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Hepatoprotective effect of *Sida cordifolia* on tartrazine-induced toxicity in albino rats

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Abstract

This study evaluates the hepatoprotective effects of aqueous (AESC) and ethanolic (EESC) extracts of *Sida cordifolia* against tartrazine-induced hepato-toxicity in albino rats. Tartrazine, a widely used synthetic food dye, is known to cause oxidative stress and hepatocellular damage. Rats exposed to tartrazine exhibited significant decrease in levels of liver glycogen, protein, amino acids, DNA, RNA and activity of acid phosphatase along with an increase in activity of alkaline phosphatase, indicating liver dysfunction. Treatment with both aqueous and ethanolic extracts of *Sida cordifolia* significantly mitigated these biochemical alterations and restored hepatic function. The findings suggest that the extracts of *Sida cordifolia*, confers a protective effect against tartrazine-induced hepatotoxicity, probably through its antioxidant and membrane-stabilizing properties, highlighting its potential as a natural therapeutic agent.

Keywords: *Sida cordifolia*, Tartrazine, Hepatotoxicity, Biochemical parameters

Introduction

Concern over the safety, possible bioaccumulation, and long-term health effects of synthetic additives has grown as a result of their widespread use in the contemporary food business ^[1]. Among these additives is tartrazine, a synthetic azo dye widely used for its vivid yellow hue in many processed foods, beverages, confections, and dairy products ^[2]. Despite being authorized for restricted usage, new scientific research indicates that tartrazine's toxic and pro-oxidant properties have the potential to upset physiological systems ^[3]. A wide range of negative effects, such as oxidative stress, mitochondrial dysfunction, DNA damage, immunological problems, and behavioural abnormalities, have been linked to tartrazine, also known as FD&C Yellow No. 5 (E102). The adverse effects of this dye can be attributed to the reductive breakdown of the azo bond that result in release of reactive amines and free radicals in liver and gut. Exposure to food additives like tartrazine significantly affects liver as it is one of the main organs involved in biotransformation. This can be observed as change in lipid peroxidation, altered enzymatic activity, and damaged hepatocellular architecture ^[4].

The worries related to the adverse effects on consumption of the food additives has attracted various scientists to explore plant products with potential to mitigate these adverse effects. Our traditional systems like Ayurveda, present intriguing therapeutic interventions using medicinal plants because of their low side effects and high phytochemical content ^[5]. *Sida cordifolia*, a popular ayurvedic herb due to its wide range of pharmacological properties has attracted scientists. Strong bioactive components found in the plant, including flavonoids, alkaloids, glycosides, and phenolic compounds, are known to contribute to its hepatoprotective, anti-inflammatory, and antioxidant properties ^[6]. *Sida cordifolia*'s hepatoprotective properties made us consider it as a natural therapeutic agent, when tartrazine induced toxicity was observed to be damaging liver in our studies. Therefore, considering that the plant will provide a strong natural defence by scavenging free radicals, stabilizing cellular membranes and re-establishing enzymatic balance ^[7], an attempt has been made in this study to explore hepatoprotective properties of *Sida cordifolia* in mitigation of tartrazine induced toxicity.

Materials and Methods**Collection of plant material**

Sida cordifolia was collected from the campus of DDU Gorakhpur University, Gorakhpur, U.P., India, 273009. It was identified by the Department of Botany, Deen Dayal Upadhyaya Gorakhpur University, Gorakhpur.

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Preparation of Plant Extract

Fresh *Sida cordifolia* plants were thoroughly cleaned and rinsed with distilled water to eliminate any adhering dust or impurities. The plant material was then shade-dried and finely ground using a mechanical grinder. For extraction, 500 grams of the powdered plant material was placed in a Soxhlet apparatus with a solvent (double-distilled water and ethanol) in a 1:10 ratio (solvent: plant powder). The extraction process was carried out for 48 hours. The resulting extracts were then concentrated using a flash evaporator. The dried residues were stored in sterilized tubes and labelled as AESC (Aqueous Extract of *Sida cordifolia*) and EESC (Ethanollic Extract of *Sida cordifolia*).

Experimental Animal

Albino rats aged 8-10 weeks, weighing 150-200 g, were used for the study. The animals were sourced from a licensed local supplier and were housed in standard polypropylene cages under controlled environmental conditions (12-hour light/dark cycle, $22 \pm 2^\circ\text{C}$, 50-60% relative humidity). All rats were acclimatized for one week prior to the commencement of the experiment, with free access to standard rodent chow and water ad libitum.

The experiment was conducted in compliance with the ethical standards for animal research with approval from the Institutional Animal Ethical Committee, Deen Dayal Upadhyaya Gorakhpur University, in accordance with the guidelines of the Committee for Purpose of control and Supervision of Experiments on animals.

Experimental protocol

Rats were randomly selected and divided into six Groups and exposed to different doses of tartrazine, plant extract and both daily for 28 days as described below:

1. **Group I:** Served as control. It was provided with normal food and water.
2. **Group II:** Was exposed to 300 mg/kg bw of tartrazine was provided orally with distilled water.
3. **Group III:** Was exposed to 400 mg/kg bw of aqueous extract of plant (AESC) orally with distilled water.
4. **Group IV:** Was exposed to 400 mg/kg bw of ethanolic extract of plant (EESC) orally with distilled water
5. **Group V:** Was exposed to both 300mg/kg bw tartrazine and 400 mg/kg bw of AESC orally with distilled water.
6. **Group VI:** Was exposed to both 300mg/kg bw tartrazine and 400 mg/kg bw of EESC orally with distilled water.
7. Doses were decided on the basis of previous studies [8,9].

Biochemical Assessment

The rats were weighed and sacrificed at specific intervals of 7th, 14th, 21st, and 28th day after treatment. From each group six animals were autopsied and liver samples were collected from both control and treated Groups, thoroughly rinsed with distilled water, dried using blotting paper to remove excess moisture. The homogenised liver tissues were subsequently used for biochemical analysis.

Liver Biochemistry: Glycogen content was determined using the Anthrone method described by Vander Vies and expressed in $\mu\text{g}/\text{mg}$ tissue [10]. Protein levels were quantified following the procedure outlined by Lowry *et al* and was expressed in $\mu\text{g}/\text{mg}$ tissue [11]. The total free amino acids were estimated using the Spies method and were expressed in $\mu\text{g}/\text{mg}$ tissue [12], while nucleic acids were measured according to Schneider's method and were expressed in $\mu\text{g}/\text{mg}$ tissue [13].

Estimation of Phosphatase Activity

The activity of acid and alkaline phosphatase in the tissue was estimated following the method of Bergmeyer (1967), as modified by Singh and Agarwal (1989), using p-nitrophenyl phosphate as the substrate [14]. Both of these parameters were expressed in μ mole substrate hydrolysed/30 minute/mg proteins

Statistical Analysis: The data is expressed as mean $\pm$ S.E. of six replicates, Student's t test and two- way ANOVA were performed to determine the significance of data.

Results

Table 1 shows the effect of oral administration of tartrazine, plant extracts and combination of plant extract and tartrazine on the liver biochemical parameters in albino rats.

A significant decrease ($p < 0.05$) in glycogen content in the rats exposed to tartrazine (Group II) was observed on 7th and 14th day whereas a highly significant ($p < 0.01$) decrease in glycogen was observed on 21st and 28th day with respect to Group I. The Rats of Group III and Group IV showed a non-significant ($p > 0.05$) change in hepatic glycogen content on 7th, 14th, 21st and 28th day with respect to Group I. A significant ($p < 0.05$) increase in glycogen content was observed in rats of Group V and Group VI on 7th and 14th day whereas highly significant ($p < 0.01$) rise in glycogen content was noticed on 21st and 28th day with respect Group II.

The protein content of the liver tissue in the tartrazine-treated (Group II) rats decreased significantly ($p < 0.05$) on 7th and 14th day, whereas on 21st and 28th, a highly significant ($p < 0.01$) decrease in protein content was noted in comparison to the Group I. On days 7, 14, 21, and 28, Group III and Group IV displayed a non-significant ($p > 0.05$) change in hepatic protein in comparison to the Group I. Rats of Group V and Group VI showed a significant ($p < 0.05$) increase in protein content on days 7 and 14, while this increase was observed to be highly significant ($p < 0.01$) on days 21 and 28 with respect to Group II.

Rats treated with tartrazine showed a significant decrease ($p < 0.05$) in total amino acids in their liver tissue on 7th and 14th day, while a highly significant ($p < 0.01$) decrease was noted on 21st and 28th day in comparison to Group I. The rats of Group III and Group IV displayed a non-significant ($p > 0.05$) change in total amino acids in the liver tissue on 7th, 14th, 21st and 28th day in comparison to Group I. A significant increase ($p < 0.05$) in total amino acids in the liver tissue on 7th and 14th day and a highly significant increase ($p < 0.01$) in total amino acids in the liver tissue on 21st and 28th day was observed in rats of Group V and Group VI with respect to Group II.

The tartrazine-treated rats (Group II) exhibited a significant decrease ($p < 0.05$) in hepatic DNA content on the 7th and 14th day, while a highly significant decrease in hepatic DNA ($p < 0.01$) was observed on the 21st and 28th day with respect to Group I. Group III and Group IV rats showed a non-significant ($p > 0.05$) change in DNA levels on all observation days (7th, 14th, 21st, and 28th) relative to the Group I. Rats of Group V and Group VI demonstrated a significant ($p < 0.05$) increase in hepatic DNA on the 7th and 14th day, with a highly significant ($p < 0.01$) increase noted on the 21st and 28th day in comparison to Group II.

The RNA content in the liver tissue of the tartrazine-treated (Group II) rats decreased significantly ($p < 0.05$) on day 7 and 14, and a highly significant ($p < 0.01$) decrease in RNA content was noted on day 21 and 28 in comparison to the Group I.

Comparing Group III and Group IV to Group I we observed that on day 7th, 14th, 21st, and 28th, there was a non-significant ($p>0.05$) change in RNA content. On days 7 and 14, rats in Group V and Group VI showed a significant ($p<0.05$) increase in RNA content, while on 21st and 28th day a highly significant ($p<0.01$) increase in RNA content was observed with respect to Group I.

Group II administered with tartrazine showed a significant ($p>0.05$) increase in the activity of acid phosphatase in liver of rats on 7th and 14th day whereas highly significant ($p<0.01$) increase in the activity of acid phosphatase was observed on 21st and 28th day with respect to Group I. Group III and Group IV showed a non-significant ($p>0.05$) change in the activity of acid phosphatase on 7th, 14th, 21st, and 28th day compared to Group I. A significant ($p<0.05$) decrease in the activity of acid phosphatase was observed in rats of Group V and Group VI

on day 7 and day 14 whereas highly significant ($p<0.01$) in was noticed on 21st and 28th day with respect to Group II.

A significant ($p<0.05$) decrease in the activity of alkaline phosphatase was observed in liver tissue of rats of Group II on 7th and 14th day, whereas a highly significant ($p<0.01$) decrease was observed on 21st and 28th day with respect to Group I. On day 7, 14, 21, and 28, Group III and Group IV displayed a non-significant ($p>0.05$) change in the activity of alkaline phosphatase in comparison to the Group I. Rats of Group V and Group VI showed a significant ($p<0.05$) increase in the activity of acid phosphatase on day 7th and 14th, whereas on 21st and 28th day we observed a highly significant ($p<0.01$) increase in the activity of acid phosphatase with respect to Group II.

Two-way ANOVA test indicates that variation in the strength of dose and exposure time, significantly influence liver biochemical parameters of rats.

Table 1: The effects of oral administration of tartrazine, AESC and EESC, tartrazine + AESC, tartrazine + EESC on liver biochemical parameters in albino rats

Parameters	Day	Control (Group I)	Tartrazine 300 mg/kg bwt (Group II)	Treatment with <i>Sida cordifolia</i>		Ameliorative Group	
				AESC (Group III)	EESC (Group IV)	Tartrazine+ AESC (Group V)	Tartrazine +EESC (Group VI)
Glycogen ($\mu\text{g}/\text{mg}$ tissue)	7 th	32.19 $\pm$ 0.37	28.77 $\pm$ 1.58*	32.48 $\pm$ 0.32	33.24 $\pm$ 0.37	29.57 $\pm$ 0.30*	30.57 $\pm$ 0.31*
	14 th	28.30 $\pm$ 0.24	27.06 $\pm$ 0.50*	28.37 $\pm$ 0.49	29.25 $\pm$ 0.50	28.11 $\pm$ 0.28*	31.27 $\pm$ 0.30*
	21 st	29.24 $\pm$ 0.28	25.49 $\pm$ 0.54**	29.34 $\pm$ 0.97	29.87 $\pm$ 0.73	28.43 $\pm$ 0.30**	32.23 $\pm$ 0.50**
	28 th	30.25 $\pm$ 0.35	26.03 $\pm$ 0.26**	30.20 $\pm$ 0.92	30.92 $\pm$ 0.25	27.71 $\pm$ 0.52**	32.98 $\pm$ 0.35**
Protein ($\mu\text{g}/\text{mg}$ tissue)	7 th	29.71 $\pm$ 0.43	28.45 $\pm$ 0.43*	28.89 $\pm$ 0.52	29.46 $\pm$ 0.24	28.93 $\pm$ 0.33*	29.28 $\pm$ 0.32*
	14 th	29.35 $\pm$ 0.32	28.24 $\pm$ 0.62*	29.33 $\pm$ 0.41	30.25 $\pm$ 0.41	30.13 $\pm$ 0.42*	31.24 $\pm$ 0.30*
	21 st	29.74 $\pm$ 0.55	26.59 $\pm$ 0.56**	30.20 $\pm$ 0.18	30.81 $\pm$ 0.18	28.48 $\pm$ 0.54**	31.87 $\pm$ 0.33**
	28 th	30.70 $\pm$ 0.33	25.74 $\pm$ 0.41**	32.86 $\pm$ 0.34	34.22 $\pm$ 0.10	30.49 $\pm$ 0.57**	31.98 $\pm$ 0.34**
Amino Acid ($\mu\text{g}/\text{mg}$ tissue)	7 th	14.26 $\pm$ 0.30	13.20 $\pm$ 0.43*	13.27 $\pm$ 0.44	14.24 $\pm$ 0.11	13.41 $\pm$ 0.28*	13.89 $\pm$ 0.29*
	14 th	14.77 $\pm$ 0.22	12.45 $\pm$ 0.35*	14.20 $\pm$ 0.18	14.19 $\pm$ 0.15	14.47 $\pm$ 0.14*	14.99 $\pm$ 0.30*
	21 st	13.69 $\pm$ 0.27	11.04 $\pm$ 0.26**	12.97 $\pm$ 0.37	13.11 $\pm$ 0.18	13.57 $\pm$ 0.24**	14.12 $\pm$ 0.25**
	28 th	13.90 $\pm$ 0.25	9.86 $\pm$ 0.13**	13.31 $\pm$ 0.21	14.48 $\pm$ 0.34	14.53 $\pm$ 0.24**	15.23 $\pm$ 0.30**
DNA ($\mu\text{g}/\text{mg}$ tissue)	7 th	14.03 $\pm$ 0.16	13.47 $\pm$ 0.14*	14.25 $\pm$ 0.16	14.37 $\pm$ 0.19	11.09 $\pm$ 0.23*	11.94 $\pm$ 0.32*
	14 th	14.45 $\pm$ 0.15	12.53 $\pm$ 0.26*	14.32 $\pm$ 0.41	15.19 $\pm$ 0.47	12.38 $\pm$ 0.40*	13.32 $\pm$ 0.34*
	21 st	13.93 $\pm$ 0.15	11.94 $\pm$ 0.43**	13.71 $\pm$ 0.30	14.06 $\pm$ 0.28	13.11 $\pm$ 0.05**	13.98 $\pm$ 0.30**
	28 th	13.32 $\pm$ 0.30	10.35 $\pm$ 0.37**	13.54 $\pm$ 0.53	13.33 $\pm$ 0.32	13.56 $\pm$ 0.25**	14.12 $\pm$ 0.34**
RNA ($\mu\text{g}/\text{mg}$ tissue)	7 th	12.41 $\pm$ 0.15	11.31 $\pm$ 0.20*	12.49 $\pm$ 0.26	12.50 $\pm$ 0.28	10.34 $\pm$ 0.28*	11.34 $\pm$ 0.28*
	14 th	11.06 $\pm$ 0.22	10.62 $\pm$ 0.17*	12.19 $\pm$ 0.17	11.81 $\pm$ 0.40	11.29 $\pm$ 0.28*	11.98 $\pm$ 0.32*
	21 st	10.82 $\pm$ 0.30	9.12 $\pm$ 0.27**	11.83 $\pm$ 0.26	11.14 $\pm$ 2.23	11.97 $\pm$ 0.29**	12.32 $\pm$ 0.34*
	28 th	11.21 $\pm$ 0.44	8.42 $\pm$ 0.19**	11.81 $\pm$ 0.29	11.42 $\pm$ 0.36	12.54 $\pm$ 0.60**	12.98 $\pm$ 0.30**
Acid phosphatase (μ mole substrate hydrolyzed /30 minutes/mg protein)	7 th	0.308 $\pm$ 0.0016	0.372 $\pm$ 0.0082*	0.319 $\pm$ 0.0061	0.310 $\pm$ 0.0018	0.349 $\pm$ 0.0141*	0.316 $\pm$ 0.0014**
	14 th	0.296 $\pm$ 0.0012	0.402 $\pm$ 0.0178*	0.298 $\pm$ 0.0013	0.294 $\pm$ 0.0006	0.302 $\pm$ 0.0015*	0.278 $\pm$ 0.0018**
	21 st	0.298 $\pm$ 0.0006	0.426 $\pm$ 0.0177**	0.283 $\pm$ 0.001	0.285 $\pm$ 0.0006	0.298 $\pm$ 0.0004**	0.269 $\pm$ 0.0012**
	28 th	0.298 $\pm$ 0.0009	0.486 $\pm$ 0.0261**	0.280 $\pm$ 0.0006	0.275 $\pm$ 0.0004	0.252 $\pm$ 0.0128**	0.248 $\pm$ 0.0018**

Alkaline phosphatase (μ mole substrate hydrolyzed /30 minutes/mg)	7 th	0.062 $\pm$ 0.0006	0.053 $\pm$ 0.008*	0.061 $\pm$ 0.0008	0.062 $\pm$ 0.0003	0.059 $\pm$ 0.0002*	0.51 $\pm$ 0.0002*
	14 th	0.065 $\pm$ 0.0007	0.046 $\pm$ 0.019*	0.065 $\pm$ 0.0007	0.064 $\pm$ 0.0003	0.061 $\pm$ 0.0017*	0.54 $\pm$ 0.0016*
	21 st	0.066 $\pm$ 0.0011	0.036 $\pm$ 0.0167**	0.067 $\pm$ 0.0004	0.070 $\pm$ 0.0005	0.060 $\pm$ 0.0010**	0.53 $\pm$ 0.0009**
	28 th	0.071 $\pm$ 0.0007	0.028 $\pm$ 0.0261**	0.069 $\pm$ 0.0006	0.071 $\pm$ 0.006	0.066 $\pm$ 0.0005**	0.56 $\pm$ 0.0010**

* indicates significant ($p < 0.05$) difference between control and Tartrazine, between control and AESC treated groups, between control and EESC treated groups.

** indicates highly significant ($p < 0.01$) difference control and Tartrazine, between control and AESC treated groups, between control and EESC treated groups.

* indicates significant ($p < 0.05$) difference between Tartrazine and Tartrazine+ AESC treated group and between Tartrazine and EESC group.

** indicates highly significant ($p < 0.01$) difference between Tartrazine and Tartrazine+ AESC treated group and between Tartrazine and EESC group.

Discussion

Liver, one of the largest organs plays a central role in various functions such as metabolism and detoxification. The liver regulates the safety and passage of all ingested substances before they enter the systemic circulation. It helps to manage almost all key metabolic tasks, like handling sugars, proteins, fats, cholesterol, and vitamins [15]. Tartrazine is metabolized to sulfanylic acid and other aromatic amines, which are known to cause hepatocellular damage. This is evidenced by histopathological changes like cell vacuolation, swelling, necrosis, and pyknosis in rat liver. These changes disrupt normal liver function and impact various biochemical parameters [16,17]. In the present study we found that tartrazine administration significantly reduces liver glycogen, protein, amino acids, DNA, RNA levels, activity of alkaline phosphatase and increases the activity of acid phosphatase in the liver when compared to the control. Decline in glycogen level was likely due to its mobilization to meet the increased energy demands of the body under stress [18]. Tartrazine is reported to significantly affect the quality of chromosomes by binding directly to the linear double standard DNA and resulting in DNA degradation [19,20,21]. Sasaki *et al.* reported DNA damage in rat stomach and colon tissues due to acute cytotoxicity or insufficient repair of DNA on exposure to high dose [22]. Hence, decrease in DNA in the present study on exposure to tartrazine can be attributed to direct and persistent DNA damage as reported by earlier researchers. Decrease in the RNA content on exposure to tartrazine might be due to binding of the dye to DNA leading to alteration in gene expression [21]. Farnum and Hawthorne have reported that consumption of tartrazine within accepted daily intake resulted in reduction of oxidative enzyme SOD1 levels due to pre-translational modifications. It can be concluded that tartrazine interferes with expression of gene by damaging or binding with DNA and alters level of RNA as well as the synthesis of proteins [23]. Further, cellular damage due to oxidative stress might be the reason for decrease in proteins and total amino acid observed in present study [24]. The damage in hepatic cells was further confirmed by observing alterations in acid and alkaline phosphatase enzyme activity. The activity of acid phosphatase, a lysosomal enzyme, increases significantly, indicating pathophysiological changes and destabilization of the lysosomal membrane. Significant decrease in the activity of acid phosphatase with respect to control in hepatocyte can be attributed to signal decreased membrane permeability or biliary blockage affecting protein production, and glucose absorption [4].

Sida cordifolia extracts, both ethanolic (EESC) and aqueous (AESC), have demonstrated significant hepatoprotective effects. Exposure to both extracts of plant at 400 mg/kg bw

were found to be safe(non-toxic) with respect to control according to the observed liver biochemical parameters. This aligns with findings from Auddy *et al.*, who reported non-toxicity of the plant extract in PC12 cells [25].

Treatment with tartrazine +AESC (Group V) and tartrazine + EESC (Group VI), the adverse effects induced by tartrazine was reversed significantly in comparison to the rats treated with tartrazine (Group II). Significant increase observed in glycogen, protein and total amino acid content in Group V and Group VI with respect to tartrazine treated Group were in accordance with findings reported by Kaur *et al.* and Ahmad *et al* [26,27]. Consistent with Silvia *et al.*'s work, our study revealed a notable increase in nucleic acid content in both the Group V and Group VI with respect to the Group II that were rats treated with only tartrazine [9]. Both Groups treated with plant extract and tartrazine indicated gradual normalization in the activities of both the alkaline and acid phosphatases with respect to tartrazine treated Group. The hepatoprotective activity of the plant extract can be attributed to the presence of potent antioxidant and anti-inflammatory phytochemicals, such as phenolics, sterols, and flavonoids that scavenge reactive oxygen species and facilitate liver regeneration [27,28]. Various researchers have reported that ethanolic extract of *Sida cordifolia* exhibits better antioxidant properties in comparison to aqueous extract [28,29,30]. Documentation of hepatoprotective effects of ethanolic as well as aqueous extracts of the plant against various hepatotoxic compounds supports our finding [31,32,33]. Hence this study indicates that both extracts can effectively mitigate liver damage caused by the exposure of tartrazine.

Conclusion

The results of this study demonstrate that extracts of *Sida cordifolia* exhibit significant hepatoprotective effects against tartrazine-induced toxicity in albino rats. The extracts of the plant effectively ameliorated the biochemical disturbances induced by tartrazine, as evidenced restoration of the level of glycogen, protein, amino acids, nucleic acids, and activity of phosphatase enzyme. Therefore, it can be concluded that *Sida cordifolia* may serve as a valuable natural therapeutic agent for mitigating hepatotoxicity induced by intake of tartrazine.

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Conflict of interest

No potential conflict of interest was reported by the authors.

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