

COMPARATIVE HEPATOPROTECTIVE ACTIVITY OF DIFFERENT EXTRACTS OF SPIRULINA AGAINST CCL₄ INDUCED LIVER DAMAGE IN RATS

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ABSTRACT

Spirulina is simple microscopic blue green algae. It belongs to cyanophyceae a procaryotic form. Present research work is to evaluate the hepatoprotective activity of spirulina. Comparative evaluation of hepatoprotective activity of petroleum ether, chloroform and methanol extracts of spirulina against carbon tetrachloride induced hepatotoxicity based on the biochemical parameters. All the test extracts administered orally for a period of 10 days showed significant hepatoprotective activity.

Keywords: spirulina, hepatotoxicity, Hepatoprotective activity, phycocyanin, phycoerythrin.

INTRODUCTION

Spirulina is simple microscopic blue green algae. It belongs to cyanophyceae a procaryotic form. The photosynthetic pigment phycocyanin and phycoerythrin makes it to be included in cyanophycean algal group. But the nature of it's nucleus (procaryotic) engrauped it in as a procaryotic organism a cyanobacterium.

In other wards spirulina is a photosynthetic procaryotic microorganism. It grows naturally in fresh, brakish sewage water and even in saline environment. It grows through photosynthesis, hence can be termed as vegetative food. It has already been affectively promoted as a natural food. Spirulina is a natural package of protein, vitamins and minerals.

The experimental intoxication induced by ccl₄ is widely used for modeling liver injury in rats. Village folk use, spirulina in liver inflammation. The present study is undertaken to observe the hepatoprotective activity of different extracts of spirulina in the induced hepatotoxicity with treatment of toxic dose of carbon tetrachloride in rats. Free radicals initiate the process of lipid peroxidation, which is generally a cause of the, inhibition of the enzyme activity^{1,2}. Both trichloro-methyl and it's peroxy radicals are capable of binding to proteins, or lipids or of abstracting a hydrogen atom from an unsaturated lipid initiating lipid peroxidation and liver damage and by doing so playing a significant role in pathogenesis of diseases³. Flavonoids, Terpinoids, Triterpinoids and steroids etc have received considerable attention in recent years due to their diverge pharmacological properties including their antioxidant and hepatoprotective activity^{4,5}. Present research work is to evaluate the hepatoprotective activity and simultaneous comparison of PEFS (Petroleum ether fraction of spirulina), CFS (Chloroform fraction of spirulina) & MFS (Methanolic fraction of spirulina).

MATERIALS AND METHODS

All the chemicals are of analytical grade. Silymarin (standard hepatoprotective drug) "Selybon" micro labs ltd. Bangalore and ALP (Alkaline phosphatase) reckon diagnostics, Baroda.

Extraction of Spirulina

Fresh spirulina were collected near our college campus and authenticated by Dr. S.K. Dash H.O.D. and professor Biological Division and bio sciences and dried the Spirulina. Then extracted in a soxhlet apparatus (Williams and Wilkin Company 2000) by using petroleum ether chloroform and methanol respectively. The percentage of yield was found to be 41, 26 & 55 respectively. It was tested positive for the presence of Alkaloids, Tannins, Proteins Flavonoids, Steroids.

Hepatoprotective activity evaluation

Healthy wistar albino rats (150 to 180 gms.) of either sex obtained from animal house CPS, mohuda. IAEC (Institutional Animal Ethical committee) has given approval for this assessment in resolution no 1 of IAEC meeting held on 6th Nov 2009. Animals were housed in a room with temperature maintained at 22 ± 2°C and humidity 55± 4%. They were fed with standard laboratory diet (Hindustan Lever, Kolkata, India) and water ad libitum throughout the investigation.

Rats were divided into 6 groups each containing 6 animals.

Group I served as a control received 1ml of NS. Carbontetrachloride 0.7ml/kg b.w., l/p mixed with liquid paraffin was administered to remaining five groups. Treatment duration was 10 days and the dose of ccl₄ was administered after every 72 hours⁶.

Group II only received the toxicant.

Group III received 100mg/kg b.w. P.O. of silymarin.



Group IV, V & VI received PEFS (pet ether fraction of spirulina) CFS (Chloroform fraction of spirulina) & MFS (Methanolifraction of spirulina) with a dose of 300 mg/kg. b.w. each to respective group. Standard as well as test drugs were given daily for 10 days. On 10th day 1hr after the last dose the blood sample were collected by

puncturing the retro-orbital plexus under light ether anesthesia. Serum was separated at 3000 rpm in cold centrifuge for different assays like⁷ SGOT Serum bilirubin⁸ cholesterol⁹ and SALP serum alkaline phosphatase¹⁰. Livers were excised and fixed in Formalin for assessment of liver weight and histopathology.

Effects of different extracts of spirulina on ccl₄ induced hepatotoxicity in rats

Groups	Biochemical parameters						Liver weight in (gm.)
	SGPT (IU/L)	SGOT (IU/L)	Billirubin mg%	Cholesterol mg%	Serlem ALPKA units/100ml	UREA mg/100ml	
Control	13.18±0.56	18.22±0.02	0.792±0.02	250.36±0.38	12.08±0.03	38.32±0.03	8.56±0.16
Ccl ₄	33.72±0.26	34.00±0.92	2.727±0.56	405.26±0.52	18.34±0.21	56.21±0.21	10.56±0.16
Silymarin	17.86±0.06	24.12±0.16	0.812±0.06	286.36±0.26	13.21±0.34	40.36±0.02	9.46±0.21
Ccl ₄ + PEFS (300 Mg/Kg)	18.62±0.68	28.60±0.72	0.908±0.02	295.12±0.23	14.27±0.30	43.27±0.04	9.49±0.22
Ccl ₄ + CFS (300 Mg/Kg)	24.21±0.21	29.20±0.41	0.916±0.03	288.31±0.62	14.12±0.20	42.18±0.02	10.11±0.04
Ccl ₄ + MFS (300Mg/Kg)	33.67±0.37	29.11±0.12	0.925±0.02	287.12±0.22	14.08±0.32	41.27±0.03	10.08±0.02

Results expressed mean ± SEM for six observations.

RESULTS AND DISCUSSION

Results indicated that all the three, extracts (PEFS, CFS, and MFS) of spirulina provide protection against the toxic effect of ccl₄ on liver. In ccl₄ induced toxic hepatitis, toxicity begins with the changes in endoplasmic reticulum, which results in the loss of metabolic enzymes located in intracellular structure¹¹. The blood sample of ccl₄ treated group rats showed drastic increase in the level of SGPT, SGOT, serum bilirubin, serum cholesterol, serum alkaline, phosphatase, urea and liver inflammation as compared to that of the control group. The rapid elevation in the levels of serum aspartate transaminase indicates the extent of liver necrosis¹². Administration of test extract showed recovery against the toxic effects of ccl₄ on comparison with standard drug silymarin.

In the assessment of liver damage by ccl₄ hepatotoxin the determination of the enzyme levels such as SGPT and SGOT is largely used. Necrosis or membrane damage releases the enzyme into circulation; therefore it can be measured in the serum. High levels of SGPT indicate liver damage such as due to viral hepatitis as well as cardiac infarction and muscle, injury. SGPT catalyzes the conversion of alanine to pyruvate and glutamate and is released in a similar manner. Therefore, SGPT is more specific to liver and is these better parameter for detecting liver injury.¹³ The comparative study in the test doses of the three extracts (PEFS, CFS, MFS) of spirulina showed significant inhibition of the Biological parameters however it is not equal to that of the inhibition which was brought back by the standard drug silymarin towards the normal where as the test drugs to word the normal but not equivalent to that of the standard drug. In this connection also PEFS brings down the SGPT, SGOT, Billirubin and liver out more comfortably in comparison to CFS and MFS. In case of cholesterol, serum ALP & urea, MFS brings down more comforting bally than that of the CFS and PEFS.

CONCLUSION

All the three extracts possess he significant hepatoprotective activity. However it is not equal to that of the standard drug silymarin but PEFS significantly reduced the SGPT, SGOT, Billirubin and liver weight. Similarly MFS significantly reduced the cholesterol, serum alkaline phosphatase and urea level in ccl₄ induced liver damage group of rats.

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