Advances in Bioresearch
Adv. Biores., Vol 12 (3) May 2021: 24-29

Print ISSN 0976-4585; Online ISSN 2277-1573
Journal's URL:http://www.soeagra.com/abr.html
CODEN: ABRDC3
DOI: 10.15515/abr.0976-4585.12.3.2429

Advances in Bioresearch

ORIGINAL ARTICLE

Evaluation of hepatoprotective effect of *Cassia occidentalis* against Paracetamol-induced hepatic damage in rats

Hemant Vinayak Deore *, Ravindra Banilal Patil, Swapnil Balwant Deshmukh
DCS's A. R. A. College of Pharmacy, Nagaon, Dhule, Maharashtra, India, 424005
*Email id:- hemantdeore24@rediffmail.com

ABSTRACT
Most of the metabolic and physiological processes of our body as well as the detoxification of various drugs and xenobiotic chemicals occur in the liver. During this detoxification process, the reactive chemical intermediates damage the liver severely. There are several commercially available drugs, consumption of which results in idiosyncratic drug reaction mediated hepatotoxicity. Drug induced hepatotoxicity is a burning problem in this regard and several drugs are withdrawn from the market due to their hepatotoxic nature. Today, a worldwide search of non-hepatotoxic drugs, especially potent hepatoprotective drugs has led towards the screening of numerous herbal products. Pharmaceutical companies and scientific communities have started to consider the therapeutic efficiency of plant based hepatoprotective remedies. Different herbs are mentioned in various ethnopharmacological practices possessing hepatoprotective capacities and around the globe, such herbs are still used by people to cure certain liver diseases. The present study To evaluate the hepatoprotective activity of aerial parts of Cassia occidentalis against Paracetamol-induced hepatotoxicity. Hepatoprotective activity of the Methanolic extract of Cassia occidentalis plant was investigated in rats by inducing toxicity with Paracetamol. The plant extract has been shown to possess significant protective effect by lowering the level of AST, ALT, ALP, LDH and bilirubin. The Methanolic extract of Cassia occidentalis at a dose of 50mg/kg, 100mg/kg, and 200mg/kg showed significant hepatoprotective activity which was comparable to that of a standard hepatoprotective agent (Silymarin). The present work aim was to measure the hepatoprotective activities of the Methanolic extract of Cassia occidentalis in albino wistar rats.
Keywords: *Hepatoprotective,Paracetamol, Methanolic extract ,Cassia occidentalis etc.*

Received 24.02.2021 Revised 20.04.2021 Accepted 03.05.2021

How to cite this article:
H V Deore, R B Patil, S B Deshmukh. Evaluation of hepatoprotective effect of *Cassia occidentalis* against Paracetamol-induced hepatic damage in rats. Adv. Biores. Vol 12 [3] May 2021. 24-29

INTRODUCTION

Liver is the important organ concerned with the biochemical activities in the human body. It has great capacity to detoxicate toxic substances and synthesize useful principles. Therefore, damage to the liver inflicted by hepatotoxic agent is of grave consequences. There is an ever increasing need of an agent which could prevent it from such damage.[1] The detoxification of the harmful chemicals occurs in the liver, which in turn,

Results in various hepatic diseases. Other factors causing liver damage include chronic alcoholism, viral infections, hepatocarcinoma, etc., Drug induced hepatotoxicity (DIHT) resulting in liver damage has turned into a major medical concern in recent years. During DIHT, formation of proinflammatory cytokines and reactive free radicals from the hepatic

neutrophils and Kupffer cells cause severe oxidativestress.[1]. In view of severe undesirable side effect of synthetic agents, there is growing focus to follow systematic research methodology and to evaluate the scientific basis for the traditional herbal medicines which are claimed to possess hepatoprotective activity [2].

Cassia occidentalis is a tropical plant used in African and Asian traditional medicines to treat or improve several diseases and conditions, in particular cardiovascular disorders [3,4]. As in various other countries, in Cameroun, roasted seeds are used as coffee substitutes, while other parts of the plants are used by traditional healers to treat metabolic and cardiovascular diseases. Interestingly, experimental

evidence supports applications in traditional medicine. Phytochemical studies of *Cassia occidentalis* leaves revealed the presence of many pharmacologically active families of a molecule, including tanins, saponins, cardiac glycosides, terpenoides and anthroquinones, terpenes, and inorganic elements [5,6]. Extracts of this plant were reported antifungal, antiviral [7,8], antibacterial, anthelminutetic [9,10], antispasmodic, analgesic, antipyretic, anti-inflammatory [11,12], and hepatoprotective properties in humans and experimental models [13,14]. Verma and colleagues (2010) showed the effect of methanolic extract of *Cassia occidentalis* for the management of alloxan-induced diabetic rats [15], and Sreejith and colleagues reported anti-allergic, anti-inflammatory and antilipid peroxiding effects [16].
The present work aim was to measure the hepatoprotective activities of the Methanolic extract of *Cassia occidentalis* root in albino wistar rats.

MATERIALS AND METHOD

Plant collection

The fresh plant of *Cassia occidentalis*was collected from the region of Taluka Yawal, District Jalgaon, India. The selected plant was authenticated by Dr. D. A. Dhale, Asst. Professor, PG & Research Dept. of Botany SSVPS's, L. K. Dr. P. R. Ghogrey Science College, Dhule, Maharashtra. Barks were dried at room temperature to avoid loss of chemical constituents and milled with the aid of grinding machine.

Extraction methodology [17]

The root of the plant were thoroughly washed with tap water, dried at room temperature and transformed to a coarse powder. The powder was extracted with solvents like a Petroleum ether (60-80^{o}C), Chloroform, Methanol, Water-methanol, and water separately by Soxhlet extraction method. Finally, the extracts were evaporated and dried under vacuum and tray dryer to obtain thick sticky extract.

Animals

Healthy adult swiss albino wistar rats of either sex weighing between 160 to 180 gm were used for acute toxicity study and hepatoprotective activity.

Toxicity study: (OECD 423). [18]

Acute toxicity study was performed according to Organisation for Economic Co-operative and development guidelines No. 423. Albino wistarrats of either sex were divided into six groups with six animals each. Plant extract was adminuteistered orally as single doses to rats at different dose levels of 50, 250, 500, 1000, 1500, and 2000 mg/kg b.w. Animals were observed individually during the first 30 minutes and periodically during 24 hours, with special attention given during the first 4 hours and daily thereafter, for a total 14 days

Experimental procedure.

Paracetamol induced liver damage:[19]

Procedure:

Albino wistar rats (150-250g) were used. All the animals were randomly divided into the six groups each group consists of 6 animals and they received the treatment as follows
Group I: Normal (Distilled water p.o.)
Group II: Toxicant (On 5th day Paracetamol 3g/kg, 1% CMC p.o.)
Group III: Standard (Silymarin 50mg/kg p.o. + on 5th day Paracetamol 3g/kg, p.o.).
Group IV: Extract treated (Methanolic extract of *Cassia occidentalis* 50 mg/kg p.o. + On 5th day Paracetamol 3g/kg, p.o.).
Group V: Extract treated (Methanolic extract of *Cassia occidentalis* 100 mg/kg p.o. + On 5th day Paracetamol 3g/kg, p.o.).
Group VI: Extract treated (Methanolic extract of *Cassia occidentalis* 200 mg/kg p.o. + On 5th day Paracetamol 3g/kg, p.o.)
The vehicle (Distilled water) or extractof*Cassia occidentalis* were administered orally for 7 days. Paracetamol suspension (1% CMC) was administered in a dose of 3g/kg p.o on 5th day. (19) 48 hours after Paracetamol administration, blood was obtained from all groups of rats by puncturing the retro-orbital plexus. The blood samples were allowed to coagulate for 45 minutes at room temperature. Serum was separated by centrifugation at 3000 rpm at room temperature for 20 minutes and subjected to biochemical investigations viz. ALT, AST, ALP, LDH, TB, and DB.
The livers of all animals were removed and processed for histological investigations.

Histopathological studies

Small portions of the liver from each of the six animals in all of the groups were preserved in 10% buffered formol saline (P^{H}7.4). The paraffin sections were then prepared and stained with haemotoxylin-eosin dye for observing the liver damage.

Statistical analysis

The data represent mean S.E.M. Result were statistically by one-way ANOVA followed by dunnet's test. The minimum level of significance was set at $p<0.005$.

RESULTS

Paracetamol induce Hepatotoxicity

The adminuteistration of Paracetamol resulted in a marked increase in serum AST, ALT, ALP, LDH,total bilirubin. The protective actions of root of *Cassia occidentalis* on hepatotoxicity induced by Paracetamol are summarized in Tables 1 and 2. Maximum hepatoprotective activity was observed at 200mg/kg dose level of *Cassia occidentalis*, which was comparable to that of Silymarin

Table 1: Effect of Methanolic extract of *Cassia occidentalis* on liver weight and liver volume in Paracetamol induced hepatotoxicity

Group	Body weight (gm)	Liver weight (gm)	Liver volume(ml)
Normal	265	8.5	8
Paracetamol treated	224	11.5	10.5
STD+ Paracetamol	261	9.5	8
50 mg/kgMethanolic extract of *Cassia occidentalis*+ Paracetamol 3g/kg	233	11	10
100 mg/kgMethanolic extract of *Cassia occidentalis*+ Paracetamol 3g/kg	254	10	9
200 mg/kgMethanolic extract of *Cassia occidentalis*+ Paracetamol 3g/kg	266	9	9.5

Table 2. Effect of Methanolic extract of *Cassia occidentalis* on various biochemical parameters in Paracetamol induced hepatotoxicity

Group	SGOT (I.U./L)	SGPT (I.U./L)	ALP (I.U./L)	LDH (I.U./L)	T.B. (%mg)	D.B. (%mg)
Normal	65.10± 1.2*	46.09± 0.6*	187.2± 1.7*	140.3 ± 1.3*	0.17± 0.006	0.17± 0.004**
Paracetamol Treated3g/kg	170.1± 1.3**	130.8 ±3.2	350.4± 3.2*	269.5 ± 1.4*	1.07± 0.1*	0.7± 0.016
STD + Paracetamol 3g/kg	96.10 ±1.1	74.10 ±0.8	211.2± 0.9*	178. ± 3.5*	0.38± 0.01*	0.25± 0.004
50 mg/kg Methanolic extract of *Cassia occidentalis*+ Paracetamol 3g/kg	137.4 ±1*	110.1 ±0.6	315.3± 1.2*	231.8 ± 1.6*	0.7± 0.03**	0.51 ± 0.01*
100 mg/kgMethanolic extract of *Cassia occidentalis*+ Paracetamol 3g/kg	116.1 ±0.9	97.0 ±0.7**	280.2± 2.4*	210.7± 1.8*	0.5 ±0.02	0.43± 0.01*
200 mg/kgMethanolic extract of *Cassia occidentalis*+ Paracetamol 3g/kg	104.5 ±1	86.18 ±0.3	241.7 ±2.6*	198.2 ± 0.4*	0.46 ±0.01	0.31± 0.01*

Values are expressed as mean±S.E.M. (n=6)

*P<0.05, **P<0.01, when compared with the Paracetamol treated group (one-way ANOVA followed by Dunnett test)

Histological profile of the control animals showed normal hepatocytes with well preserved cytoplasm, prominent nucleus,nucleolus, and central vein. There was no sign of inflammation,fatty change or necrosis in these animals (Fig. 1). In animals treated with Paracetamol only, liver sections showed marked necrosis and inflammatory cell infiltration in the centrizonal area. Inflammatory cells were also observed in the portal triad (Fig. 2). Pretreatment with *Cassia occidentalis*at 50 and 100mg/kg dose showed a reduction of necrosed area and inflammatory infiltrates in the centrizonal area with the disappearance of inflammatory infiltrate around portal triad (Figs. 4 and 5). *Cassia occidentalis*at 200mg/kg dose showed a greater reduction of the necrosed area and sparse inflammatory cell infiltration around the central vein (Fig. 6) as compared to 50 and 100mg/kg dose.

Histopathological changes of Paracetamol induced hepatotoxicity in albino wistar rat liver (H and E × 100)

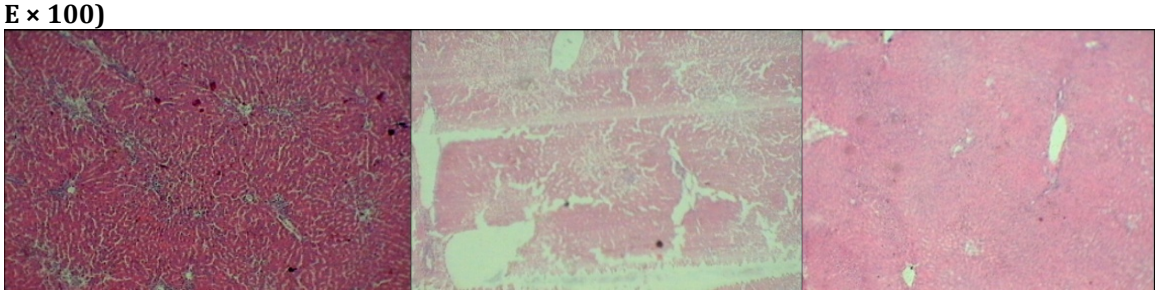

Fig.

Figure: 1
Normal liver
Normal histology showing prominuteent central vein, portal triads and normal hepatocytes.
(H and E X 100)

Figure: 2
Toxicant control Paracetamol (3g /kg) Focal areas of liver cell degeneration, lymphocytic infiltration, fatty degeneration
(H and E X 100)

Figure: 3
Standard silymarin (50mg/kg)
Small areas of cell degeneration
And almost normal histology
(H and E X 100)

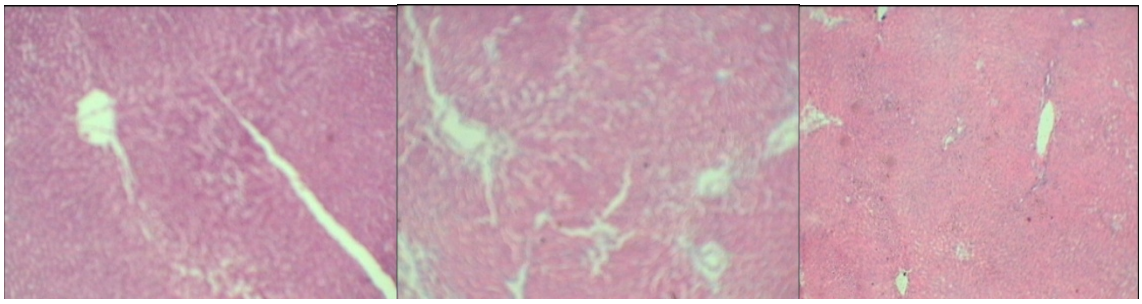

Figure: 4
Methanolic extract of *Cassia occidentalis*50 mg/kg
Small areas of cell degeneration
(H and E X 100)

Figure: 5
Methanolic extract of *Cassia occidentalis*100 mg/kg
Small areas of cell degeneration and Lymphocytic infiltration
(H and E X 100)

Figure: 6
Methanolic extract of *Cassia occidentalis*200mg/kg
Normal central vein Portal triad
Occasional areas of cell degeneration
(H and E X 100)

DISCUSSION

Damage to the structural integrity of the liver is reflected by an increase in the level of serum transaminases and bilirubin because these are cytoplasmic in location and are released into circulation after cellular damage. [20] The present study has also demonstrated that the Vivo hepatoprotective activity against liver injury induced by Paracetamol. Paracetamol (N-acetyl p-amino phenol) a widely used analgesic and the antipyretic drug is known to cause hepatotoxicity in experimental animals and humans at high doses. [21] It is mainly metabolized in the liver to excretable glucuronide and sulfate conjugates. However, hepatotoxicity of Paracetamol has been attributed to the formation of toxic metabolites when a part of Paracetamol is activated by hepatic Cytochrome p 450 to a highly reactive metabolite N-acetyl-p-benzoquinoneimine, which is normally conjugated with GSH and excreted in the urine as conjugates. Overdose of Paracetamol leads to mitochondrial dysfunction followed by acute hepatic necrosis. [22]

In our present investigation rats treated with Paracetamol induced hepatotoxicity developed significant hepatic damage which was observed through a substantial increase in the concentration of SGOT, SGPT, ALP, LDH and bilirubin. Treatment of rats with Methanolic extract of *Cassia occidentalis*prior to and concomitant with the challenge of Paracetamol produced an alleviation of the hepatic injury to a considerable extent which was reflected by the ability of the extract to lower the elevated serum enzymes levels resulting from the administration of Paracetamol alone. The increased levels of SGOT and SGPT in

serum are indicative of cellular leakage and loss of functional integrity of cell membrane in liver . In view of this, the extract mediated reduction in levels of SGOT, and SGPT towards the respective normal values is an indication of stabilization of plasma membrane as well as repair of hepatic tissue damage caused by Paracetamol. This effect is in agreement with the commonly accepted view that serum levels of transminases return to normal with healing of hepatic parenchyma and the regeneration of hepatocytes.
Alkaline phosphatase (ALP) is the prototype of these enzymes that reflect the pathological alteration in biliary flow. The use of ALP in chemical induced liver dysfunction has been investigated in our study. Paracetamol induced elevation of this enzymatic activity in serum is in line with high level of serum bilirubin content. The extract mediated suppression of the increased ALP activity with the concurrent depletion of raised bilirubin level suggests the possibility of the extract being able to stabilize biliary dysfunction in rat liver during chronic hepatic injury with Paracetamol.[23]
Thus, the present study revealed a significant in activities of SGOT, SGPT, ALP, LDH and serum bilirubin level on exposure to Paracetamol indicating considerable Hepatocellular injury. Administration of Methanolic extract of *Cassia occidentalis* at 50 mg/kg ,100 mg/kg and 200mg/kg dose level attenuated the increased level of the serum enzymes, produced by Paracetamol and caused a subsequent recovery towards normalization almost like that of Silymarin treatment.

CONCLUSION

Based onthe above results, it can be concluded that the active principles present in root callus extract of *Cassia occidentalis* offered better antihepatotoxic action as compared to the active principles present in natural root extract against Paracetamol induced hepatic damage. However, more elaborate work is required to establish the efficacy of root extract by isolating and identifying the active constituents present in the natural root and root callus extracts which are responsible for the antihepatotoxic activity.

ACKNOWLEDGMENT

Authors are thankful to DCS's A.R.A. College of Pharmacy for providing experimental facility. Corresponding author also thankful to Mr. U.P.Joshi for helping in plant extracts

CONFLICTS OF INTEREST

The authors declare no conflict of interest.

REFERENCES

1. Achliya, G.S., Wadodkar, S.G., Dorle, A.K.(2004). Evaluation of hepatoprotective effect of Amalkadi Ghrita against carbon tetrachloride-induced hepatic damage in rats. Journal of Ethnopharmacology, 2: 229-232.
2. Priyankar D, Manas Ranjan Sah1, Arnab Sen.(2013). Hepatotoxicity and present hepatoprotective scenario.International Journal of Green Pharmacy, 265-273
3. Seethapathy GS, Ganesh D, Santhosh Kumar JU, Senthilkumar U, Newmaster SG, Ragupathy S. (2014). Assessing product adulterationin natural health products for laxative yielding plants, Cassia, Senna, and Chamaecrista, in Southern India using DNA barcoding.Int J Leg Med,http://dx.doi.org/10.1007/s00414-014-1120-z.
4. Yadav JP, Arya V, Yadav S, Panghal M, Kumar S, Dhankhar S. (2010).Cassia occidentalis L.: a review on its ethnobotany, phytochemical and pharmacological profile. Fitoterapia, 81: 223-230.
5. Bukhari NA, Siddique I, Perveen K, Siddiqui I, Alwahibi MS. (2014).Synthetic seed production and physio-biochemical studies in Cassia angustifolia Vahl. – a medicinal plant. Acta Biol Hung, 65: 355-367.
6. Epifano F, Fiorito S, Locatelli M, Taddeo VA, Genovese S. (2015). Screening for novel plant sources of prenyloxyanthraquinones: Senna alexandrina Mill. and Aloe vera (L.) Burm. F. Nat Prod Res, 29: 180-184.
7. Chen L, Yang Y, Yuan P, Yang Y, Chen K, Jia Q, (2014). Immunosuppressive effects of A-type procyanidin oligomers from Cinnamomum tamala. Evid Based Complement Alternat Med,365-258.
8. Cong Q, Shang M, Dong Q, Liao W, Xiao F, Ding K. (2014).Structure and activities of a novel heteroxylan from Cassia obtusifolia seeds and its sulfated derivative. Carbohydr Res, 393: 43-50.
9. Shao F, Chen HJ, Liu RH, Hou YC, Ren G, Huang HL.(2013). Effects of heishunpian total alkaloids on Cassia acutifolia induced mice diarrhea and contraction of isolated intestinal smooth muscle in rats. Zhong Yao Cai, 36: 1805-1809.
10. Somova LO, Nadar A, Rammanan P, Shode FO. (2003). Cardiovascular, antihyperlipidemic and antioxidant effects of oleanolic and ursolic acids in experimental hypertension. Phytomedicine, 10: 115-121.
11. Nakamura S, Xu F, Ninomiya K, Nakashima S, Oda Y, Morikawa T.(2014). Chemical structures and hepatoprotective effects of constituents from Cassia auriculata leaves. Chem Pharm Bull (Tokyo), 62: 1026-1031.
12. Purushotham KN, Annegowda HV, Sathish NK, Ramesh B, Mansor SM. (2014).Evaluation of phenolic content and antioxidant potency in various parts of Cassia auriculata L.: a traditionally valued plant. Pak J Biol Sci, 17: 41-48.

13. Silva CR, Monteiro MR, Rocha HM, Ribeiro AF, Caldeira-de- Araujo A, Leitao AC.(2008). Assessment of antimutagenic and genotoxic potential of senna (Cassia angustifolia Vahl.) aqueous extract using in vitro assays. Toxicol Vitro, 22: 212-218.
14. Matsuzawa N, Takamura T, Kurita S, Misu H, Ota T, Ando H.(2007). Lipid-induced oxidative stress causes steatohepatitis in mice fed an atherogenic diet. Hepatology, 46: 1392-1403.
15. Wang X, Li Q, Shen L, Yang J, Cheng H, Jiang S.(2014). Fumigant, contact, and repellent activities of essential oils against the darklingbeetle, Alphitobius diaperinus. J Insect Sci,14: 75.
16. Kirtikar K. R, Basu B. D, Basu M. L. (1956). Indian Medicinal Plants Allahabad, India, 3: 2322-2324.
17. Ottu OJ, Atawodi SE, Onyike E. (2013). Antioxidant, hepatoprotective and hypolipidemic effects of Methanolic root extract of *Cassia singueana*in rats following acute and chronic carbon tetrachloride intoxication. Asian Pacific Journal of Tropical Medicine. 609-615
18. Organization for Economic Cooperation and Development (OECD). (2006). OECD Guidelines forTesting of Chemicals (Internet). France: OECD Publishing; 2006 july 10.Section 4, Health Effects: Test No.423: Acute Oral Toxicity: Acute Toxic Class MethodAvaible from: http://www.oecdbookshop.org/oecd/index.asp/lange. (Last accessed on 2009 Mar 22).
19. Chattopathyay R.R.(2003).Possible mechanism of hepatoprotective activity of *Azadirachta indica* leaf extract: Part II.J. Ethnopharmacol,2003:217-219.
20. Venkateswaran S. Viswanthan, P, Venugopal P. (1997). Protective effect of Livex , a herbal formulation against erythromycin estolate induced hepatotoxicity, Journal ofethnopharmacology, 57:161-167.
21. Tieppo, J,Vercelino R, Dias, A,Marroni, C.A. **(**2005) Common bile duct ligation as a model of hepatopulmonary syndrome and oxidative stress. Arq Gastroenterol, 42: 244-248.
22. Kumar G, Banu, G.S, Kannan V, Pandian M. (2004). Antihepatotoxic effect of β-carotine on Paracetamol induced hepatic damage in rats, Ind.J. Exp. Biology,43: 351-355.
23. 23 Bhakta T, Mukharji P. Mukharji K. (1999). Evaluation of hepatoprotective activity of Cassia fistula leaf extract. Journal of Ethnopharmacology, 66: 277-282.