

# Toxicity Assessment, Evaluation of Antioxidant and Hepatoprotective Activity on *Cordia obliqua* Fruit Extracts

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## ABSTRACT

**Background:** *Cordia obliqua* Willd plant is a genus of flowering plants in the borage family, Boraginaceae. It is also known as clammy cherry. Very little research was carried out for identification of its medicinal importance when compared to other *Cordia* species. **Objective:** To determine the safe dose and to explore the in vivo antioxidant and hepatoprotective activity of *Cordia obliqua* fruits. **Methods:** As per our previous study the ethanolic and aqueous extracts were rich in phytoconstituents and exhibited good in vitro antioxidant effect. So the ethanolic and aqueous extracts were used for evaluation of activity. Acute toxicity study (LD<sub>50</sub>) was conducted according to OECD guidelines. For hepatoprotective activity paracetamol induced hepatotoxicity was studied using standard drug like Silymarin. The antioxidant potential of the plant extracts were tested using three tests viz, Reduced GSH, Catalase and SOD activity. **Results:** Acute toxicity studies showed the non-toxic nature of *Cordia obliqua* fruit extract upto dose of 3000mg/kg body weight. Administration of Paracetamol to rats increased the levels of marker enzymes like ALT, AST and ALP. Increase in the levels of these enzymes in serum indicates damage to the liver cells. Pretreatment with aqueous and ethanolic extracts of *Cordia obliqua* decreased the levels of ALT, AST, ALP and increased levels of total protein, total bilirubin, direct bilirubin and comparisons histology of cells of extract which are an indication for the hepatoprotective activity. **Conclusion:** The fruits of *Cordia obliqua* are safe and effective in treatment of hepatic disorders and prevent oxidation of cells.

**Key words:** Hepatotoxicity, Paracetamol, Silymarin, *Cordia obliqua*.

## INTRODUCTION

Liver is the second largest and vital organ in the body which plays a vital role in metabolism and maintenance of homeostasis in human system. Toxic liver injury produced by drugs and chemicals are similar to natural liver disease. Continuous use of agents like Rifampicin, Isoniazid, Paracetamol, Ethyl alcohol, Carbon tetrachloride, Ranitidine and Oral contraceptives of hormonal origin and chemicals used as food preservatives and agrochemicals are threatening the integrity of liver.<sup>1,2</sup> The effects of liver disease on hepatic metabolism of drugs are complex and difficult to predict, particularly when multiple drugs are administered simultaneously.<sup>3</sup> Undiscerning use of anti-malarial drugs<sup>4</sup>, Anti-tubercular drugs<sup>5</sup>, analgesics, oral contraceptives<sup>6</sup>, Pharmaceuticals (mainly antibiotics, chemotherapeutics and CNS active drugs).<sup>7</sup> Toxins in food (especially aflatoxin<sup>8</sup>, Viruses, bacteria, mushrooms, parasites like amoebas etc. may also infect the liver.<sup>9</sup> According to American Gastroenterological institutes official journal "Gastroenterology" recent survey has reported that they are 13% deaths has reported due to antibiotics and stated has "Idiosyncratic Drug Induced Liver Injury".

Herbal plants which contain wide variety of phytoconstituents which acts as a protective barrier for the liver and includes-monoterpenes, terpenoids, flavonoids, phenolic compounds, carotenoids, alkaloids, glycosides, organic acids,

lipids<sup>10</sup> Several Indian medicinal plants have been extensively used in the Indian traditional system of medicine for the management of liver disorders. In India over 40 polyherbal commercial formulations reputed to have hepatoprotective action is being used. Scrutiny of the literature indicates that 160 phyto-constituents from 101 plants belonging to 52 plant families have antihepatotoxic activity.<sup>11</sup>

In the present study, the fruits of *Cordia obliqua* were selected for evaluation of pharmacological activity and to identify the chemical constituents. *Cordia obliqua* Willd plant is a genus of flowering plants in the borage family, Boraginaceae. It contains about 300 species of shrubs and trees, which are found worldwide, mostly in warmer regions. It is a medium-sized deciduous tree and very vigorous in growth. Many of the species are commonly called manjack, Clammy Cherry, glue berries, sebesten, or snotty gobblers. According to traditional system, it possesses anthelmintic, purgative, diuretic, expectorant, antipyretic, hepatoprotective and analgesic action. The fruits are edible and used as pickle, vegetable, raw, cooked. They lessen thirst and the scalding of the urine, remove pain from the joints and the burning of the throat and are also effective in treating the diseases of the spleen.<sup>12</sup> It was found that fruits are traditionally used for the treatment of hepatic disorders.<sup>13,14</sup>

The leaves of *Cordia obliqua* were investigated for hepatoprotective.<sup>15</sup> Analgesic, antipyretic and anti-inflammatory activities.<sup>16</sup> Since the fruits were

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not investigated and reported for its hepatoprotective activity, this prompted us to select the fruits of *Cordia obliqua*.

## MATERIALS AND METHODS

### Collection and authentication of plant

*Cordia obliqua* fruits were collected from Chidambaram in month of September, herbarium was prepared & submitted. Identification (Specimen voucher- HPU:102/2019) has been done by Dr P Shivakumar Singh, Head Department of Botany, Palamuru University, Mahabubnagar, Telangana, India.

### Preparation of extracts

The fruits of *Cordia* were collected; shade dried, segregated, pulverized by a mechanical grinder and passed through a 40 mesh sieve. The powdered plant materials were stored in an air-tight container. Extraction of dried coarse powder of fruits was carried out by Soxhlet apparatus with different solvents such as Petroleum ether, Benzene, Chloroform, Acetone, Ethanol and finally macerated with Chloroform water by increasing order of polarity. The solvents were evaporated under reduced pressure to get semisolid masses and placed in desiccator for further use. The percentage yield of ethanol and water extract was found 12.76 and 34.78 w/w and it was dark brown in color. As per our previous studies the ethanolic and water extracts has exhibited in vitro antioxidant activity due to maximum number of Phyto-constituents as well as maximum total phenol content. So, the ethanol and aqueous extracts were selected for screening of biological activity.

### Preliminary phytochemical screening

Several phytochemical tests were performed for testing different chemical groups present in the extracts. The chemical tests for various phytoconstituents present in the extracts were carried out as described. Alkaloids were tested by Mayer's, Wagers, Hager's and Dragendorff's reagent, phenols were identified by lead acetate and ferric chloride solution, saponins by foam test, steroids were identified by Libermann-Burchard and Salkowski test, cardiac glycosides by Keller-Kiliani's and Legal's test, tannins by ferric chloride solution, Sugars were identified by Molisch's and Fehling's reagent, glycosides by acid hydrolysis followed by Molisch's test.<sup>17-19</sup>

### Experimental animals

Wistar rats of either sex with 8-12 weeks age, weighing 120-140g, were procured from the Sai Nath agency, Hyderabad, Telangana, India. The animals were set aside in cages, 2 per cage, with twelve: twelve hours light and dark cycle at  $25 \pm 2$  °C. The rats were maintained on their particular diets and water ad libitum. All experimental Procedure done with standard procedure prescribed by CPCSEA, India and protocol approved by Institutional Ethical Committee with IAEC/COP/19-20/02).

### Chemicals and drugs

The chemicals used in study were Silymarin and Paracetamol which were collected from Micro labs, Bangalore. 2-thiobarbituric acid and Ellman's reagent was purchased from Sigma Aldrich, Bangalore. All other solvents and chemicals of all the drugs and chemicals used were of Pharmaceutical grade.

### Determination of acute oral toxicity (LD<sub>50</sub>)

The acute oral toxicity study<sup>20</sup> of fruit extracts of *Cordia obliqua* was determined in female albino mice (120-140 g) maintained under standard husbandry conditions. The animals were fasted 4 h prior to the experiment and Up and Down procedure (OECD Guidelines No. 425) method was adopted for acute oral toxicity studies. Animals

were administered with single doses of each extract and observed for their physical behavior and mortality during 48 h study period (short term toxicity). Based on the short term profile of extracts the doses for the next animals were determined. All the animals were observed for long term toxicity (7 days). The LD<sub>50</sub> studies of the test extracts were conducted with 180 mg/ kg and use dose progression factor 3.2 single animals dose till body weight change or mortality and we met our criteria at dose level of 3000 mg/kg body weight.

### Paracetamol induced hepatotoxicity

Wistar rats weighing between (120-140 g) were divided into five groups of six rats in each group. Group A was served as normal control which was given with vehicle only, Group B with Paracetamol (2000 mg/Kg p.o), Group C with Silymarin (25 mg/Kg p.o) that serves as standard, Animals in groups D, E treated with 300 mg/kg of aqueous and ethanolic extracts respectively. B, C and D were intoxicated with Paracetamol (2 gm/Kg p.o) for 3 days and from 4<sup>th</sup>-10<sup>th</sup> day with different doses of *Cordia obliqua* in related groups respectively. On the 11<sup>th</sup> day, after recording thiopentone sodium sleeping time in all groups of animals and they were anaesthetized with ether. Blood was collected through cardiac puncture, later sacrificed by overdose of ether. Livers removed were washed with saline, weighed and stored in 10 % Formaldehyde for histological studies.<sup>21</sup>

### Histopathological studies

For light microscopic evaluation, liver tissues were fixed in 10% buffered formalin and then thin sections (4  $\mu$ m) were subsequently stained with hematoxylin/eosin for general histological features determination.<sup>22</sup> Sections were studied under light microscope Leica DM 500.

### In Vivo antioxidant activity on paracetamol induced hepatotoxicity in rats

The antioxidant potential of the plant extracts were tested using three tests viz, Reduced GSH, Catalase and SOD activity. Accurately weighed 1g of the liver tissue was taken and homogenized in 9 ml of 1.15% KCl with a Homogenizer. After successful homogenization, homogenized liver was kept in a freezer until it got frozen and later kept at room temperature to become liquefied. The same procedure was repeated for two freeze-thaw cycles. The homogenated liver was centrifuged at 3000 rpm at 4°C for 30 min. The supernatant of the liver homogenate was separated and used for assays. The activity of GSH and Catalase was determined by the method of Sinha<sup>23</sup> and Superoxide dismutase (SOD) activity by Markuland.<sup>24</sup>

### Statistical analysis

The obtained values in all five groups were expressed as mean with SD from 6 animals, subjected to statistical analysis using one-way analysis of variance followed by Dunnett's *t* test to verify significant difference if any among the groups. *P* < 0.05\*, 0.01\*\*, and 0.001\*\*\* was considered statistically significant.

## RESULTS

### Preliminary phytochemical analysis

Preliminary phytochemical investigation of *Cordia obliqua* fruit extracts was carried out in our previous study to identify different primary and secondary metabolites. Petroleum ether, benzene, chloroform and acetone extracts showed the presence of steroids and cardiac glycosides; alkaloids in ethanolic extract, saponin; phenols and tannins in ethanolic and aqueous extracts, carbohydrates in chloroform, acetone, ethanolic and aqueous extracts, gums and mucilage in aqueous extract.

## The acute oral toxicity study of fruit extracts of *Cordia obliqua*

Acute toxicity studies showed the non-toxic nature of the *Cordia obliqua* fruit extract upto dose of 3000 mg/kg body weight which is 10 times more than the dose used in the study. The studies followed OECD guidelines. Based on the observation there was no change in physical behavior and mortality of any animals when observed for 48 hr. There was no lethality or any toxic reactions found at any of the doses selected until the end of the study period.

## Effect of aqueous and Ethanolic fruit extracts of *Cordia obliqua* on serum biochemical parameters

ALT is an enzyme found in the liver that helps convert proteins into energy for the liver cells. When the liver is damaged, ALT is released into the bloodstream and levels increase. Aspartate transaminase (AST) is an enzyme that helps metabolize amino acids. Like ALT, AST is normally present in blood at low levels. An increase in AST levels may indicate liver damage, disease or muscle damage. Alkaline phosphatase (ALP). ALP is an enzyme found in the liver and bone and is important for breaking down proteins. Higher-than-normal levels of ALP may indicate liver damage or disease, such as a blocked bile duct, or certain bone diseases. Bilirubin is a substance produced during the normal breakdown of red blood cells. Bilirubin passes through the liver and is excreted in stool.

Elevated levels of bilirubin (jaundice) might indicate liver damage or disease or certain types of anemia.<sup>25</sup> The serum levels of Aspartate aminotransferase (AST), Alanine aminotransferase (ALT) and alkaline Phosphatase (ALP) was significantly increased and the levels of total bilirubin, direct bilirubin and total protein were significantly decreased in paracetamol intoxicated rats when compared to normal control group (Tables 1 and 2). The group which received fruit extracts of

*Cordia obliqua* at 300mg/kg body weight of both the extracts showed a significant increase in the mean levels of AST, ALT, ALP, TP, total bilirubin and direct bilirubin.

## Histopathology of liver

In normal group (control) liver sections showed the normal lobular architecture of the liver (Figure1). Histological studies revealed that liver show moderate vacuolar degeneration, severe congested vessels. (Paracetamol) (Figure2). Histology of liver showed no micro vascular fatty changes and mild central venous congestion.(Silymarin) (Figure3). In this group mild perivascular edema, moderate inflammatory cell infiltration was observed, (Ethanolic Extract) (Figure 4). Liver section showed very mild perivascular edema and very mild vacuolar degeneration and hemorrhage (Aqueous Extract) (Figure 5).

## In Vivo antioxidant activity on paracetamol induced hepatotoxicity in rats

The activities of GSH, SOD and CAT in liver were significantly lower in Paracetamol induced hepatotoxicity rats compared to control rats. Administration of *Cordia obliqua* fruit extract at both doses showed a significant increase in GSH, SOD and CAT activity when compared to paracetamol intoxicated group. The in vivo antioxidant assay showed that the extract increased the activity of serum superoxide dismutase (SOD) and catalase and glutathione (GSH). Catalase is a ubiquitous enzyme that catalyzes the decomposition of hydrogen peroxide, a reactive oxygen species, which is a toxic product of both normal aerobic metabolism and pathogenic ROS production.<sup>26,27</sup>

The SOD catalyzes the dismutation of superoxide to hydrogen peroxide and oxygen, thereby reducing the likelihood of superoxide anion reacting with nitric oxide to form reactive peroxynitrite.<sup>27</sup> Glutathione is largely known to minimize the lipid peroxidation of

**Table 1: Paracetamol Induced hepatotoxicity in fruit extract of *Cordia obliqua*.**

S.No	Direct bilirubin	Total Bilirubin	Total Protein	Albumin	ALP	ALT	AST
Normal	0.72 ± 0.29	1.19 ± 0.16	6.52 ± 1.07	5.15 ± 0.76	41.6 ± 3.64	14.91 ± 2.52	59.38 ± 3.51
Paracetamol intoxicated 2000 mg/kg	0.74 ± 0.19	1.14 ± 0.22	4.78 ± 0.29	3.16 ± 0.48	100.2 ± 8.34	40.8 ± 1.82	94.44 ± 4.91
Standard Silymarin treated 25mg/kg	0.74 ± 0.05*	0.86 ± 0.20*	8.20 ± 0.05	4.66 ± 0.27*	57.6 ± 8.44*	25.26 ± 1.95*	65.8 ± 3.49*
Alcoholic extract 300 mg/kg	1.25 ± 0.12*	2.8 ± 0.13*	8.09 ± 0.08*	5.02 ± 0.05	79 ± 3.93*	32.97 ± 3.74*	78.8 ± 8.18*
Aqueous extract 300 mg/kg	1.23 ± 0.13*	2.74 ± 0.19*	9.03 ± 0.8*	5.42 ± 0.62*	64.2 ± 3.89*	22.35 ± 2.96*	63.22 ± 6.48*

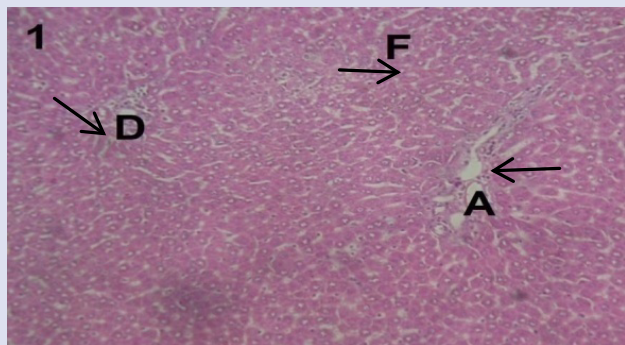
All Values are expressed as mean ± SEM (n=6), One Way Analysis of Variance. The result was significant at  $p < .05$ . \*Data differed significantly at  $p < 0.05$  when compared with the Paracetamol intoxicated group in the relevant column.

**Table 2: Effects on liver antioxidant enzymes level in Paracetamol induced hepatotoxicity in rats.**

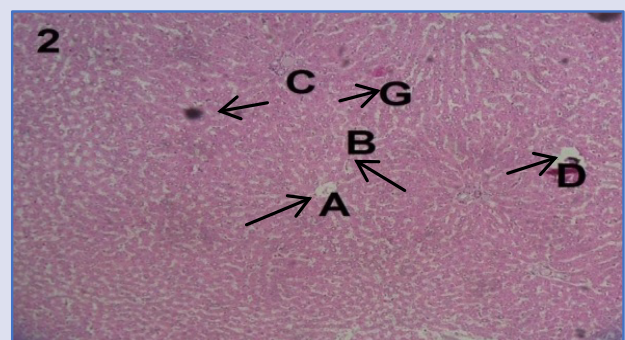
S.No	GSH (µg/mg protein)	CAT (µmoles H <sub>2</sub> O <sub>2</sub> /min/mg protein)	SOD (Unit/mg protein)
Normal	1.97 ± 0.15	4.06 ± 0.26	2.11 ± 0.20
Paracetamol intoxicated 2000 mg/kg	0.90 ± 0.44	1.19 ± 0.47	0.37 ± 0.07
Standard Silymarin treated 25mg/kg	1.74 ± 0.84*	3.83 ± 0.46	1.86 ± 0.16*
Alcoholic extract 300 mg/kg	1.58 ± 0.38*	3.18 ± 0.21*	1.07 ± 0.12*
Aqueous extract 300 mg/kg	1.39 ± 0.13*	2.51 ± 0.20*	0.94 ± 0.35*

All Values are expressed as mean ± SEM (n=6), One Way Analysis of Variance. The result was significant at  $p < 0.05$   
\*Data differed significantly at  $p < 0.05$  when compared with the Paracetamol intoxicated group in the relevant column.

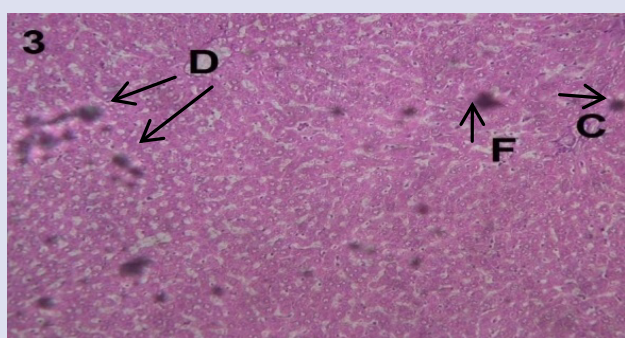




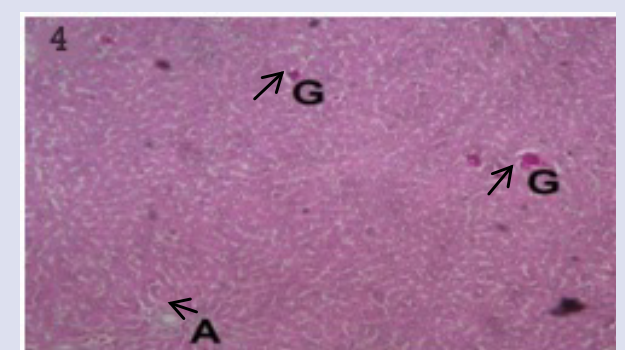
**Figure 1:** Histology of normal hepatic tissue.



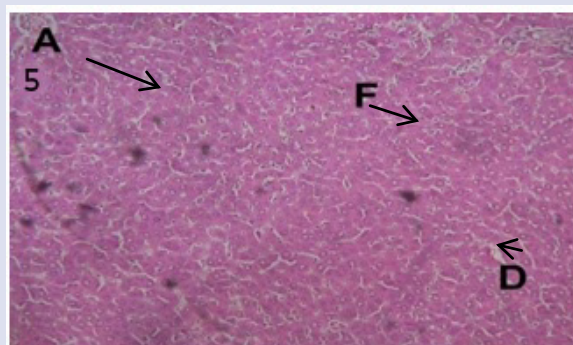
**Figure 2:** PCM induced damage in hepatic tissue.



**Figure 3:** Effect of Silymarin on PCM induced hepatic damage.



**Figure 4:** Effect of *Cordia obliqua* Ethanolic fruit extract on PCM induced hepatic damage.



**Figure 5:** Effect of *Cordia obliqua* aqueous fruit extract PCM induced hepatic damage.

A: Central Vein, B: Total Degeneration, C: Sinusoids, D: Portal Triad, E: Portal Inflammation, F: Plates of Hepatocytes, G: Pyknosis

cellular membranes and other such targets that is known to occur with oxidative stress. N-acetyl-cysteine is a by-product of glutathione and is popular due to its cysteine residues and the role it has on glutathione maintenance and metabolism.<sup>28</sup>

The increased serum activities of Catalase, GSH and SOD as observed in this study suggest that the extract has an *in vivo* antioxidant activity and is capable of ameliorating the effect of ROS in biologic system.<sup>29,30</sup>

## DISCUSSION

The study was carried out to evaluate the hepatoprotective and *in vivo* antioxidant activity of both aqueous and ethanolic fruit extracts of *Cordia obliqua* in paracetamol induced hepatotoxicity in Wistar Albino Rats. The results of present study showed that the extracts safe dose was 3000mg/kg. Liver injury is manifested in terms of increase in levels of serum aminotransferases, modest hepatic infiltration by both lymphocytes and eosinophils and slight focal hepatocellular necrosis also causes liver cholestasis associated with increased plasma bilirubin and alkaline Phosphatase. Paracetamol is a non-steroidal anti-inflammatory drug (NSAID), most commonly indicated in fever and mild to moderate pain like headache and myalgia. It is one of the safest NSAID in therapeutic dose and is well tolerated. PCM induces liver injury through the action of its toxic metabolite, N-acetyl-p-benzoquinone imine, produced by the action of Cytochrome P-450. Depletion of GSH causes the remaining quinone to bind to cellular macromolecules leading to cell death. Damage induced in the liver is accompanied by the increase in the activity of some serum enzymes. However, toxic doses of paracetamol cause severe hepatotoxicity characterized by abnormality of hepatic function and degeneration, necrosis or apoptosis of hepatocytes that leads to liver failure in both human and animals. Paracetamol has been taken as test model to screen the anti-hepatotoxicity of indigenous drugs.

In the present study it was observed that Chronic administration of Paracetamol to rats increased the levels of marker enzymes like ALT, AST and ALP as these are stored in the liver cells and increase in the levels of these marker enzymes in serum indicate damage to the liver cells. Pretreatment with aqueous and ethanolic fruit extracts of *Cordia obliqua* decreased the levels of ALT, AST, ALP and increased levels of total protein, total bilirubin and direct bilirubin is an indication for the hepatoprotective activity of these extracts against drug induced hepatotoxicity.

## CONCLUSION

The acute toxicity assessment revealed the safe dose of fruits and evaluated the hepatoprotective property of *Cordia obliqua* fruit extracts in paracetamol induced oxidative stress and hepatotoxicity in rats. The study correlated the hepatoprotective activity of *Cordia obliqua* fruit extract in both the extracts,; both the extracts has showed better hepatoprotective activity and it may be due to the presence of Alkaloids, Phenols and Tannins which exhibited free radical-scavenging and antioxidant activities.

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## CONFLICTS OF INTEREST

The authors have no conflicts of interest.

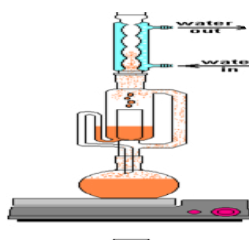
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## GRAPHICAL ABSTRACT



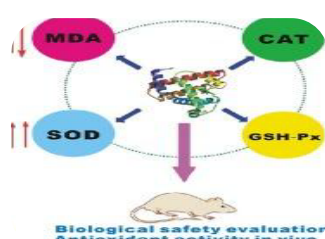
Fruit selection



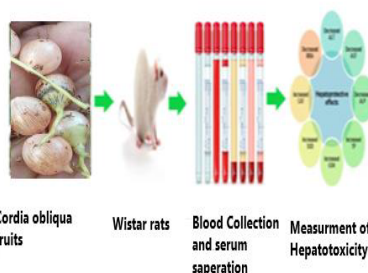
Extraction

Qualitative analysis methods		
Sl.No.	Phytoconstituents	Test
1	Alkaloids	Dragendorff's test
2	Carbohydrates	Benedict's test
3	Reducing sugars	Fehling's test
4	Saponins	Emulsion test
5	Phenols	Sublimation test
6	Flavonoids	Shimada's test
7	Terpenoids	Libermann-Bouchard's test
8	Proteins	Biuret test
9	Enzymes	Lead acetate test
10	Cardiac glycosides	Lead acetate test
11	Protein Amino acids	Ninhydrin test
12	Starch	Iodine test
13	Alkaloids	Dragendorff's test
14	Carbohydrates	Benedict's test
15	Reducing sugars	Fehling's test
16	Saponins	Emulsion test
17	Phenols	Sublimation test
18	Flavonoids	Shimada's test
19	Terpenoids	Libermann-Bouchard's test
20	Proteins	Biuret test
21	Enzymes	Lead acetate test
22	Cardiac glycosides	Lead acetate test
23	Protein Amino acids	Ninhydrin test
24	Starch	Iodine test

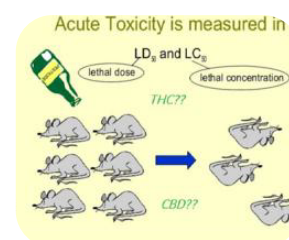
Phytochemical Screening



Estimation of In vivo Antioxidant activity



Evaluation of Hepatoprotective activity



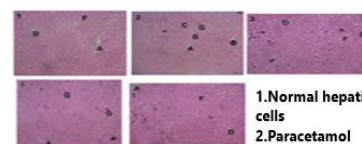
Toxicity assesment  
3000 mg/kg body

Antioxidant Enzyme	Control	Paracetamol	Cordia obliqua
MDA	0.50 ± 0.04	0.50 ± 0.04	0.50 ± 0.04
SOD	0.50 ± 0.04	0.50 ± 0.04	0.50 ± 0.04
CAT	0.50 ± 0.04	0.50 ± 0.04	0.50 ± 0.04
GSH Px	0.50 ± 0.04	0.50 ± 0.04	0.50 ± 0.04

Effects on liver antioxidant enzymes level in Paracetamol induced hepatotoxicity in rats

Sl.No	Control	Paracetamol	Cordia obliqua
1	0.72 ± 0.29	0.72 ± 0.29	0.72 ± 0.29
2	0.72 ± 0.29	0.72 ± 0.29	0.72 ± 0.29
3	0.72 ± 0.29	0.72 ± 0.29	0.72 ± 0.29
4	0.72 ± 0.29	0.72 ± 0.29	0.72 ± 0.29
5	0.72 ± 0.29	0.72 ± 0.29	0.72 ± 0.29
6	0.72 ± 0.29	0.72 ± 0.29	0.72 ± 0.29
7	0.72 ± 0.29	0.72 ± 0.29	0.72 ± 0.29
8	0.72 ± 0.29	0.72 ± 0.29	0.72 ± 0.29
9	0.72 ± 0.29	0.72 ± 0.29	0.72 ± 0.29
10	0.72 ± 0.29	0.72 ± 0.29	0.72 ± 0.29
11	0.72 ± 0.29	0.72 ± 0.29	0.72 ± 0.29
12	0.72 ± 0.29	0.72 ± 0.29	0.72 ± 0.29
13	0.72 ± 0.29	0.72 ± 0.29	0.72 ± 0.29
14	0.72 ± 0.29	0.72 ± 0.29	0.72 ± 0.29
15	0.72 ± 0.29	0.72 ± 0.29	0.72 ± 0.29
16	0.72 ± 0.29	0.72 ± 0.29	0.72 ± 0.29
17	0.72 ± 0.29	0.72 ± 0.29	0.72 ± 0.29
18	0.72 ± 0.29	0.72 ± 0.29	0.72 ± 0.29
19	0.72 ± 0.29	0.72 ± 0.29	0.72 ± 0.29
20	0.72 ± 0.29	0.72 ± 0.29	0.72 ± 0.29

Paracetamol Induced hepatotoxicity in fruit extract of Cordia obliqua



1.Normal hepatic cells  
2.Paracetamol  
3.Silymarin  
4.Ethanol extract  
5.Aqueous extract  
A : Central Vein, B : Total Degeneration, C : Sinusoids, D : Portal Triad, E : Portal Inflammation, F : Plates of Hepatocytes, G : Pyknosis

Histopathology of liver

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