Indian Journal of Pharmacy and Pharmacology 2022;9(1):67–74

Content available at: https://www.ipinnovative.com/open-access-journals

Indian Journal of Pharmacy and Pharmacology

Journal homepage: https://www.ijpp.org.in/

Original Research Article

Antioxidant and hepatoprotective effects of *ougeinia dalbergioides* benth against paracetamol and ccl$_4$ induced liver damage in rats

Sarita Karole[1], Deepak Kumar Jain[2,*], Sahil Abbas[3], Nisha Abbas[3,4], Pradeep Kare

[1]*Dept. of Pharmacy, Oriental College of Pharmacy, Bhopal, Madhya Pradesh, India*
[2]*Dept. of Medicinal Chemistry Research Laboratory, SLT Institute of Pharmaceutical Sciences, Guru Ghasidas Central University, Bilaspur, Chhattisgarh, India*
[3]*Dept. of Pharmacy, College of Pharmacy, Agra, Uttar Pradesh, India*

ARTICLE INFO

Article history:
Received 03-12-2021
Accepted 28-12-2021
Available online 23-03-2022

Keywords:
Hepatic diseases
Antioxidant
Hepatoprotective activity
Ougeinia dalbergioides

ABSTRACT

Hepatic diseases are flattering ordinary day by day and pose grave health threats to the life of humans. In sort to treat these diseases, the awareness of man is diverting towards herbal drugs, which are much safer and cost effectual than synthetic drugs. The aspire of this work was to explore antioxidant and hepatoprotective activity of ethanolic and aqueous extract of *Ougeinia dalbergioides* (*O. dalbergioides*) bark for treatment of liver damage induced by CCl_4 and paracetamol (PCM) in rats. In vitro antioxidant activities aqueous and ethanolic extracts were also checked using 1-1-diphenyl-2-picryl hydrazine (DPPH), nitric oxide (NO), ABTS scavenging method. *O. dalbergioides* at chosen oral doses of 250 mg/kg and 500 mg/kg levels of serum marker enzymes such as ALP, SGPT, SGOT, total bilirubin and direct bilirubin was reduced which indicate major hepatoprotective effects, when contrasted with silymarin and negative control. All the parameters wereobserved at oral dose of 500 mg/kg with extreme effects. The significant anti-oxidant activity was also demonstrated by the extracts. The preliminary screening of phytochemical *O. dalbergioides* indicates the presence of phenolics, tannins, flavonoids, carbohydrate and phytosterols. It is conceivable that extract of *O. dalbergioides* has activity of hepatoprotective against CCl_4 and paracetamol induced hepatotoxicity, due to the scavenging of free radical mechanisms demonstrated by phenolics and flavonoids, thus asserting its medicinal therapeutic role in liver diseases.

1. Introduction

The sequence of liver disorders influenced by the oxidative stress.[1] Liver disease prevents by the cellular antioxidant systems by diminishing oxidative stress.[2] Cell membranes, DNA and proteins injured because of high oxidative stress. Thus, it imparts toxicity, like muscular dystrophy andliver cancers. Some adverse effects are related with presentlyaccessible synthetic antioxidants. Consequently, natural antioxidants are being recognized because their improved effectiveness and safety[3–6] Liver is the major internal organ of body having hepatocytes which are its functional entities. Liver independently performs up to 500 functions, typically by interacting with other systems and organs. The chief job of liver is to detoxify toxic substances from body and to metabolize ingested substances (nutritional supplements, food, alcohol or medicines). Along with it, liver controls glycogen storage and breakdown of red blood cells; produce plasma proteins and synthesize hormones.[7] The damage produced in liver is hepatotoxicity and it is resulting by chemicals.[8] Hepatotoxicity is also defined as obliterationor malfunction to liver because of xenobiotic or drugs known as hepatotoxins when they are given in overeat.[9] Liver injury

* *Corresponding author.*
E-mail address: jaindeepak2022@gmail.com (D. Kumar Jain).

https://doi.org/10.18231/j.ijpp.2022.012

can also be induced as a consequence of oxidative stress which is persuaded by stimulation of enzymes in CYP 450 system likeCYP_2E.[10] Hepatotoxicity injures liver in both gentle and severe forms. The capability of any compound, formulation or product to stop damage to the liver is known as hepato protection or anti-hepatotoxicity.CCl_4 induced hepatotoxicity accrues in parenchymatic cells of liver and cytochrome P450- dependent monooxygenases in liver metabolized it to CCl_3 * radicals, thus rapidly injured the hepatocytes.[11] Paracetamol is an extensively used analgesic drug but over dosage of its causes hepatotoxicity and nephrotoxicity as it is detoxified in the liver in the form of sulphate or glucuronide which escorts to creation of reactive N-acetyl p-benzoquinonimine metabolite which binds to cellular macromolecules covalently andbegin cell damage.[12] Numerousdrugs which are synthetic being administered in the executive of liver mayhems havinggrave side effects, because of which there is an increasing interest to trailuniversal study method and to evaluate technical basis for conventionalnaturaldrugs that are conserved to retain hepatoprotective activity.[13] There is an integer of herbal preparations in Ayurveda for management of hepatic diseases in the deficiency of a dependable liver defensive drug in contemporary remedy.[14] Numerous studies have defensible that phytotherapeutic agents who possess antioxidant activity are effectual in producing hepatoprotection against PCM and CCl_4 induced hepatotoxicity.[15]

2. Materials and Methods

2.1. Plant material

The barks of *O. dalbergioides* were obtained from the Medicinal Garden of Oriental College of Pharmacy Raisen Rd, Patel Nagar, Bhopal, MP 462022 in March 2019. The barks were identified by Dr. Padma Shrivastava, Professor (Botany), Govt. P.G College, Mathma Gandhi Road, Pipalani, Berkheda, P.O. Bhel, Huzur (T), Bhopal (Dist.), MP 462022. A receipt sample (Accession no.650) of the genuine *O. dalbergioides* barks has been placed in the herbarium of the College.

2.2. Drugs and chemicals

liquid paraffin, CCl_4 and sucrose was procured from Qualigens Fine Chemicals, Mumbai, India.Silymarin,2,2-Diphenyl-1-(2,4,6-trinitrophenyl)hydrazyl**,** 2,2'-azinobis-(3-ethylbenzothiazoline-6-sulfonic acid) , sodium nitroprusside, potassium ferricyanide, ascorbic acid was procured from Sigma Aldrich Company (St. Louis, MO, USA). Paracetamol acquired as gift sample by Ranbaxy Lab Pvt. Ltd. Dewas. Assessment of marker enzymes for liver functions such as SGOT, SGPT, total bilirubin, ALP were performed using kits supplied by Span Diagnostic Ltd., Surat, India. Other required chemicals and reagents used duringexperimentations were of analytical grade and accessible commercially via renowned sellers.

2.3. Extraction

Parts of herbal plant (barks) of *O. dalbergioides* were dried in gloom at room temperature. The dehydrated barks in shade were coarsely grounded and extracted using soxhlet apparatus with petroleum ether (60-75°C). The process of extraction was stopped after defatting of the material. Initially 250gm of plant powered was packed into the thimble and 2 liters of solvent (Water, Ethanol) used for extraction was poured into flask (Round Bottom) separately. The soxhlet extraction process was executed for 18-24hrs till the obtained liquid in siphon tube shows clear nature. Afterward under low pressure extracted liquid was evaporated to obtain dried powder of extract. At the end% yields of the dried extracts were determined.[16]

2.4. Qualitative phytochemical analysis of extract

The *O. dalbergioides* plant extract obtained was subjected to the preliminary phytochemical analysis following standard methods.[17,18] The collected extract was monitored for identification of different active principles such as flavonoids, phenolic compounds, saponins, tannins, alkaloids, glycosides, carbohydrates, fixed oils or fats, amino acids and protein.

2.5. Experimental animals

Wistar rats weighing 180-240gm of either sex procured from the central animal house, Pinnacle Biomedical Research Institute (PBRI) Bhopal MP which was used in the experiment. The procured rats were housed in clean, bigcages of polypropylene in a specific temperature-controlled room 22±2°C having RH (44-55%) where 12-hr light and dark cycles are maintained. All the rats were adapted to environment of laboratory for at least one week prior to study. In a noise-free room all the experiments were carried out between 08.00 to 15.00 hr. Rats were provided with a clean drinking water ad libitum and standard rodent pellet diet purchased from Lipton India, Bangalore, India. The use and care of experimental animals were severelyaccording to the guidelines given by the Institutional Animal Ethical Committee constituted as per the norms of CPCSEA, India.

2.5.1. Acute oral toxicity

Wistar rats which are healthy, fasted overnight were screened for acute toxicity identification studiesfor the determination of non-observable adverse effect dose level by using of acute toxic class method of oral toxicity as per guidelines of Organization for Economic Co-operation and Development (OECD) 423. The experimental rats (n=3) were consumed *O. dalbergioides* extract in the

maximum test dose of 2000 mg/kg and detectedcontinually for autonomic profiles, neurological, and behavioral for the period of 2hr and after for 24, 72hr and after that up to 14 days for any moribund state, lethality or expiry of animal. In another group of rats (n=3) the limit test was repeated for estimated LD50 determination and corroboration.[19]

2.6. In-vitro antioxidant activity

By using the standard methods, the ethanolic and aqueous extract of *O. dalbergioides* was screened for its in vitro antioxidant activity. The particular conc. Of the obtained extract was used in all these methods. In this, after addition of all reagents it gives a final conc. Of 50-1000μg/ml. The absorbance (Abs) of the extract or standards, but without the reagents was calculated against a blank solution. Without the extract or standards the control test was executed. IC50 value and % scavenging which is the conc. of the sample obliged for scavenging of 50% of the free radicals was determined.

2.6.1. DPPH radical scavenging method

This test was conceded out as given by.[20]In 96-well plate having 2.5ml of various concentrations (50-300μg/ml) of the extract or standards, an aliquot of 0.5ml of 0.1mM DPPH in methanol was added. At room temperature mixing of reaction mixture is carried out and detained for 20min. The Abs was checked at 517nm against a blank. By comparing Abs with that of a blank solution the scavenging activity of each fraction of free radical was determined. By using following equation the capability of DPPH radical scavenging was calculated which is expressed as % inhibition:

2.7. DPPH scavenging activity (%) = $(A_0\text{-}A_1)/A_o \times 100$

Where A_1 is the Abs of the sample and A_0 is the Abs of the control.

2.7.1. NO radical inhibition assay

NO was measured by using Griess reaction which is created from sodium nitroprusside (SNP). At physiological pH in aqueous solution the SNP impulsively creates NO which interrelates with oxygen to yield nitric ions which is determined by Griess reagent. NO scavengers compete with the oxygen leading to declined production of nitric oxide. The mixture of reaction (6ml) containing phosphate buffer saline (PBS, pH 7.4, 1ml), SNP (10mM, 4ml) and standard solution or extract (1ml) at different concentrations which was incubated at 25^{o}C for 150min.0.5ml of the reaction mixture containing nitrite ion was removed after incubation process, 1ml of sulfanilic acid reagent (0.33% in 20% glacial acetic acid) was mixed and kept aside for 5min. After completion ofdiazotization reaction, 1ml of NEDD was added, stirred and kept aside for 30min in diffused light. The Abs of sample was measured at 540nm against the blank solutions.[21]

2.7.2. ABTS radical scavenging activity

To 0.2ml of different concentrations of standard or extract, 0.16 ml of ABTS solutionand 1.0ml of distilled DMSO were added. The reaction mixture was mixed homogenously and incubated for at least 20 min. Abs of these samples were measured at 734nm spectrophotometrically.[22]

2.8. In vivo hepatoprotective activity

2.8.1. CCl4induced hepatotoxicity in rats

The dilution of CCl_4 was carried out using liquid paraffin (1:1) prior to consumption. The experimental rats were alienated into 7 groups, every group containing 6 rats. The rats were then screened to either one of the following treatments for next 9 days

1. **Group 1:** Control; distilled water (1ml/kg, po)
2. **Group 2:** Toxicant; distilled water for 9 days + CCl_4 (1ml/kg, po) on ninth day.
3. **Group 3:** Standard; silymarin (100mg/kg/day, po) for 9 days + CCl_4 (1ml/kg po) on ninth day.
4. **Group 4:** Test; aqueous extract (250mg/kg/day, po) for 9 days + CCl_4 (1ml/kg po) on ninth day.
5. **Group 5:** Test; aqueous extract (500mg/kg/day, po) for 9 days + CCl_4 (1ml/kg po) on ninth day.
6. **Group 6:** Test; ethanolic extract (250mg/kg/day, po) for 9 days + CCl_4 (1ml/kg po) on ninth day.
7. **Group 7:** Test; ethanolic extract (500mg/kg/day, po) for 9 days + CCl_4 (1ml/kg po) on ninth day.

Rats were fastened 12 hr before CCl_4administration to increase the acute liver damage in rats of groups 2-7. The rats were sacrificed after the 24hr of administrations of CCl_4. Blood samples from sacrificed rats were collected and the serum was used for assay of various marker enzymes such as SGOT, SGPT, ALP, and serum bilirubin.[23]

2.8.2. PCM induced liver damage in rats

The similar procedure was followed which is mentioned above other than the liver was damage using PCM (1g/kg po) which is diluted with sucrose solution (40% w/v). PCM was consumed in 3 equal doses on day 9 and animals were sacrificed after the 48hr of consumption of PCM. Blood sample from animals were collected and serum was used for assay of marker enzymes such as SGOT, SGPT, ALP, and serum bilirubin.[23]

2.8.3. Biochemical studies

At the 9^{th} day of the experimental studies, blood sample was withdrawn by using micro-capillary method from the retro-orbital plexus of ratby giving light ether anesthesia. A glass capillary is inserted by using a slight thumb pressure

into the medial canthus of the eye (30 degree angle to the nose) to enter the plexus/sinus by puncturing tissues. When the plexus is punctured, blood will withdraw through the glass capillary tube which was collected in 1.5ml Eppendorff tubes. The glass capillary tube is then softly removed and wiped with sterile cotton. Bleeding can be blocked by applying light finger pressure on the eye.The collected blood samples were allowed to clot for 45min at RT. Serum was separatedfrom blood by centrifugation at 2500 rpm for 15 min at 30°C and used for the determination of different biochemical parameters namely SGOT, SGPT, ALP and serum bilirubinusing semi-autoanalyser.[24–28]

2.9. Statistical analysis

The multiple comparisons with the Dunett-t-test were followed by one-way analysis of variance (ANOVA) to compare various parameters between the groups. A p value P< 0.01was considered significant.

3. Results

The outcomes of qualitative analysis of phytochemicals of the raw powder barks of *O. dalbergioides* are exposed in Table 1. Ethanolic and water extracts of barks of *O. dalbergioides* indicates the occurrence of phenolics, flavonoids, carbohydrate, tannins and Phytosterols. ABTS and scavenging activity of DPPH radical of aqueous and ethanolic extract of *O. dalbergioides* was given in Table 2. The extract showed potent activity against radical scavenging in the both methods in conc. dependent way (50-300μg/ml). The value of IC50 of extract was equivalent to the standards (ascorbic acid) used. The aqueous extract showed lower IC50 value than ethanolic extract in both models. Ethanolic extract of O. dalbergioides demonstrated a strong activity ofnitric oxide scavenging which was equivalent to aqueous extract of O. dalbergioides. The IC50 value of ethanolic extract of O. dalbergioides was less than aqueous extract of O. dalbergioides but close to ascorbic acid. IC50 values of standards and extract were shownin Table 2. Acute oral toxicity studies illustrated that the extract of *O. dalbergioides* was secure up to a dose of 2,000 mg/kg of body weight and NOAEL dose is greater than 2,000 mg/kg. No death or moribund state or any toxic reactions were seen up to the end of the observation time period of 14 days.Alterations in the activities of serum enzymes ALP, SGOT and SGPT and total bilirubin in the serum of PCM (1gm/kg po) and CCl_4-induced (1ml/kg po) liver injuries in experimental rats as confirmation from Table 3, 4. The serum marker enzyme levels ALP, SGOT, SGPT and bilirubin were shows considerable increasein CCl_4 and PCM — induced liver injuries in rats when contrasted with control group (P< 0.01).Although, management with aqueous and ethanolic extract at oral dose 250 and 500 mg/kg for 9days showed noteworthy hepatoprotective activity in terms of serum ALP, SGOT, SGPT and level of total bilirubin in CCl_4 and PCM-induced liver injuries in experimental rats as contrasted to control groups. Silymarin (100mg/kg) also major decline in the serum enzymes level and bilirubin level in PCM and CCl_4-treated groups as contrasted with respective control group.

Table 1: Phytochemical evaluation of *O. dalbergioides* bark extracts

S. No.	Constituents	Ethanol extract	Aqueous extract
1.	Alkaloids	-	–
2.	Glycosides	–	–
3.	Flavonoids	+	+
4.	Saponins	–	–
5.	Phenolics	+	–
6.	Proteins and amino acids	–	–
7.	Carbohydrate	+	+
8.	Tannins	+	–
9.	Phytosterols	+	–

4. Discussion and Conclusion

Free radicals are defined as the chemical species which holds one or more unpaired electrons. They are extremely unstable and cause destructionof other molecules by removing electrons from them to conquer stability. They are formed into the system and are potentially damaging, highly reactive and fleeting chemical species. These radicals are necessary for detoxification, chemical signaling, energy supply and immune function therefor they are incessantly generated in the body of human. Endogenous antioxidant enzyme system regulates free radicals, but because of more generation of free radicals by contact to ecological oxidant elements such as UV radiation, smoking of cigaretteetc or a malfunction in mechanism of antioxidant defense or impairment to cellular structures, the risk enhance for numerous disorders such as liver diseases, cardiovascular disorders, ulcerative colitis, mild congestive impairment, parkinson's disease, alzheimer's disease, cancer and inflammation.[29]Fascinatingly, the human body has defense mechanisms counter tooxidative stress induced by free radical, which includesrepair and defensive mechanisms, i.e. physical and antioxidant defense. Enzymatic antioxidants likecatalase, glutathione peroxidase, superoxide dismutase, etcas well as non-enzymatic antioxidants likeascorbic acid, phenolic compounds, flavonoids, carotenoids, etc act by one or more than one mechanisms like scavenging of free radicals, reducing activity, quenching of singlet oxygen and potential complexing of pro-oxidant metals. It is potential to decrease the threats of chronic disorders and prevention of disease progression by either increasing the

Table 2: DPPH, ABTS, NO radical scavenging activity of *O. dalbergioides* extract (mean±SEM)

IC50 (μg/ml)* Drug/Std.	DPPH radical scavenging activity		ABTS radical scavenging assay		Nitric oxide radical scavenging assay	
	Aq. Extract	**Eth. Extract**	**Aq. Extract**	**Eth. Extract**	**Aq. Extract**	**Eth. Extract**
O. dalbergioides	230.02±4.23	249.98±5.88	175.66±2.55	225.15±3.64	263.72±2.42	215.03±5.86
Ascorbic acid	198.08±1.02	198.08±1.02	159.97±3.38	159.97±3.38	200.42±5.90	200.42±5.90

*Mean of three determinations

Table 3: Effect of aqueous and ethanol extract of *O. dalbergioides* on enzyme SGOT, SGPT, ALP and total bilirubin levels in serum of CCl_4 induced hepatotoxicity (Each values represent the Mean ± SEM)

Treatment	Dose (po, mg/kg)	SGOT(U/L)	SGPT(U/L)	ALP(U/L)	Direct Bilirubin(mg/dl)	Total Bilirubin(mg/dl)
Vehicle control	—	96.02±1.79**	81.53±3.30**	224.85±1.90**	0.11±0.03**	1.5±0.13**
CCl_4 Control	–	309.69±1.96	250.75±4.72	479.54±4.75	2.27±0.06	3.47±0.16
CCl_4 +Silymarin	100	124.32±3.10**	100.58±0.73**	258.43±5.90**	0.64±0.19**	2.18±0.09**
CCl_4 + Aqueous extract	250	198.05±4.14**	180.06±2.45**	387.66±3.03**	1.9±0.03**	3.02±0.11**
CCl_4 + Aqueous extract	500	158.05±5.15**	121.08±3.49**	295.96±4.00**	1.3±0.04**	2.96±0.04**
CCl_4 + Ethanolic extract	250	187.34±3.27**	175.3±1.68**	389.21±1.88**	1.85±0.20**	3.86±0.01**
CCl_4 + Ethanolic extract	500	147.94±3.28**	111.3±1.79**	289.41±2.89**	0.85±0.10**	2.86±0.02**

(n=6). ** P< 0.01)

Table 4: Effect of aqueous and ethanol extract of *O. dalbergioides* on enzyme SGOT, SGPT and total bilirubin levels in serum of paracetamol induced hepatotoxicity (Each values represent the Mean ± SEM)

Treatment	Dose (po, mg/kg)	SGOT(U/L)	SGPT(U/L)	Total Bilirubin(mg/dl))	Direct Bilirubin(mg/dl)
Vehicle control	—	130±2.08**	52.39±0.41**	1.14±0.02**	0.62±0.03**
PCMControl	–	275.1±1.82	163.12±1.57	3.35±0.13	2.57±0.02
PCM+Silymarin	100	145±1.26**	65.04±0.52**	1.17±0.01**	1.75±0.03**
PCM + Aqueous extract	250	250±1.26**	153.25±1.54**	3.02±0.01**	1.79±0.03**
PCM + Aqueous extract	500	212±1.29**	81.29±1.57**	2.74±0.02**	1.32±0.02**
PCM+ Ethanolic extract	250	258±2.41**	125.12±0.33**	2.98±0.01**	1.64±0.01**
PCM+ Ethanolic extract	500	209±2.91**	75.19±0.73**	2.22±0.01**	1.29±0.03**

(n=6). ** P< 0.01)

body's natural antioxidant protection or complementing with establishedantioxidants. Therefore detection of herbal antioxidants is a major thrustarea.[30] Different in vitro models were used to study antioxidant activity of the aqueous and ethanolic extract of *O. dalbergioides*. Subsequently, free radicals are of various chemical bodies, to corroborate its antioxidant activity it is important to test extract against several free radicals. IC50 values found were compared with the standard samples. ABTS radical scavenging activity is reasonablynewmethod, which includes a severe radical, chemically generated and is regularly used to screenversatilemixtures of antioxidant like plant beverages, extracts and biological fluids. The capability in aqueous and organic media and the stability in a large pH range increasedconcentration in the use of $ABTS^{+}$ for judgment of antioxidant activity.[31] In ABTS method, the extract disclosedefficient antioxidant activity which is equal to the standard. Here, direct role of phenolic compounds in free radical scavengingwas shown by the extract's radical scavenging activity. The DPPH has been widely accepted, stable free radical, which is used as tool for determiningfree radical scavenging activity of antioxidants. DPPH accepts hydrogen radical or an electron to become a stable diamagnetic fragment.[32] The reduction ability of DPPH radical is estimated by the reduction in absorbance at 517 nm persuaded by antioxidants. The investigationalinformations of the extract exposed that the extract have the free radicals scavenging activity. From the outcomes we observe a relationship in the DPPH radical scavenging activity which is dose dependent. The contribution of free radicals, mainly their enhancedgeneration, seems to be a characteristic of most of the human disorders including cancer and heart diseases. It has been establish that flavonoids, tannins, tocopherols, ascorbic acid, cysteine, aromatic amines and glutathione decrease and by their hydrogen donating ability it decolorize the DPPH. Phenolic compounds and Flavonoids of *O. dalbergioides* extracts are perhaps intricate in its activity of antiradical.[33] Nitric oxide produced during its reduction in presence of oxygen or with various superoxides, such as N_2O_4, NO_2, N_3O_4 is highly reactive. These radicals are accountable for changing the functional performance and structure of numerous components of cell. The *O. dalbergioides* extracts showed improved activity with oxygen to react with nitric oxide and thus blocks the production of anions and the activity is comparable to the standards. The secondary metabolites of the plant may have properties to offset the outcome of nitric oxide generation and in turn may be significantly interested in avoiding the adverse effects of extreme nitric oxide production in the human body. Additionally scavenging activity also helps to arrest the reactions orchains initiate by surplus formation of nitric oxide that is hazardous to the health of human. Nitric oxide is also concerned for cancer, inflammation and other diseases.In the appraisal of liver injury by CCl_4 and PCM hepatotoxin, the estimation of levels of different enzymes such as SGOT and SGPT is mainly used. Membrane damage or necrosis discharges the enzyme into blood circulation; therefore, it can be determined in serum. Increased levels of SGOT shows liver injurieslike viral hepatitis as well as cardiac infarction and muscle damages. SGPT catalyses the alteration of alanine to glutamate and pyruvate which is released in a similar way. So, SGPT is highlyspecific to the liver, thus better parameter for identifying liver damages.[34,35] Our outcomes using the model of CCl4 andPCM — induced liver toxicity in the rats proved that *O. dalbergioides* extracts at oral dose 250 and 500mg/kg caused remarkablereduction of SGOTandSGPT levels. Bilirubinand ALPlevels in serum are related to the function of liver cell. Increase in serum level of ALP is due to increased synthesis in presence of increasing biliary pressure.[36] Our results confirmed that *O. dalbergioides* extracts at 250 and 500mg/kg caused noteworthy inhibition of ALP and bilirubin levels. Efficient manage of bilirubin level and alkaline phosphatase activity points towards an early development in the secretory mechanism of the hepatic cell. In present years, interest has been focused on the role of biotransformation of chemicals to highly reactive metabolites that begin cellular toxicity. Many compounds, including clinically useful drugs can cause cellular damage through metabolic activation of the chemical to extremely reactive compounds such as free radicals, carbenes and nitrenes. CCl_4 has perhaps been studied more expansively both biochemically and pathologically than any extra hepatotoxin. CCl_4 hepatotoxicity depends on the reductive dehalogenation of CCl_4catalysed by Cyt 450 in the liver cell endoplasmic reticulum foremost to the generation of an unstable complex CCl_3 radical. This trichloromethyl radical reacts quickly which is reported as an extremely reactive species. These free radicals assault microsomal lipids leading to its peroxidation and also covalently attach to microsomal lipids and proteins eventually initiating a site of secondary biochemical processes which is the final cause for the unfolding of the panorama of pathological consequences of CCl_4 metabolism.[37] Phytochemical investigated on *O. dalbergioides* have reported the presence of hydroxlupeol, betulin, lupeol and isoflavanones such as homoferreirin, dalbergioidin and ougenin.[38,39] A number of scientific reports indicated certain flavonoids, triterpenoids and steroids have defensive effect on liver due to its antioxidant properties.[40–42] Presence of those compounds in *O. dalbergioides* may be accountable for the defensive effect on PCM and CCl_4-induced liver damage in rats. In conclusion, the results of this study reveal that *O. dalbergioides* has a potent hepatoprotective action upon PCM and CCl_4-induced hepatic damage in rats. Our results demonstrate that the hepatoprotective effects of *O. dalbergioides* may be due to its antioxidant and free

radical scavenging properties. Additional, investigation is in progress to determine the precise phytoconstituents that is accountable for its hepatoprotective effect.

5. Source of Funding

None.

6. Conflict of interest

None.

References

1. Ajith TA, Hema U, Aswathy MS. Zingiber officinale Roscoe prevents acetaminophen-induced acute hepatotoxicity by enhancing hepatic antioxidant status. *Food Chem Toxicol*. 2007;45(11):2267–72. doi:10.1016/j.fct.2007.06.001.
2. Najmi AK, Pillai KK, Pal SN, Aqil M. Free radical scavenging and hepatoprotective activity of jigrine against galactosamine induced hepatopathy in rats. *J Ethnopharmacol*. 2005;97(3):521–5. doi:10.1016/j.jep.2004.12.016.
3. Sundaram R, Murugesan G. Hepatoprotective and antioxidant activity of a mangrove plant Lumnitzera racemosa. *Asian Pac J Trop Biomed*. 2011;1(5):348–52. doi:10.1016/S2221-1691(11)60078-6.
4. Adewusi EA, Afolayan AJ. A review of natural products with hepatoprotective activity. *J Med Plant Res*. 2010;4(13):1318–34. doi:10.5897/jmpr09.472.
5. Didem DO, Nilufer O, Fatma EE. Hepatoprotective effect of Vitis vinifera L. leaves on carbon tetrachloride-induced acute liver damage in rats. *J Ethnopharmacol*. 2007;112(1):145–51. doi:10.1016/j.jep.2007.02.013.
6. Asha VV, Sheeba MS, Suresh V, Wills PJ, Fitoterapia. Hepatoprotection of Phyllanthus maderaspatensis against experimentally induced liver injury in rats. *Fitoterapia*. 2008;78(2):134–41.
7. Ghori SS, Khan M, Rahman A, Bangladesh S. Amelioration of carbon tetrachloride- and paracetamol- induced hepatotoxicity in rats by Ficus dalhousiae. *J Pharmacol*. 2014;9:588–94. doi:10.3329/bjp.v9i4.20515.
8. Greenhough S, Hay DC. Pharmaceutical Medicine. *Pharm Med*. 2012;26(2):85–9. doi:10.1007/bf03256896.
9. Victor J, Senior N. Drug-Related Hepatotoxicity. *Engl J Med*. 2006;354(7):731–9. doi:10.1056/nejmra052270.
10. Okuda M, Li K, Beard MR. Mitochondrial injury, oxidative stress, and antioxidant gene expression are induced by hepatitis C virus core protein. *Gastroenterology*. 2002;122(2):366–75. doi:10.1053/gast.2002.30983.
11. Vermeulen NP, Bessems JG, Straat RVD. Molecular Aspects of Paracetamol-Induced Hepatotoxicity and its Mechanism-Based Prevention. *Drug Metab Rev*. 1992;24(3):367–407. doi:10.3109/03602539208996298.
12. Amin A, Hamza AA. Hepatoprotective Effects Of Hibiscus, Rosmarinus And Salvia On Azathioprine-induced Toxicity In Rats. *Life Sci*. 2005;77(3):266–78.
13. Rao GM, Rao CV, Pushpangadan P, Shirwaikar A. Hepatoprotective effects of rubiadin, a major constituent of Rubia cordifolia Linn. *J Ethnopharmacol*. 2006;103(3):484–90. doi:10.1016/j.jep.2005.08.073.
14. Kumar A. Pharmacological And Phytochemical Aspects Of Lichen Parmelia Perlata: A Review. *Int J Res Pharm Chem*. 2012;2(1):92–102.
15. Barros AO, Souza D, Aranha RS, Esp. Aranha ESP. *Int J Pharm Pharm Sci*. 2014;6:71–6.
16. Mukherjee PK. Quality control of herbal drugs. *Business Horizons*. 2007;2:629–53. doi:10.1016/b978-0-12-813374-3.00017-x.
17. Nayak A, Garg M, Jain S, Khan MA, Jain DK, Khan N, et al. In-vitro and in-vivo anti-inflammatory activity of Plumeria indica Linn flowers. *J Pharm*. 2019;8(3):94–104.
18. Joshi S, Parkhe G, Aqueel N, Dixit N, Jain DK. Viscoplastic analysis of metal cutting by finite element method. *Pharmacologyonline*. 2019;1:27–33.
19. Jonsson M, Jestoi M, Nathanail AV. Application of OECD Guideline 423 in assessing the acute oral toxicity of moniliformin. *Food Chem Toxicol*. 2013;53:27–32. doi:10.1016/j.fct.2012.11.023.
20. Parejo I, Codina C, Petrakis C, Kefalas P. Evaluation of scavenging activity assessed by Co(II)/EDTA-induced luminol chemiluminescence and DPPH· (2,2-diphenyl-1-picrylhydrazyl) free radical assay. *J Pharmacol Toxicol Methods*. 2000;44(3):507–12. doi:10.1016/s1056-8719(01)00110-1.
21. Srinivasan R, Chandrasekar M, Nanjan MJ, Suresh B. Antioxidant activity of Caesalpinia digyna root. *J Ethnopharmacology*. 2007;113(2):284–91.
22. Huang MH, Huang SS, Wang BS. Antioxidant and anti-inflammatory properties of Cardiospermum halicacabum and its reference compounds ex vivo and in vivo. *J Ethnopharmacol*. 2011;133(2):743–50. doi:10.1016/j.jep.2010.11.005.
23. Roy CK, Kamath JV, Asad M. Hepatoprotective activity of Psidium guajava Linn. leaf extract. *Indian J Exp Biol*. 2006;44(4):305–11.
24. Sorg DA, Buckner B. A Simple Method of Obtaining Venous Blood from Small Laboratory Animals. *Proc Soc Exp Biol Med*. 1964;115(4):1131–3. doi:10.3181/00379727-115-29134.
25. Reitman S, American FS. A Colorimetric Method for the Determination of Serum Glutamic Oxalacetic and Glutamic Pyruvic Transaminases. *J Clin Pathol*. 1957;28(1):56–63. doi:10.1093/ajcp/28.1.56.
26. King J. The Hydrolases-Acid and Alkaline Phospatase. *Pract Clin Enzymol*. 1965;1(3):82–92. doi:10.3923/rjes.2007.82.92.
27. Kind PR, King EJ. Estimation of plasma phosphatase by determination of hydrolysed phenol with amino-antipyrine. *J Clin Pathol*. 1954;7(4):322–8. doi:10.1136/jcp.7.4.322.
28. Jendrassik L, Grof P, Biochem J. Folic Acid Alleviates Oxidative Stress and Hyperhomocysteinemia Involved in Liver Dysfunction of Hypothyroid Rats. *Am J Biomed Res*. 1938;2(4):82–91. doi:10.12691/ajbr-2-4-3.
29. Rajkapoor B, Burkan ZE, Senthilkumar R. Oxidats AD Huma Diseases: Role of Atioxidat Medicial Plats - A Review. *Pharmacologyonline*. 2010;1:1117–31.
30. Ali SS, Kasoju N, Luthra A. Indian medicinal herbs as sources of antioxidants. *Food Res Int*. 2008;41(1):1–15. doi:10.1016/j.foodres.2007.10.001.
31. Huang MH, Huang SS, Wang BS, Wu CH, Sheu MJ, Hou WC, et al. Antioxidant and anti-inflammatory properties of Cardiospermum halicacabum and its reference compounds ex vivo and in vivo. *J Ethnopharmacol*. 2011;133(2):743–50. doi:10.1016/j.jep.2010.11.005.
32. Kalaivani T, Mathew L. Free radical scavenging activity from leaves of Acacia nilotica (L.) Wild. ex Delile, an Indian medicinal tree. *Food Chem Toxicol*. 2010;48(1):298–305. doi:10.1016/j.fct.2009.10.013.
33. Halliwell B, Gutteridge J. Free Radicals in Biology and Medicine. Oxford; 2007. doi:10.1093/acprof:oso/9780198717478.001.0001.
34. Hofseth LJ. Nitric oxide as a target of complementary and alternative medicines to prevent and treat inflammation and cancer. *Cancer Lett*. 2008;268(1):10–30. doi:10.1016/j.canlet.2008.03.024.
35. Willianson EM, Okpako DT, Evans FJ. Selection, Preparation and Pharmacological Evaluation of Plant Material. vol. 1; 1996. p. 238.
36. Moss DW, Butterworth PJ. Enzymology and medicine. Pitman medical. vol. 1; 1974. p. 464.
37. Wei YH. Oxidative stress and mitochondrial DNA mutations in human aging. *Proc Soc Exp Biol Med*. 1998;217(44205):53–63. doi:10.3181/00379727-217-44205.
38. Mukherjee DK, Barua AK, Bose PK, Cul1 S. Rediscovering cancer: from mechanism to therapy. vol. 29; 1963. p. 151–2.
39. Balakrishna S, Ramanathan JD, Seshadri TR, Frs, Venkataramani B. Constitution of o-dimethyl latifolin. *Proc Royal Soc*. 1962;268:1–1.

40. Defeudis FV, Papadopoulos V, Drieu K. Ginkgo biloba extracts and cancer: a research area in its infancy. *Fund Clin Pharmacol*. 2003;17(4):405–17. doi:10.26226/morressier.59d5184ed462b80296ca3fff.
41. Takeoka GR, Dao LT. *J Agric Food Chem*. 2003;51:496–501.
42. Banskota AH, Tezuka Y, Adnyana IK. Hepatoprotective Effect of Combretum quadrangulare and Its Constitutents. *Biol Pharm Bull*. 2000;23(4):456–60. doi:10.1248/bpb.23.456.

Author biography

Sarita Karole, Professor

Deepak Kumar Jain, Associate Professor

Sahil Abbas, Associate Professor

Nisha Abbas, Assistant Professor

Pradeep Kare, Research Scholar

Cite this article: Karole S, Kumar Jain D, Abbas S, Abbas N, Kare P. Antioxidant and hepatoprotective effects of *ougeinia dalbergioides* benth against paracetamol and ccl_4 induced liver damage in rats. *Indian J Pharm Pharmacol* 2022;9(1):67-74.