Research Article

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NEUROPROTECTIVE EFFECT OF BIOFLAVONOIDS AGAINST CARBON TETRACHLORIDE INDUCED NEUROTOXICITY IN RATS

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ABSTRACT

The present study was undertaken to explore the neuroprotective potential of bioflavonoids against carbon tetrachloride (CCl_4) induced neurotoxicity in rats. Neurotoxicity was induced by CCl_4 (3ml/kg body weight) in wistar rats. Blood biochemical, hematological parameters and histopathological studies were carried out to assess the neuroprotective effect. Administration of CCl_4 has induced significant neurotoxicity in rats, which was evident from enhanced levels of potassium and decreased levels of sodium. Pretreatment with quercetin (50 mg/kg dose orally) significantly reversed CCl_4 induced neurotoxicity than the silymarin (50 mg/kg dose orally). From the obtained results it can be concluded that quercetin exerted a significant neuroprotective effect against CCl_4 induced neurotoxicity in rats than silymarin ($p < 0.001$) for sodium and potassium in blood biochemical parameters, packed cell volume, mean corpuscular hemoglobin count and red blood cells as well in attenuation of pathological changes in brain tissues.

Keywords: Neurotoxicity, bioflavonoid, carbon tetrachloride, quercetin, silymarin

INTRODUCTION

Flavonoids comprise a large group of chemical compounds with a basic diphenylpropane structure. They were divided into several subclasses like flavonols, flavons, flavanols and flavanons based on their functional groups. These compounds naturally occur in various plant parts (fruits, vegetables, nuts, seeds, flowers) and essential constituent of diet as well. Epidemiological studies indicated that diets rich in flavonoids were associated with reduced incidence of several chronic diseases including hepatic, renal diseases, asthma, type II diabetes and certain types of cancer. The neuroprotective properties of flavonoids were multi-faceted involving antioxidant and anti-inflammatory effects. The antioxidant property of flavonoids was thought, until relatively recently, to underlie the majority of their protective cellular effects. However, it is becoming increasingly apparent that flavonoids also influence cellular function by modulating the activity of many enzymes including the inhibition of protein kinases and lipid kinases[1].

Silymarin is a compound which is obtained from the plant *Silybum marianum* (milk thistle) was an edible plant it has been used medicinally for centuries as a herbal medicine. It is a mixture of mainly three flavonolignans: silybin, silidianin, and silychristine, with silybin being the most active. This compound has been used to treat liver disorders. The compound consistuent antioxidant activity, stimulation of ribosomal RNA polymerase and subsequent protein synthesis, lead to enhanced hepatocyte regeneration[2-5].

Quercetin a major representative of the flavonol subclass, has received the considerable attention because of its overwhelming presence in foods. Quercetin and its sugar-bond or glucosylated, forms represent to 60-75% of flavonoids intake. It displayed the ability to prevent the oxidation of low-density lipoproteins (LDL) by scavenging free radicals and chelating transition metal ions. As a result, quercetin may aid in the prevention of certain diseases viz., cancer, atherosclerosis and chronic inflammation[6].

MATERIALS AND METHODS

Animals

Wister rats of either sex; weighing around 200- 250 g in the present study were the in-house breed of Chalapathi Institute of Pharmaceutical Sciences, Guntur. They were exposed to an alternate light and dark cycle of 12 hour period. During this period the animals were provided with a standard diet and water ad libitum. The animals were acclimatized to the laboratory conditions for at least 5 days before the neuro toxicity test. The experimental protocol was approved by the Institutional Animal Ethics Committee (IAEC) of Chalapathi Institute of Pharmaaceutical Sciences, Guntur, Andhra Pradesh (Approval No. 09/IAEC/CIPS/2016-17; dt 05/04/2016) and care of the animals was taken as per guidelines of the committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA), Ministry of Forest, Environment, Climate Change, Government of India.

Drugs and reagents

Silymarin, quercetin was purchased from Sigma Aldrich, Bangalore, India. Carbon tetrachloride (CCl_4) and formalin were obtained from the Chalapathi Institute of Pharmaceutical Sciences. CCl_4 was administered intraperitonially (i.p.) to induce neurotoxicity in wistar rats[7,8].

Experimental groups

The efficacy of silymarin was compared with quercetin by evaluating in vivo neuroprotective activity in rats against CCl_4 induced neurotoxicity[9,10,11].

Four groups, each comprising of five wistar rats, were employed in the study. Group I (Control group): Rats were administered normal saline (0.9% w/v), orally for 14 days. Group II (CCl_4 -treated control group): Rats were administered CCl_4 (3 ml/kg,

i.p.) on the day 14. Group III (Silymarin + CCl₄- treated group): Rats were treated with silymarin (50 mg/kg, orally) for 14 days. On the 14th day silymarin was administered 60 min prior the administration of CCl₄. Group IV (Quercetin + CCl₄-treated group): Rats were treated with quercetin (50 mg/kg, orally). The remainder of the procedure was similar to that of Group III. The blood samples were collected by cardiac puncture under deep anesthesia (terminal blood sampling using a 23G needle vertically into the sternum) produced by the administration of ketamine and xylaxine. After sufficient exsanguination, the brain of the treatment animals was isolated after cervical dislocation. The blood samples and brain were immediately subjected to hematological, blood biochemical and histopathological studies respectively. The carcass of the dissected animal was disposed by deep burial as per the Standard Operating Procedures of CPCSEA, Government of India. The histopathological brain study samples were prepared by the hematoxylin and eosin staining techniques.

Statistical analysis

The results of the study expressed as mean ± standard error of means (S.E.M.). The data of neurotoxicity studies were statistically analyzed by one-way analysis of variance (ANOVA) followed by Tukey's multiple range test using Graph pad Prism version 6.0. A p-value (<0.05) was considered to be statistically significant.

RESULTS

Various pharmacological studies employed in the present study did not show any significant mortality. Further, no significant difference was observed between the results obtained from rats of either sex.

**Effect on blood biochemical and hematological parameters**

The treatment group relatively to control group received significant impact on blood biochemical and hematological parameters. The neurotoxic agent CCl₄ drastically reduced the sodium levels where as increased potassium level. In turn the lymphocytes count was also decreased. Silymarin and quercetin treatment groups showed significant neuroprotective effect ($p<0.05$) against CCl₄ group (Table 1& 2). Administration of silymarin (50 mg/kg, orally) to CCl₄ treated animals showed significant difference in sodium, total count, differential count and in PCV. Treatment with quercetin (50 mg/kg, orally) significantly decreased the CCl₄ induced rise in blood biochemical and hematological parameters (Table 1 & 2).

Table 1: Blood biochemical parameters of various treatment groups

S.No.	Parameter	Group I	Group II	Group III	Group IV
1.	Sodium (Na⁺) (mmol/lit)	135.4±1.5	116.6±5.44	134.8±1.88	132.4±2.29
2.	Potassium (K⁺) (mmol/lit)	7.24±0.2	9.8±0.09	10±0	8.84±0.13

Table 2: Hematological parameters of various treatment groups

S.No	Parameter	Group I	Group II	Group III	Group IV
1.	Hemoglobin (mg/dl)	13.56±0.21	16.4±0.39	13.41±0.76	10.55±1.12
2.	Total Count(mm³)	3800± 291.54	2440± 156.84	1540± 166.13	3500± 164.31
3.	Differential Count (%)				
	a) Polymorphs	30±4.29	36.6±1.28	31.6±2.16	36±1.92
	b) Lymphocytes	62.8±4.72	55±2.05	60.6±3.39	55±1.58
	c) Eosinophils	2.2±0.86	4.4±1.02	2.6±0.51	5.8±0.86
	d) Monocytes	2.4±0.81	5±0.71	2.4±0.81	3±0.63
4.	Packed Cell Volume (%)	42.4±0.92	50.2±0.86	38.2±2.31	31±3.57
5.	Mean Corpuscular Hemoglobin Count (%)	31±0.32	32.8±0.37	34.4±0.51	30.8±0.37
6.	Platelet Count (lakhs/mm³)	2.94±0.22	4.86±0.57	3.44±0.65	3.36±0.71
7.	Red Blood Cells (million/mm³)	7.3±0.26	8.87±0.17	7.22±0.44	5.46±0.72
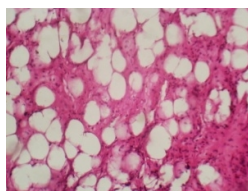
Control
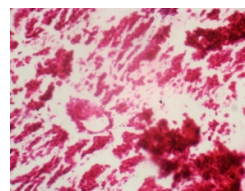
Positive control

Silymarin

Quercetin

Figure 1: Histology slides of the isolated brain of various treatment groups showing haematoxylin and eosin stained cells

Histopathological studies

In relation with group I, group II animals showed significant damage in brain tissue. The significance of p value is <0.05. The group II showed foci of necrosis and inflammation, where as in group I normal parenchyma was observed. From the figure (c) necrosis was observed in group III animals. The group IV animals showed significant recovery of brain tissue when compared with group III (Figure 1).

DISCUSSION

Carbon tetrachloride induced neurotoxicity employed in the present study is one of the most widely accepted models to evaluate neurotoxicity of the animals. In the present study, CCl_4 induced neurotoxicity showed a significant increase in observed blood biochemical parameters except sodium ($p<0.002$) when compared to control group. The hematological parameters showed increased levels except lymphocytes (p <0.271) and total count ($p<0.003$).

Pretreatment with silymarin attenuated CCl_4 induced neurotoxicity when compared to positive control group which is evident from significant increased in potassium (0.0001) and hematological parameters. The total count showed a significant decrease in total count ($p<0.0003$) and packed cell volume showed significant decrease ($p<0.001$)

In hematological parameters silymarin showed no significant difference against quercetin except mean corpuscular hemoglobin count ($p<0.0002$). In blood biochemical parameters the silymarin showed significant difference in potassium ($p<0.0001$).

Pretreatment with quercetin showed significant difference in sodium ($p<0.0504$) and potassium ($p<0.0001$). Group IV showed a significant decreased in observed hematological parameters except hemoglobin ($p<0.9916$), platelet count ($p<0.1413$) and differential count ($p<0.4419$) when compared with CCl_4 group.

Therefore, from the above findings, it is evident that quercetin abolished CCl_4 induced neurotoxicity. The neuroprotective properties of flavonoids are multi-faceted involving antioxidant and anti- inflammatory effects. The antioxidant property of flavanoids was thought, until relatively recently, to underlie the majority of their protective cellular effects. However, it is becoming increasingly apparent that flavonoids also influence cellular function by modulating the activity of many enzymes including the inhibition of protein kinases and lipid kinases. Therefore, it may be concluded that quercetin exerts its beneficial effect in CCl_4- induced neurotoxicity by virtue of its ability to prevent the damage of cell membranes and secondary free radicals, antioxidative, and anti-inflammatory actions. Nevertheless, further studies are needed to explore the full potential of quercetin in neuroprotective effect.

CONCLUSION

From the obtained results it can be concluded that quercetin exerted a significant neuroprotective effect against CCl_4 induced neurotoxicity in rats than silymarin ($p<0.001$) for most of the blood biochemical parameters, mean corpuscular hemoglobin count as well in attenuation of pathological changes in brain tissues.

REFERENCES

1. Jinu K V, Archana R, Kumar Sai Sailesh, Mukkadan J K. A comprehensive review on neuroprotective effects of pomegranate. Int. J. Res. Ayurveda Pharm. Mar - Apr 2016;7(2):48-51 http://dx.doi.org/10.7897/2277-4343.07255
2. Jiang HH, Yan FS, Shen L, Ji HF. Silymarin versus silibinin: differential antioxidant and neuroprotective effects against H_2O_2-induced oxidative stress in PC12 cells. Nat Prod Commun 2016; 11(5): 633-6.
3. Jung UJ, Jeon MT, Choi MS, Kim SR. Silibinin attenuates MPP+-induced neurotoxicity in the substantia nigra in vivo. J Medicinal Food. 2014; 17(5): 599-605.
4. Borah A, Paul R, Choudhury S, Choudhury A, Bhuyan B, Das Talukdar A, et al. Neuroprotective potential of silymarin against CNS disorders: insight into the pathways and molecular mechanisms of action. CNS Neurosci Ther 2013; 19(11): 847-53.
5. Pérez-HJ, Carrillo-SC, García E, Ruiz-Mar G, Perez-Tamayo R, Chavarria A. Neuroprotective effect of silymarin in a MPTP mouse model of parkinson's disease. Toxicology 2014; 319:38-43.
6. Costa LG, Garrick JM, Roquè PJ, Pellacani C. Mechanisms of neuroprotection by quercetin: counteracting oxidative stress and more. Oxid Med Cell Longev 2016; 24: 1- 10.
7. Naseem M, Parvez S. Hesperidin restores experimentally induced neurotoxicity in wistar rats. Toxicol Mech Methods 2014; 24(7): 512-9.
8. Ismail AF, Moawed FS, Mohamed MA. Protective mechanism of grape seed oil on carbon tetrachloride-induced brain damage in γ-irradiated rats. J Photochem Photobiol B 2015; 153: 317-23.
9. Al-Olayan EM, El-Khadragy MF, Omer SA, Shata MT, Kassab RB, Abdel Moneim AE. The beneficial effect of cape gooseberry juice on carbon tetrachloride - induced neuronal damage. CNS Neurol Disord Drug Targets 2016; 15(3):344-50.
10. Zaib S, Khan MR. Protective effect of cucurbita pepo fruit peel against CCl_4 induced neurotoxicity in rat. Pak J Pharm Sci 2014; 27(6):1967-73.
11. Llorens J, Crofton KM. Enhanced neurotoxicity of 3, 3'-iminodipropionitrile following carbon tetrachloride pretreatment in the rat. Neurotoxicology 1991; 12(3):583-94.

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