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PM Ghadge
MVSc Scholar of Veterinary Pharmacology and Toxicology, College of Veterinary and Animal Sciences, Parbhani, Maharashtra, India

SR Rajurkar
Department of Veterinary Pharmacology and Toxicology College of Veterinary and Animal Sciences, Maharashtra Animal and Fishery Sciences University, Nagpur Maharashtra, India

ND Jadhav
Department of Veterinary Pharmacology and Toxicology College of Veterinary and Animal Sciences, Maharashtra Animal and Fishery Sciences University, Nagpur Maharashtra, India

GR Gangane
Department of Veterinary Pharmacology and Toxicology College of Veterinary and Animal Sciences, Maharashtra Animal and Fishery Sciences University, Nagpur Maharashtra, India

PB Kansatwad
Department of Veterinary Pharmacology and Toxicology College of Veterinary and Animal Sciences, Maharashtra Animal and Fishery Sciences University, Nagpur Maharashtra, India

Corresponding Author:
PM Ghadge
MVSc Scholar of Veterinary Pharmacology and Toxicology, College of Veterinary and Animal Sciences, Parbhani, Maharashtra, India

Hepatoprotective effect of *Cassia fistula* against carbon tetrachloride induced hepatotoxicity in wistar rats: Pathomorphological studies

PM Ghadge, SR Rajurkar, ND Jadhav, GR Gangane and PB Kansatwad

Abstract
Experimental trial was conducted to evaluate hepatoprotective effects of *Cassia fistula* in carbon tetrachloride induced hepatotoxic wistar rats and its pathomorphological study for a period of 28 days. The study was conducted on 48 Wistar rats through six groups. Group I was taken healthy control. The group II, III, IV, V and VI being the treatment groups were given carbon tetrachloride for induction of hepatotoxicity for a period of 28 days. Group III treated with standard drug Silymarin and group IV, V and VI with different doses of aqueous extract of *Cassia fistula.* Toxicated rats show Behavioural changes, decrease in body weight and increase in relative organ weight. Gross and histopathological examination revealed mild to moderate changes in liver, kidney, lung, brain and testes in CCl_4 treated rats. Daily dosing of aqueous extract of leaves of *Cassia fistula* showed ameliorative against CCl_4 toxicated rats.

Keywords: *Cassia fistula,* carbon tetrachloride, pathomarphological

Introduction
Chronic liver diseases one of the primary health issues all over the world with liver cirrhosis and drug induced liver injury. Untreated liver diseases may results in liver failure and turn fetal. Among all digestive diseases liver diseases are recognized as 2nd leading cause of mortality in world. Untreated liver diseases may results in liver failure and turn fetal. The liver is considered one of the most important organs that act as a metabolic center for nutrients such as carbohydrates, proteins, lipids and the excretion of waste metabolites. Many chemicals have a strong effect on damaging liver cells such as carbon tetrachloride, thioacetamide etc. (Mohamed Saleem *et al.*, 2010) [13].

Liver injury in rats due to Carbon tetrachloride (CCl_4) was first reported in 1936 (Cameron *et al*., 1936) [3]. The course of CCl_4-induced hepatotoxicity is determined by the partial oxygen pressure in the tissues, due to the low partial pressure of oxygen in the tissue which leads to the predominant formation of CCl_3* and $CHCl_2$* radicals and to the covalent bond with the metabolites (De Groot *et al*., 1988 [7] Masuda and Nakamura, 1990) [12]. High oxygen partial pressure shifts the CCl_4 metabolism to form the CCl_3-OO * radical followed by lipid peroxidation and forces the cell from steatosis to apoptosis or fatty liver (Kiezka and Kappus, 1980; De Groot *et al*., 1988) [10, 7].

"Green medicines" are healthier and safer than synthetic ones. The entire world population is transformed into natural drugs. Acceptance by the public and the medical profession is increasing as understanding of the mechanisms by which herbs can positively influence health and quality of life. (Dawada *et al*., 2012) [6]. *Cassia fistula* Linn. (Cassia) The family of the Caesalpiniaceae commonly known as Amulthus and popularly known in English as "Indian Laburnum" has been widely used in the Ayurvedic system of medicine for various disorders. (R. K. Gupta, 2010) [8].

Considering these facts, the present study was planned to evaluate the hepatoprotective effect of *Cassia fistula* in carbon tetrachloride induced hepatotoxic Wistar rats: Pathomorphological studies.

Material and Methods
The plant material was identified by taking help of Botanist from Department of Agriculture Botany, VNMKV, Parbhani and was used in present study.

The aqueous extract of *Cassia fistula* leaves was prepared and same was used for hepatoprotective study in Wistar rats. Carbon tetrachloride was procured from chemical supplier. Standard drug Silymarin was purchased from the market.

Experimental animals

The present study was conducted on 48 Wistar rats of either sex, age 4-6 weeks and having 180-200 g body weight. All the rats were procured from the Laboratory Animal House, Department of Veterinary Pharmacology and Toxicology, College of Veterinary & Animal Sciences, Parbhani. The Wistar rats was housed in standard laboratory conditions in polypropylene cages, provided with food and water ad-libitum in the experimental room of Laboratory animal house, Department of Veterinary Pharmacology and Toxicology, College of Veterinary and Animal Sciences, Parbhani. The Institutional Animals Ethics Committee (IAEC) approved the experimental protocol as per the guidelines of Committee For The Purpose of Control and Supervision of Experiments on Animals (CPCSEA) with Resolution no. IAEC/40/19 dated 02/03/2019.

Cassia fistula leaves extract

Aqueous extract of *Cassia fistula* leaves was prepared by cold extraction method. Leaves of the plant were allowed to dry completely under shade. Shed dried leaves were ground to powder with the help of an electrically operated grinder. Then 20% of aqueous solution was made by dissolving 200 grams of powder in 1 litre of distilled water. It was mixed thoroughly and allowed to soak for 48 hours at 4^0 C in refrigerator. It was shaken intermittently with an electrical operated flask shaker. Thus, resulting solution was first filter by muslin cloth and than by whatsman filter paper onto glass plates. They were allowed to dry and aqueous extract of *Cassia fistula* was obtained. Resulting extract were scraped off the glass plate and stored in a plastic container at 4^0C for dosing of rats during experiment.

Induction of hepatotoxicity

Total 48 Wistar rats were used for the present investigation. Amongst 40 rats from II to VI induced hepatotoxicity by daily intraperitoneal administration of carbon tetrachloride 0.1 ml and liquid paraffin 0.1 ml, i. e. @ 0.2 ml/rats. (Slater, 1978) [19].

Experimental design

The 48 Wistar rats were divided into 6 different groups, each group comprised of 4 male and 4 female rats as detailed below.

Group I: Healthy control

Group II: Treated with CCl_4 in liquid paraffin 1:1 @ 0.2 ml/rat intraperitoneal route

Group III: Treated with CCl_4 in liquid paraffin 1:1 @ 0.2 ml/rat intraperitoneal route and standard drug Silymarin @ 25 mg/kg body weight orally.

Group IV: Treated with CCl_4 in liquid paraffin 1:1 @ 0.2 ml/rat intraperitoneal route and aqueous extract of *Cassia fistula* @ 200 mg/kg body weight orally,

Group V: Treated with CCl_4 in liquid paraffin 1:1 @ 0.2 ml/rat intraperitoneal route and aqueous extract of *Cassia fistula* @ 400 mg/kg body weight orally,

Group VI: Treated with CCl_4 in liquid paraffin 1:1 @ 0.2 ml/rat intraperitoneal route and aqueous extract of *Cassia fistula* @ 600 mg/kg body weight orally.

Daily dosing was carried out for the experimental period of 28 days

Collection of Sample

Rats were sacrificed on day 28 of experiment by using excess dose of inhalation anesthesia (di-ethyl ether) at the end of experiment. Relative organ weights, Gross necropsy examination were performed. Liver, kidney, lungs, brain, ovaries and testes were collected in 10% formalin for histopathological examination.

Parameters studied

Various types of parameters were studied, these include general observations (behavioral changes, body weight and organ weight) pathological observations (gross pathology and histopathology) were performed.

Behavioral changes

All the experimental animals in the treatment groups were daily examined for any abnormal behavioral changes. if any and compared with control group animals.

Body weight

The body weights were taken on day 0^{th}, 7^{th}, 14^{th}, 21^{th} and day 28^{th} i.e. on the termination day of the experiment.

Relative Organ weight

At the end of the study, the relative organ weights of liver, kidney, lungs, brain, ovaries and testes of experimental rats were noted.

Pathomorphological study

Gross necropsies of the organs were observed for the presence of any abnormal gross pathological change. Liver, kidney, lungs, brain, ovaries, and testes tissue were collected in 10% formalin. After fixation, the tissue pieces were processed as per the standard procedure (Culling, 1974) [5].

Statistical analysis

The data obtained from various parameters from all groups were analyzed as per the method suggested by (Panse and Sukhatme, 1967) [15] using factorial randomized block design (FRBD) or completely randomized block design (CRD).

Results and Discussion

The present experiment was designed to assess the hepatoprotective effect of *Cassia fistula* in carbon tetrachloride induced hepatotoxic Wistar rats. The results are interpreted in the present topic.

Behavioral Changes

All experimental rats appeared alert, active and healthy during period of acclimatization without displaying any unfavorable manifestations suggested adequate acclimatization. All the rats were observed daily during the experimental period, experimental rats of treatment groups did not revealed any observable behavioral changes, except in group 2, carbon tetrachloride control group animals. In which rats showed dullness, restlessness, reduced feed intake.

Body weigh

The details of Average body weight (gm/week) of all the groups on 0th, 7th, 14th, 21st and 28th day of experiment are summarized in Table 1 and fig.1

The mean Average body weight in group II animals on day 7th, 14th, 21st and 28th were observed to be significantly decreased when compared to group I (healthy control) animals.

Table 1: Value of Average body weight (gm/week) in experimental rats in different groups at different intervals of study

Groups of rat	Average body weight of rats (gm/week)					Stat	CD
	Intervals of study						
	0 day	7th day	14th day	21st day	28th day		
I	$187.67^{ap} \pm 2.80$	$189.08^{apq} \pm 2.64$	$191.12^{aq} \pm 2.64$	$196.10^{ar} \pm 2.64$	$202.18^{as} \pm 2.69$	S	At 5% 2.189 At 1% 2.877
II	$187.85^{ap} \pm 1.76$	$186.85^{ap} \pm 1.65$	$184.30^{bq} \pm 1.38$	$182.22^{bqr} \pm 1.42$	$180.43^{br} \pm 1.46$	S	
III	$187.85^{ap} \pm 2.28$	$188.13^{ap} \pm 2.30$	$190.66^{aq} \pm 2.37$	$196.29^{ar} \pm 2.77$	$202.52^{as} \pm 2.69$	S	
IV	$188.67^{ap} \pm 2.61$	$188.95^{ap} \pm 2.62$	$190.20^{ap} \pm 2.60$	$195.23^{aq} \pm 2.53$	$201.11^{ar} \pm 2.50$	S	
V	$187.70^{ap} \pm 2.14$	$187.96^{ap} \pm 2.18$	$190.42^{aq} \pm 2.29$	$195.43^{ar} \pm 2.28$	$201.76^{as} \pm 2.30$	S	
VI	$188.77^{ap} \pm 1.89$	$189.03^{ap} \pm 1.89$	$191.47^{aq} \pm 1.82$	$196.17^{ar} \pm 1.71$	$203.08^{as} \pm 1.79$	S	
Stat	NS	NS	S	S	S		
CD	At 5% - 2.398 At 1% - 3.152						

Different superscripts a, b, c shows significance difference between different groups on specific day (p<0.05)
Different superscripts p, q, r, s shows the significance difference between different day in a specific group (p<0.05).

The mean Average body weight in group III, V and VI animals on day 7th, 14th, 21st and 28th did not show any significant variation when compared to group I (healthy control) animal on same day however when compares to group II the body weight significantly increased due to administration of aqueous extract of *Cassia fistula*. Results obtained from Group VI showed maximum recovery.

Result obtained from group IV does not showed maximum recovery as compared to other treatment group but when compare to group II there was significantly increased in body weight.

Similar results were reported by Lakra *et al*. (2016) [11] Adewole *et al*. (2007) [1].

Relative Organ weight

The Relative organ weights (gm) of all the groups of experiment are summarized in Table 2 and fig.2.

Table 2: Value of Relative organ weight (gm) of liver, kidney, lung, brain, testis and ovaries in experimental rats

Groups of rat	Relative organ weight of experimental rats (gm)					
	Liver	Kidney	Lung	Brain	Testis	Ovaries
I	$3.66^{c} \pm 0.25$	$0.84^{c} \pm 0.02$	1.07 ± 0.09	0.72 ± 0.03	2.02 ± 0.08	0.19 ± 0.01
II	$5.87^{a} \pm 0.15$	$1.27^{a} \pm 0.07$	1.17 ± 0.10	0.82 ± 0.05	2.53 ± 0.18	0.17 ± 0.01
III	$3.73^{c} \pm 0.19$	$0.83^{c} \pm 0.05$	0.94 ± 0.04	0.72 ± 0.02	2.11 ± 0.20	0.21 ± 0.02
IV	$4.67^{b} \pm 0.18$	$1.03^{b} \pm 0.06$	1.02 ± 0.08	0.78 ± 0.02	2.31 ± 0.30	0.18 ± 0.01
V	$4.08^{bc} \pm 0.24$	$0.89^{bc} \pm 0.06$	0.97 ± 0.04	0.75 ± 0.03	2.21 ± 0.19	0.23 ± 0.01
VI	$3.76^{c} \pm 0.21$	$0.85^{c} \pm 0.05$	0.98 ± 0.06	0.73 ± 0.03	2.10 ± 0.11	0.20 ± 0.02
CD values	0.601	0.162	-	-	-	-
Statistics	S	S	NS	NS	NS	NS

*****Means bearing similar superscripts in column and rows do not differ significantly (P<0.05)

The values of relative weight of liver in group II animals was observed to be significantly elevated when compared group I (control). The value of relative weight of liver in group III, V and VI animals were observed to be significantly lower when compared to group II due to administration of aqueous extract of *Cassia fistula*. However, in group IV was observed to be significantly decline when compared to group II however observed to significantly increase when compared to III and VI values. Similar results reported by Chang *et al*. (2009) [4] Lakra *et al*. (2016). [11].

The values of relative weight of kidney in group II animals was observed to be significantly elevated when compared group I (control). In group III, V and VI animals were observed to be significantly lower when compared to group II values due to administration of aqueous extract of *Cassia fistula*. In group IV was observed to be significantly decreased when compared to group II however, significantly increased when compared to group I, III and VI values. Similar results reported by Adewole *et al*. (2007) [1] in CCl_4 induced kidney damage in rats.

The relative weight of lung, brain, testes and ovaries does not vary significantly.

Gross Pathology

The gross pathological evaluation showed congestion, enlargement of liver (hepatomegalae), increased fragility and focal necrosis of liver from the rats of group II (CCl_4 treated rats). The earlier reports in respective gross pathological examination in CCl_4 toxicated rats published by Uehara *et al*. (2013) [20] goes parallel with present observation. However, kidney, lung, brain, testes and ovaries did not showed any appreciable gross pathological changes in any of rats from control as well as treatment groups.

Histopathological Investigation

The histoarchitecture of liver from rat of control were within normal histological limits. The histoarchitecture study of liver revealed congestion, minimal to severe, focal to diffuse, fatty changes and necrosis. There was dilation of central vein, mononuclear cell infiltration as well as vacuolar degenerative changes in hepatic parenchyma of rats of group II which were toxicated with CCl_4. (plate1). However, treatment related restoration of histoarchitecture of liver was noted and same was evidence with less intense histopathological changes in rats of groups III, IV, V and VI also the restoration appeared to be treatment and dose dependent (plate 2).The earlier reports in respective histopathological examination of liver in CCl_4 toxicated rats and restoration of histoarchitecture of liver by *Cassia fistula* published by Bhakta *et al*. (1999) [2] Dawada *et al*. (2012) [6] goes parallel with present observation.

In CCl_4 toxicity, trichloromethyl free radical was produced which is toxic reactive metabolite. This toxic metabolite binds covalently to the lipid membrane of the adipose tissue and causes peroxidative degradation which results in fats from the adipose tissue are translocated and accumulated in the hepatocyte. (Singh *et al*., 2014) [18].

The histomorphological studied of the kidney from rats of group II showed mild to moderate, focal to multifocal cystic degenerative changes, vacuolar degenerative changes, congestion, necrotic changes and occasional hyaline caste in lumen of convuluted tubules. (plate 3). The earlier reports in respective histopathological examination of kidney in CCl_4 toxicated rats published by Adewole *et al*. (2007) [1] Saber *et al*. (2012) [16] goes parallel with present observation. Similar but less intense histopathological changes were noted in rats of group III, IV, V and VI and the restoration appeared to be treatment and dose dependant. However, the section of the kidney from the rats of the control group found to be within histological limits.

The histopathological study of section of lung from rats of group II showed congestion and inflammatory cell infiltration with variation in extends of intensity, more intense pneumopathy (plate 4). The earlier reports in respective histopathological examination of lung in

CCl_4 toxicated rats published by Khan *et al*. (2012) [9] goes parallel with present observation.

The brain on histopathology revealed mononuclear cell infiltration and vacuolation in the rats of group II (plate 5). The earlier reports in respective histopathological examination of brain in CCl_4 toxicated rats published by Simeonova *et al*. (2019) [17] goes parallel with present observation. However, the section of brain from all other treatment of control group did not showed any appreciable histopathological changes.

The histopathological examination of testes from rats of toxicated non treated group showed minimal to mild loss of spermatogoneal cells, increase in interstitial cells and distortion of basement membrane at places. (plate 6) The earlier reports in respective histopathological examination of testes in CCl_4 toxicated rats published by Ojo *et al*. (2016) [14] goes parallel with present observation. The sections of testes from treated rats were with less intensity and the same were found to be dose dependent.

The sections of ovaries from the control as well other experimental groups did not showed any appreciable histopathological change.

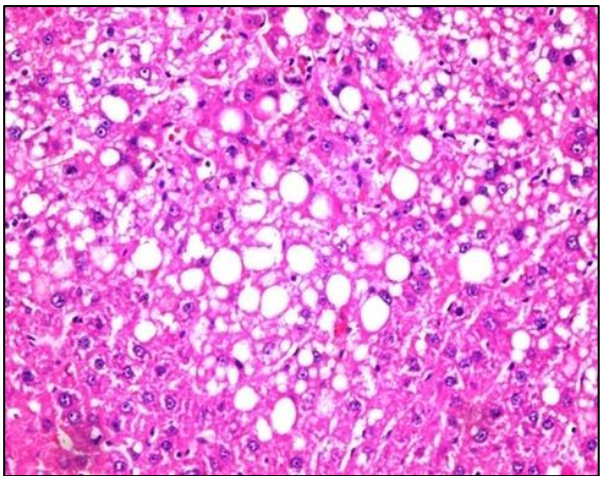

Plate 1: Section of liver with fatty changes and necrosis in rat of group II (400X) H and E stain

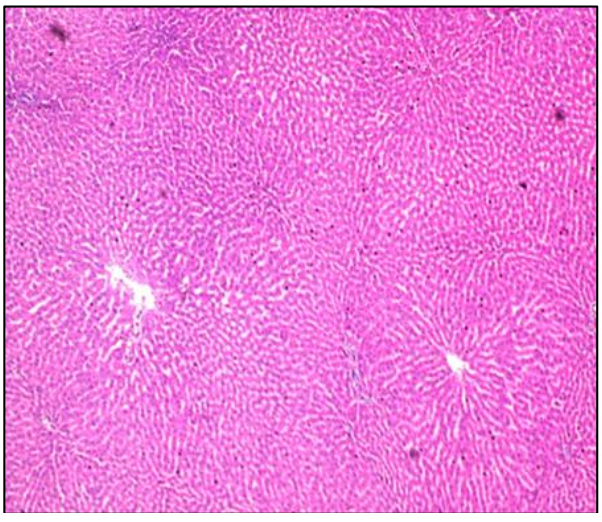

Plate 2: Section of Liver with mononuclear cell infiltration in rat of group VI (100X) H and E stain

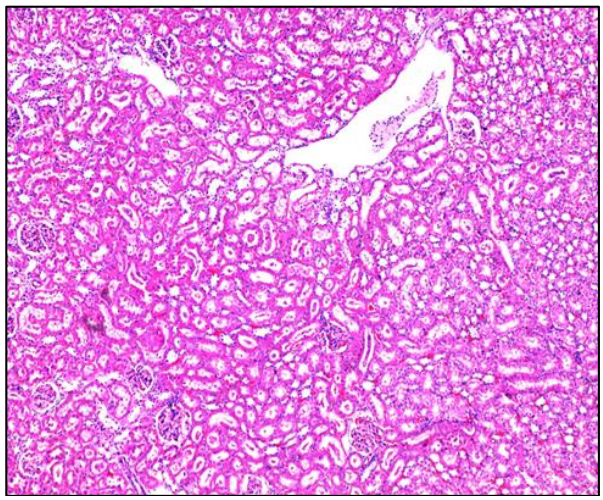

Plate 3: Note cystic degenerative changes, congestion and MNC infiltration in kidney from rat of group II (100X) H and E stain

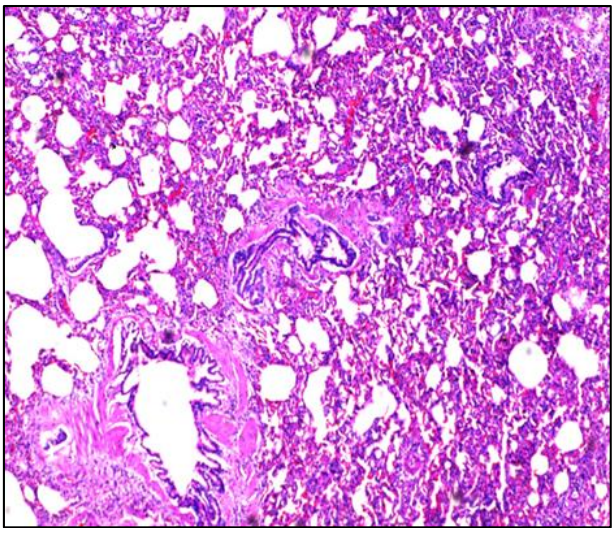

Plate 4: Section of lungs from rat of group II showing congestion and severe inflammatory cell infiltration. (100X) H and E stain

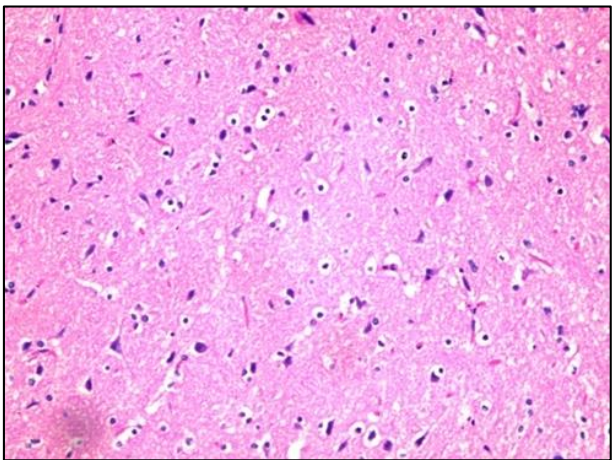

Plate 5: Section of brain showing MNC infiltration and vacuolation from rat of group II (400X) H and E stain

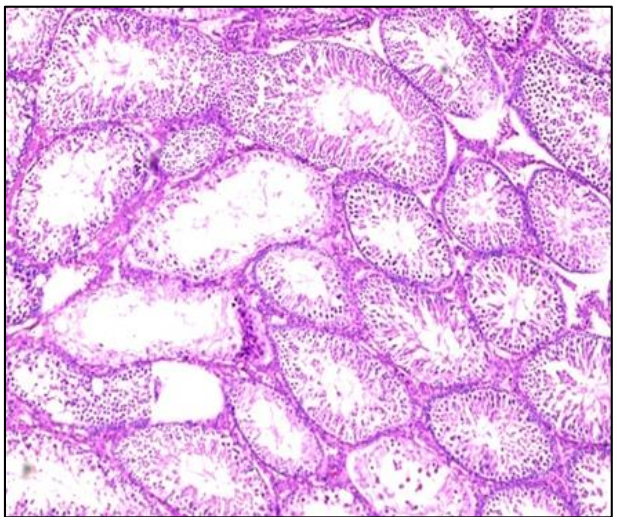

Plate 6: Note occasional loss of spermatogonial cell in testes from seminiferous tubule in rat of group II (100X) H and E stain

Conclusion

From present investigation it can be concluded that, Use of carbon tetrachloride at the dose rate of 0.1 ml/ rat by intraperitoneal injection for 28 days can induce hepatotoxicity in wistar rats. The Carbon tetrachloride showed damaging results in gross and histopathological observations where-as the treatment with *Cassia fistula* leaves extract at the dose rate of 600 mg/kg body weight showed promising results.

References

1. Adewole SO, Salako AA, Doherty OW, Naicker T. Effect of Melatonin on Carbon Tetrachloride Induced Kidney Injury in Wistar Rats. African Journal of Biomedical Research. 2007; 10:153-164.
2. Bhakta T, Mukherjee PK, Mukherjee K, Banerjee S, Mandal SC, Maity TK *et al*. Evaluation of hepatoprotective activity of *Cassia fistula* leaf extract. Journal of Ethnopharmacology. 1999; 66:277-282.
3. Cameron GR, Thomas JC, Karunarathe WAE. The pathogenesis of liver injury in carbon tetrachloride and thioacetamide poisoning. J Path Bact. 1936; 41:297.
4. Chang C, Chen Y, Yang S, Huang G, Tsi D, Huang C *et al.* Effect of Schisandrin B and Sesamin Mixture on CCl_4-induced Hepatic Oxidative Stress in Rats. Phytother Res, 2009; 23:251-256.
5. Culling CFA. Handbook of histopathological and histochemical techniques, 3rd end., Butterworth and Co. Ltd. 1974, 209-221.
6. Dawada S, Zade V, Dabhadkar D, Pare S. Hepatoprotective Activity of *Cassia fistula* root against Carbon tetrachloride-Induced Hepatic Injury in rats (Wistar). International Journal of Pharma Sciences and Research (IJPSR) 2012; 3(4):368-378.
7. De Groot H, Littauer A, Hugo Wissemann D, Wissemann P, Noll T. Lipid peroxidation and cell viability in isolated hepatocytes in a redesigned oxystat system: Evaluation of the hypothesis that lipid peroxidation, preferentially induced at low oxygen partial pressure, is decisive for CCL_4 liver cell injury. Arch. Biochem. Biophys. 1988; 264:591-599.
8. Gupta RK. Medicinal & Aromatic plants, CBS publishers & distributors, 1st edition, 2010, 116-117.
9. Khan RA. Protective effect of *Launaea procumbens* (L.) on lungs against CCl_4–induced pulmonary damage in rats. Complementary and Alternative Medicine 2012; 12(133):1-7.
10. Kiezcka H, Kappus H. Oxygen dependence of CCl_4-induced lipid peroxidation *in vitro* and *in vivo*. Toxicol. Lett. 1980; 5:191-196.
11. Lakra PP, Hemalatha S, Sridhar R, Mangala Gowri A, Kumar K, Sahoo D *et al*. Evaluation of hepatoprotective potential of *Cassia fistula* in N-Diethylnitrosamine induced hepatocarcinogenesis in Wistar rats. Indian J Vet. Pathol. 2016; 40(3):218-223.
12. Masuda Y, Nakamura Y. Effects of oxygen deficiency and calcium omission on carbon tetrachloride hepatotoxicity in isolated perfused livers from phenobarbital-pretreated rats. Biochem. Pharmacol 1990; 40:1865-1876.
13. Mohamed Saleem TS, Chetty CM, Ramkanth S, Rajan VST, Mahesh Kumar K, Gauthaman K *et al*. Hepatoprotective Herbs–A Review. Int. J Res. Pharm. Sci. 2010; 1(1):1-5.
14. Ojo OA, Ojo AB, Ajiboye B, Akintayo C, Oyinloye BE, Ojo AA *et al.* Carbon Tetrachloride-induced oxidative stress in Wistar rat brain: Neurocurative potential of *Ficus asperifolia* (Miq). Journal of Chemical and Pharmaceutical Science. JCPS 2016; 9(3):1334-1338.
15. Panse UG, Sukhatme PV. Statistical Methods for Agril. Workers, ICAR Publications, New Delhi, 1967.
16. Saber AS, Hawazen AL. Protective Effect of Rosemary (*Rosmarinus officinalis*) Leaves Extract on Carbon Tetrachloride Induced Nephrotoxicity in Albino Rats. Life Science Journal. 2012; 9(1):779-785s.
17. Simeonova R, Vitcheva V, Kondeva Burdina M, Popov G, Shkondrov A, Manov V *et al.* Alcesefoliside protects against oxidative brain injury in rats. Revista Brasileira de Farmacognosia 2019; 29:221-227.
18. Singh D, Arya PV, Sharma A, Aggarwal VP, Dobhal MP, Gupta R *et al.* Antioxidant Potential of Plumieride against CCL_4 -Induced Peroxidative Damage in Rats. Antioxidant. 2014; 3:798-813.
19. Slater TF. Biochemical studies on liver injury. Academic Press, New York, 1978, 1-44.
20. Uehara T, Ainslie G, Kutanzi K, Pogribny IP, Muskhelishvili L, Izawa T *et al*. Molecular Mechanisms of Fibrosis- Associated Promotion of Liver Carcinogenesis, toxicological sciences. 2013; 132(1):53-63.