Haryana Vet. (December, 2024) **63(2),** 265-268

Short Communication

REVERSAL OF CARBON TETRACHLORIDE INDUCED HEPATIC DAMAGE BY MORIN IN RATS

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Received: 15.05.2023; Accepted: 27.09.2023

SUMMARY

The purpose of this study was to ascertain morin's function in rat liver injury caused by carbon tetrachloride. Eighteen male wistar strain albino rats, weighing between 150 and 200 grams, were assigned six per group at random. Group II and III received CCl_4@ 1ml/kg B.wt in olive oil (1:1) I/P twice a week in the second and third weeks, whereas Group-I, acting as the control, was given 1% DMSO orally for three weeks. Furthermore, morin @ 30 mg/Kg B.wt was given orally to Group-III rats for three weeks. Hepatic tissue homogenate and histomorphological analysis of the liver were used to determine the extennt of CCl_4-induced damage, Biochemical tests such as ALT, AST, ALP, bilirubin, total protein, albumin, SOD, CAT, GSH, GPx and TBARS were estimated as biomarkers of hepatic damage. Increased levels of ALT, AST, ALP, and TBARS and decreased levels of GSH, SOD, CAT, GPX and total protein were indicative of oxidative damage mediated by CCl_4. Comparing the morin treated group to the CCl_4-induced hepatotoxic group, the former displayed improvement in antioxidant markers, a significant decrease in malondialdehyde levels, and a reversal of altered hepatic biomarkers. The results of the experiment indicated that morin's antioxidant action protected rats against CCl_4-induced hepatic damage.

Keywords: Hepatotoxicity, Morin, SOD, TBARS

How to cite: Sri Harshini, V., Srividya, G., Ravi Kumar, P., Naveen Swaroop, M., Samatha, V., Anjaneyulu, P. and Sai Prathyusha, G. (2024). Reversal of carbon tetrachloride induced hepatic damage by Morin in rats. *Haryana Vet.* **63(2)**: 265-268.

The body's vascular, secretory, immunological, metabolic, and xenobiotic detoxification processes are all significantly impacted by the liver. Around 1.2% of global mortality is attributed to it, making it the eleventh most common cause of death and the fifteenth most common cause of illness. (Cheemerla and Balakrishnan, 2021). Carbon tetrachloride induced hepatotoxicity in rats was widely accepted model to investigate the drugs for hepatoprotective effects (Gosh, 2015). Lipid peroxidation and oxidative stress are the primary causes of acute liver damage in CCl4-induced toxicity. Recently, there has been a greater focus on using natural products as a source for creating novel therapeutic agents. Morin has a variety of pharmacological and biochemical properties, such as anti-oxidant, metal ion chelating ability, anti-inflammatory (Zhang *et al.*, 2011), cardioprotective (Al-Numair *et al.*, 2012) and anticancer properties (Sivaramakrishnan and Devaraj, 2010). Morin shields erythrocytes, hepatocytes, endothelial cells and myocytes against oxidative damage by controlling the activity of metabolic enzymes (Kitagawa *et al.*, 2004). In light of this, the objective of current investigation was to ascertain morins role in hepatic damage caused by carbon tetrachloride in rats.

Eighteen adult male wistar strain albino rats, weighing between 150 and 200 grams and three to four months of age, were used in the experiment. Analytical grade chemicals were utilized to estimate different biochemical parameters.

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Experimental design

The institutional animal ethics committee, N.T.R. College of Veterinary Science, Gannavaram authorized the experimental protocol vide Ref. No. 3/IAEC/NTR CVSC/21 dated 12/03/2022. The rats in groups-II and III were administered with CCl4 @ 1 ml/kg body weight in olive oil (1:1) I/P twice a week in the second and third weeks, while group-I, which served as the control, received 1% DMSO orally for three weeks and olive oil I/P twice a week. For three weeks, Group-III rats were given 30 mg/kg body weight of morin orally. Blood was drawn via the retrobulbar venous plexus twenty-four hours following the final CCl4 therapy. Serum and plasma were separated for biochemical estimations. Rats were sacrificed as per the CCSEA guidelines and livers were collected for biochemical and histopathological examination at the conclusion of the experiment.

Experimental procedures

Serum samples of various groups were used to estimate the liver function indicators, such as ALT, AST, ALP, and bilirubin. whereas, total protein and albumin were estimated through plasma using kits. Bovine serum albumin (BSA) was used as the standard method to measure the total protein content in the liver tissue homogenate using Lowry's method (1951). Antioxidant parameters were estimated using standard protocols *viz*., SOD by Madesh and Balasubramanian (1998), GSH by Sedlak and Lindsay (1968), CAT by Aebi (1983),

Table 1. Effect of morin on antioxidant parameters in CCl_4 intoxicated rats

Group	Antioxidant parameters			
	GSH** (µm of GSH/mg of protein)	SOD** (Units/mg of protein)	CAT* (mM H_2O_2 utilized/ min/mg of protein)	GPx** (Units/ml)
I	60.82±2.18^{c}	68.31±3.57^{c}	5.23±0.63^{c}	1299.49±59.63^{cl}
II	8.38±0.59^{a}	11.27±0.53^{a}	2.38±0.59^{a}	431.13±56.61^{a}
III	30.56±0.97^{b}	54.96±3.04^{b}	3.58±0.66^{bc}	1027.59±67.62^{b}

**p<0.01, *p<0.05
Column's means with different superscripts show a significant difference.

Table 2. Effect of morin on liver functional markers in CCl_4 intoxicated rats

Group	Liver functional markers			
	AST** (IU/L)	ALT** (IU/L)	ALP** (IU/L)	Total bilirubin** (mg/dl)
I	7.95±0.55^{a}	18.12±0.67^{a}	51.13±2.11^{a}	7.38±0.43^{a}
II	38.01±2.32^{c}	58.04±1.41^{c}	94.43±2.61^{c}	15.41±1.10^{c}
III	16.79±0.79^{b}	24.01±0.95^{b}	66.79±2.80^{b}	9.72±0.23^{b}

**p<0.01, *p<0.05
Column's means with different superscripts show a significant difference

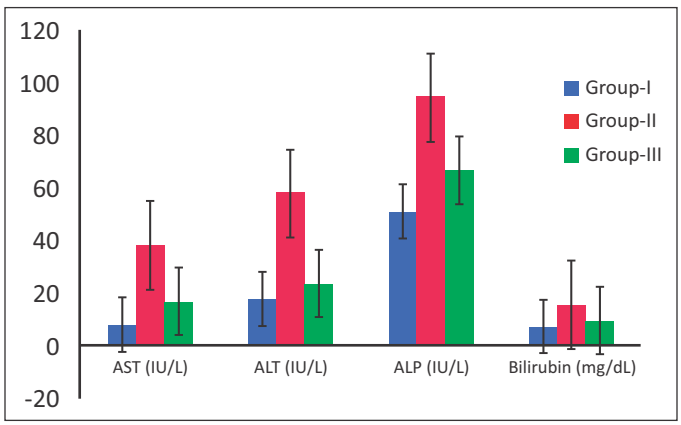
GPx by Paglia and Valentine (1967) and TBARS by Balasubramanian *et al.* (1988).

Experimental Data analysis:

Statistical software (SPSS 17.0 version) was used to analyze the data. Each group's mean ± SEM was used to express the findings of the experiment. Following one-way ANOVA, the means were compared and Duncan's multiple range test was used to determine the statistical significance of the group differences.

One of the hepatotoxins most frequently utilized in the experimental investigation of hepatic damage is carbon tetrachloride. Complex C-Cl bond breakage by CCl_4 produces oxidatively active compounds including chloromethyl free radicals (•CCl_3), which in turn react with molecular oxygen to form trichloromethyl peroxyl free radicals (CCl_3O_2•) by hepatic cytochrome P450 enzymes (Huo *et al.*, 2020). This CCl_3• alkylates lipids, proteins, and nucleic acids, which changes calcium homeostasis, steatosis, and protein synthesis.

(Weber *et al.*, 2003) whereas CCl_3O_2• can remove hydrogen from polyunsaturated fatty acids to form covalent bond with the sulfhydryl groups of various membrane molecules, such as GSH, which depletes them and results in lipid peroxidation (Bagali *et al.*, 2020). Both alkylation and lipid peroxidation are causative factors for CCl_4-induced hepatotoxicity. It would make sense that substances that counteract free radicals would have a hepatoprotective effect, given their significant role in the hepatotoxicity caused by CCl_4.



The reference values of ALT, AST, ALP, bilirubin were 7.95±0.55 IU/L, 18.12±0.67 IU/L, 51.13±2.11 IU/L and 7.38±0.43 mg/dl, respectively in Group-I rats which were presented in Table 2. Elevated levels of AST (38.01 ±2.32 IU/L), ALT (58.04±1.41 IU/L), ALP (94.43±2.61 IU/L) and bilirubin (15.41±1.10 mg/dL) in the serum of group-II rats indicated severe liver damage caused by CCl_4. Administration of morin at a dose of 30 mg/kg showed marked reduction in AST, ALT, ALP and bilirubin levels by about 56%, 58%, 30% and 37%, respectively inn relation to Group-II rats that received CCl_4 alone indicating that morin may have exhibited hepatoprotective properties and mitigated lipid peroxidation effects of CCl_4 to revert the deteriorative alterations of the liver parenchyma and concomitantly decreased the leakage of intracellular enzymes and protects the rat against CCl_4 induced hepatotoxicity which were in accordance with Lee *et al.* (2008) and Ozdemir *et al.* (2020).

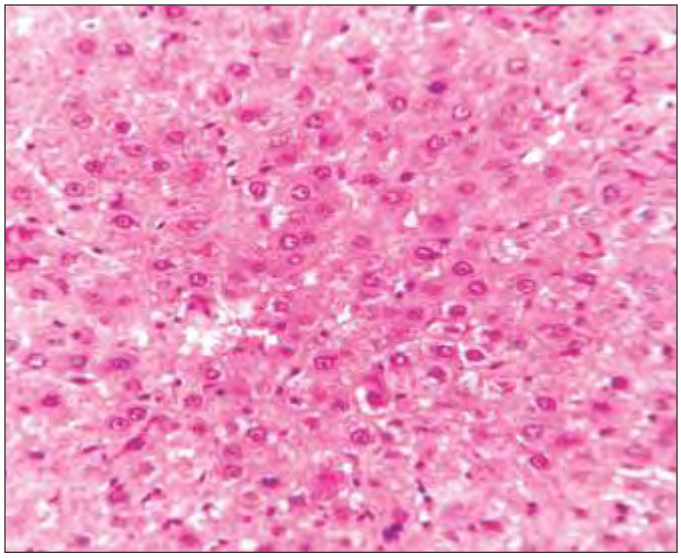


Fig. 2. Group I- Normal architecture of liver. H&E x 400

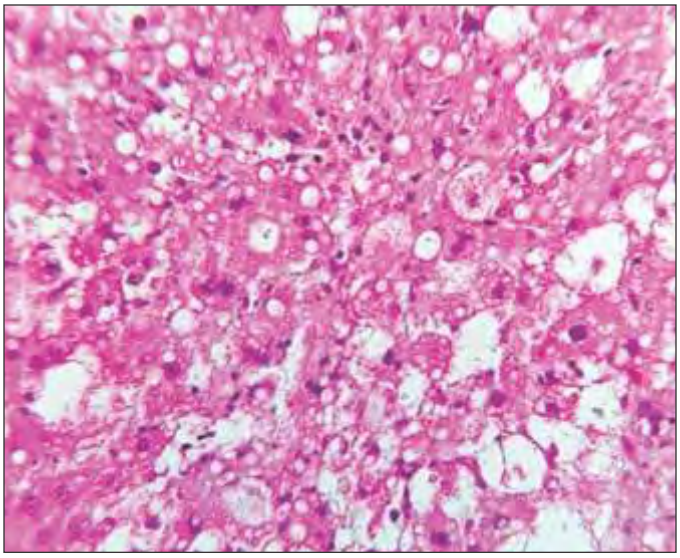


Fig. 3. Group II- liver showing necrosis of hepatocytes, severe fatty change and infiltration of mononuclear cells. H&E x 400

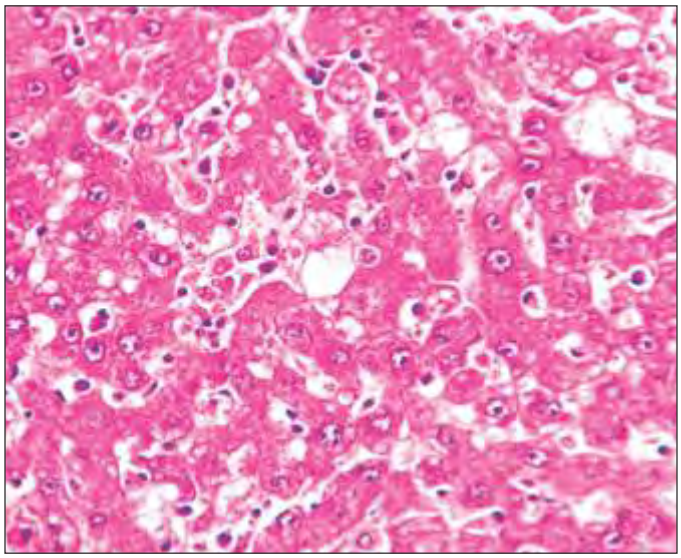


Fig. 4. Group II- hepatocytes showing tiny to big lipid droplets in cytoplasm, pushing the nucleus to one side. H&E x 400

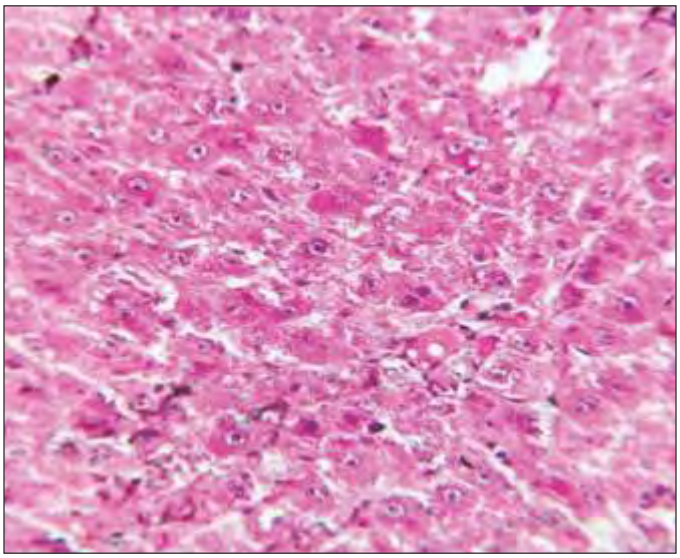


Fig. 5. Group III: mild to moderate cloudy swelling of hepatocytes. H&E x 400

The normal plasma levels of protein and albumin in the control group were 6.55±0.06 g/dl and 3.92±0.08 g/dl. In CCl_4 induced toxic group the total protein and albumin levels were reduced significantly to 5.51±0.09 g/dl and 2.48±0.10 g/dl. Rats supplemented with morin group showed raise in the total protein and albumin levels to 6.20±0.01 g/dl and 3.19±0.07 g/dl in group-III by stabilization of the endoplasmic reticulum, which results in the production of proteins and the existence of a free radical-blocking effect (Khattab, 2012).

Liver tissue antioxidant markers *viz*., GSH, SOD, CAT and GPx activities were found significantly lowered by CCl_4 in group-II rats 86%, 83%, 54% and 66% in contrast to control group. A statistically significant rise in the levels of antioxidant indicators was seen following the administration of morin at a dose of 30 mg/kg P.O. over a period of 21 days. Morin's capacity to prevent thiol groups from oxidative degradation by preventing membrane lipid oxidation may be attributed to its protective role in CCl_4 induced oxidative stress (Singaravelu *et al.*, 2020 and Tianzhu *et al.*, 2014). Increase in the CAT activity was observed in Group-III rats because morin stimulates catalase activity and scavenges intracellular H_2O_2, it has a cytoprotective effect against H_2O_2-induced DNA damage and lipid peroxidation (Zhang *et al.*, 2009).

The lipid peroxidation marker (TBARS) in liver homogenate revealed a significant increase in the MDA concentration by 83% in Group-II under the influence of carbon tetrachloride a treatment with morin restored MDA levels by around 65%, limiting liver damage and reversing lipid accumulation in the liver (Gu *et al.*, 2017). This diminution in the concentration of lipid peroxidation

products demonstrates morin's anti-lipid peroxidative properties which are mostly caused by hydroxyl groups at the 2' and 4' positions of morin (Choudhury *et al.*, 2017).

CONCLUSION

The experiment findings indicated that because of its antioxidant action, morin provided protection to rats against oxidative damage caused by CCl_4. Further studies may be conducted to determine the mode of action of morin and safety studies in different livestock species to make it applicable as novel drug in treatment regimen of various disease conditions related to oxidative stress.

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