergioidin , homoferreirin and ougenin[5-7]. Yet there is paucity of information regarding the activity of *O. oojeinensis* in hepatic protection. This study was undertaken to fill the lacuna in this regard.

## Materials and methods

Standard Chemical kits for Determination of SGOT, SGPT, ALP, ACP and Bilirubin, $CCl_4$ and 5% CMC.

**Plant materials:** The bark of *O. oojeinensis* was collected from the Lamber forest Raipur, Chhattisgarh in the month of May. The collected material was authenticated by Dr. P. Jayaraman, Botanist, Plant Anatomy Research Centre (PARC), Chennai. The plant was also compared with herbarium specimen maintained at Minor Forest Produce (Trading and Development) Co-Op. Fed. Ltd., Shankar Nagar, Raipur, Chhattisgarh, by Expert Medicinal Plant, Mr. S.N.Khotele. Dried powder of bark was exhaustively extracted successively in soxhlet apparatus, using petroleum ether, choloroform, ethylacetate, ethanol and distilled water repectively. The extracts were then made to powder by using rotary evaporator under reduced pressure. Bark of *O. oojeinensis* yielded 0.62%, 0.45%, 0.57%, 4.50% and 3.7% w/w powdered extract with petroleum ether, choloroform, ethylacetate, ethanol and distilled water respectively. Suspension of ethanolic extract was prepared using 5% Carboxy methyl cellulose (CMC) and were divided in two doses 100 mg/kg and 200 mg/ kg and subjected for hepatoprotective activity in $CCl_4$ induced hepatotoxicity.

**Experimental animals :** Male wistar albino rats having weight 180-230gm were kept in qurantine for 10days under standard husbandry conditions ($27.3^{o}$, Relative humidity 65 ±10%) for 12 hrs in dark and light cycle respectively and were given standard food and water *ad. libitum*. The study was permitted by the Institution Animal Ethical Committee with Reg. No. CPCSEA/265.

**Acute oral toxicity study:** Acute oral toxicity was performed by following OECD guideline – 420 fixed dose procedure for ethanolic extract and it was found that dose increasing upto 2000 mg/kg body wt. shown no toxicity or mortality in experimental rats. The $LD_{50}$ of the ethanolic bark extract as per OECD guidelines – 420 is greater then 2000 mg/kg[8].

**Experimental design**: Assessment of hepatoprotective activity was carried out on wistar albino rats. The animals were segregated into five groups of six animals each. Group I served as normal control receiving 5% CMC (10ml/kg). All other groups received $CCl_4$ (3ml /kg i.p.) with equal volume of olive oil (50% v/v) for two successive days. Group II animals were maintained as $CCl_4$ group, while group III, IV and V animals were treated orally for seven days with suspension of ethanolic extract (100 mg/kg), ethanolic extract (200 mg/kg) and reference drug silymarin (25 mg/kg), respectively. After the drug treatment all the animals were sacrificed by cervical dislocation.

Blood was collected from the carotid artery and was allowed to clot for 45 min at room temperature; serum was separated by centrifugation at 2500 rpm for 15 min, used for the estimation of various biochemical parameters[9-11].

**Biochemical estimation:** Biochemical parameters such as glutamate oxaloacetate transaminase (GOT), glutamate pyruvate transaminase (GPT), alkaline phosphatase (ALP), acid phosphatase (ACP) and serum bilirubin were determined[12-16].
**Histopathology :** the liver from each animal was removed after dissection, liver lobes were fixed for for 48 hours in 10% formalin and were embedded in parafifin. Subsequently, five section (T.S.) were cut on a microtome and stained with haematoxylin and eosin. These section were observed under microscope for histopathologival changes and finally, compared with that of normal system.

**Statistical analysis:** Results of estimation of biochemical and functional parameters have been reported as mean value ± SEM. The variation in a set of data has been estimated by performing one way analysis of variance (ANOVA). Individual comparisons of group mean values were done using Turkey's test (Sigma stat 3.5). P values $<0.05$ were considered statistically significant.

## Results

In the present studies, rats treated with $CCl_4$, developed significantly liver damage as observed from the elevated serum levels of hepato-specific enzymes as well as severe alteration in other biochemical parameters. Values of the biochemical parameters were observed to be increased in the $CCl_4$ treated rats.
Treatment with both doses of ethanolic extract of *O. oojeinensis* barks decreased the $CCl_4$ induced alteration in SGOT, SGPT, ALP, ACP, total bilirubin and direct bilirubin in blood. It is found that the test samples offer protection against toxin as evidenced by remarkable reduction in all biochemical parameters ($P<0.05$). Histopathological studies provided supportive evidence to the biochemical analysis. The control group showed the normal parenchymal architecture with cords of hepatocytes, portal tracts and central veins. Centrilobular necrosis accompanied by fatty changes and ballooning degeneration were observed in the remaining hepatocytes in the livers of rats treated with carbon tetrachloride. The toxin mediated histological changes in the liver section of rats of test groups were much less intensity than those observed in the rats of CCl4 treated group.

## Discussion

The degree of hepatotoxicity developed by $CCl_4$ can be observed by elevated level of bio chemical parameters which is attributed to the generation of trichloromethyl free radical during metabolism by hepatic microsomes which in turn cause peroxidation of lipid of cellular membrane. Hepatocellular necrosis leads to very high level of GOT and GPT released from liver in the blood. Among the two GPT is better index of liver injury, as liver GPT activity represents 90% of total enzyme present in the body. ALP activities on the other hand are related to the functioning of the hepatocytes, increase in its activity is due to increased synthesis in presence of increased biliary pressure.

**Table 1: Effect of different ethanolic extracts of *Ougeinia oojeinensis* barks against $CCl_4$ induced hepatotoxicity in albino rats.**

| Treatment | GOT (U/L) | GPT (U/L) | ALP (U/L) | ACP (U/L) | Bilirubin (mg/100 ml of blood | |
|---|---|---|---|---|---|---|
| | | | | | Direct (mg/dl) | Total (mg/dl) |
| Normal Control CMC 10ml/kg | 48.84 ± 0.97 | 152.30 ± 0.76 | 172.20 ± 0.61 | 162.21 ± 0.89 | 0.30 ± 0.01 | 0.41 ± 0.01 |
| $CCl_4$ 1ml/kg i.p | 1249.00* ± 2.84 | 2041.00* ± 0.95 | 396.90* ± 1.51 | 256.32* ± 0.77 | 2.49* ± 0.55 | 3.13* ± 0.71 |
| Ethanolic extract (100 mg/kg oral) | 81.93** ± 1.38 | 195.30** ± 0.74 | 208.50** ± 1.15 | 210.80** ± 0.94 | 0.52** ± 0.01 | 0.61 ** ± 0.01 |
| Ethanolic extract (200 mg/kg oral) | 52.75** ± 1.01 | 162.70** ± 0.73 | 181.00** ± 0.94 | 176.30** ± 0.65 | 0.38** ± 0.01 | 0.42** ± 0.01 |
| Silymarin (25 mg/kg) | 50.32** ± 0.75 | 157.40** ± 0.72 | 177.70** ± 0.95 | 172.10** ± 0.92 | 0.31** ± 0.01 | 0.40** ± 0.01 |

**Values are expressed as mean ± SEM, n = 6 in each group. *P<0.05 compared to control group, **P<0.05 compared to $CCL_4$ treated group.**

Reduction in the level of SGOT and SGPT towards the respective normal value is an indication of stabilization of plasma membrane as well as repair of hepatic tissue damages caused by carbon tetrachloride. This effect is an agreement with the view that serum level of transaminase return to normal with healing of hepatic parenchyma and regeneration of hepatocytes. Suppression of increased ALP activity with current depletion of raised bilirubin level, suggest the stability of the biliary dysfunction in rat liver during chronic hepatic injury with $CCl_4$[17].

The administration of ethanolic extract of bark of *O. oojeinensis* decrease the $CCl_4$ induced elevated enzyme levels suggest the protection of structural integrity of hepatocyte cell membrane or regeneration of damaged liver cell by the extracts. The effectiveness of the normal functional conditions of the liver is indicated by the decreased level of serum bilirubin.

The present study revealed that the ethanolic extracts of both doses possess significant protective effect against hepatotoxicity induced by Carbon tetrachloride

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