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Study Toxic Effects of AZO Dye Tartrazine on the Adrenal Gland and Cardiovascular System of Albino Rats and the Protective Antioxidant Effect of Rosemary

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Abstract:

Introduction: Food additives are products that are added to the basic foodstuffs to improve their appearance, flavor, taste, color, texture, nutritional value, and shelf life. Tartrazine, which is a synthetic food additive, is a shining yellow color azo pigment. Rosemary is an aromatic evergreen shrub with leaves similar to hemlock needles. Rosemary extract could be useful for replacing or even decreasing synthetic antioxidants in foods.

Aim of the study: To assess the toxic effects of the food additive (Azo Dye Tartrazine) on the adrenal gland and cardiovascular system. And to evaluate the protective effect of rosemary on albino rats

Material and methods: The present study was conducted in the period from October to November 2023. The weight of the albino rats used in the experiment ranged from 150 to 200 grams. 80 male & female albino rats were separated randomly into four groups (20 rats each) as follows: Group (1), Control group (Rats were fed normal food and drank distilled water), Group (2), Tartarizine-treated group (tartarizine was given to the rats orally (300 mg/kg/day), Group (3), Rosemary group (rats were administered antioxidant rosemary orally (100 mg/kg/day), Group (4), Rats received [tartrazine (300 mg/kg/day) + rosemary (100 mg/kg/day). All rats received the treatment orally for 30 days.

Results: Compared to the control group, the group given tartrazine had significantly higher levels of body weight, serum cortisol, lactate dehydrogenase, cardiac troponin 1, and testosterone. However, when rosemary was added to the tartrazine, significantly, these levels dropped, also there are also significant histopathological changes in the heart &adrenal gland.

Conclusion: Because of its antioxidant qualities, rosemary may reduce the toxic effects of tartrazine, and it is thought to be given to people if they need to be shielded from the hazardous effects of tartrazine. The cardiovascular system and adrenal glands are at risk from tartrazine's harmful effects.

Keywords: Adrenal gland; Albino rats; Cardiovascular system; Rosemary; Tartrazine.

1. Introduction

Additives to foods are ingredients added to basic food products to enhance their nutritional value, shelf life, texture, color, flavor, and appearance. It is essential to the current ample and healthy food supply [1]. Tartrazine is a synthetic food additive characterized by its vibrant yellow hue. It is a more resilient and cost-effective alternative compared to natural food coloring ingredients [2].

Tartarazine has been associated with adverse allergic responses and has shown a detrimental effect on animal learning and memory skills. Numerous rat toxicity studies have shown various detrimental effects on kidneys, heart, and blood cells, as well as irritability, restlessness, and sleep disruptions in youngsters who ingested tartrazine via colored meals. The combination of tartrazine and sodium benzoate has the potential to cause hyperactivity and thyroid cancers in many children [3].

Tartrazine could be regarded as toxic due to its possible oxidative impairment induced by depletion of GSH, the main antioxidant for the cell, and a significant increase in MDA levels, where the researchers strongly believe that the usage of these possibly toxic colors in food needs to be re-evaluated [4].

The phenolic acids, including rosmarinic acid, and abietane diterpenes are the main sources of rosemary extract's antioxidant capabilities (*Rosmarinus officinalis* L.) [5].

Toxins in the hormonal system concentrate on the adrenal gland, and inhibiting adrenal steroidogenesis in humans might be fatal. However, there are still a few methods for evaluating adrenal toxicity [6]. This study aimed to assess the toxic effects of the food additive (Azo Dye Tartrazine) on the adrenal gland and cardiovascular system and to evaluate the protective effect of rosemary on albino rats.

2. Subjects & Method

2.1. Subjects

Sample size was eighty male &female albino rats, with a weight range of 150–200 grams. The animals were sourced from a Fayoum animal breeding farm. The study was done in the Faculty of Science at Fayoum University. Animals were handled in accordance with the experimental research ethics regulations set by the

research ethics committee number (M 646 on 12 February 2023 at the Faculty of Medicine in Fayoum University.

The animal accommodations were kept at ambient temperature (22°C ±6°C), utilized a 12:12 light-dark period, and had sufficient ventilation.

They received nourishment on a regular basis. To aid with their adjustment, the rats were housed in the proper settings for an entire week prior to the experiment.

Following the guidelines laid forth by [7], the experimental food consisted of the following components: 5% fat, 65% carbohydrates, 20.3% protein, 5 percent fiber, 3.7% salt combination, and 1 percent vitamin mixture. The animals were also given tap water and a standard baseline diet.

2.2 Methods

Since tartrazine and rosemary are utilized in solid form, we made two forms of each. (One low and the other high) By dissolving the solid in distilled water, low doses of tartrazine and rosemary were 15 and 8 mg/kg, respectively, while high doses were 500 and 100 mg/kg bw, respectively. Rosemary: Dried rosemary (*Rosmarinus officinalis* L.) leaves. The rosemary extracts were prepared according to the method described before [8]. This method uses a dehydrator to dry rosemary. Rosemary leaves, Set to 95-115 degrees and dry for at least 4 hours. It can take as long as 24 hours to dry rosemary in a dehydrator. Rats were separated randomly into four groups (20 rats each) as follows:

- Group (1), Control group (Rats were fed normal food and drank distilled water).
- Group (2), Rartarizine-treated group (tartarizine was given to the rats orally (300 mg/kg/day)
- Group (3), Rosemary group (rats were administered antioxidant rosemary orally (100 mg/kg/day).
- Group (4), Rats received [tartrazine (300 mg/kg/day) + rosemary (100 mg/kg/day). All rats received the treatment orally for 30 days.

Selection of the mentioned group doses is based on [4]. The blood samples were centrifuged at 3000 revolutions per minute for 20 minutes to obtain serum. To conduct biochemical analysis, the serum supernatant was isolated and then frozen at -80 °C.

2.3 Biochemical analysis

Cardiac biomarkers were measured:

- cTnI (cardiac troponin level): normal level of Cardiac Troponin in serum between 0 and 0.04 ng/ml.
- LDH (lactate dehydrogenase enzyme): normal level in serum up to 140u/l [4].

Adrenal gland biomarkers were measured:

- Mineralocorticoids (aldosterone level): normal level in serum morning sample 3-15ng/dl [9].
- Glucocorticoids (cortisol level): normal serum level 5-25mcg [10].

Histopathological study

At the end of the study, the rats were weighed and anesthetized with an intraperitoneal injection of 25 mg/kg sodium thiopental. Intra-cardiac perfusion was performed using a butterfly needle inserted into the left ventricle of the heart. Initially, the intravascular perfusion by saline solution (0.9% NaCl) to wash out the blood from the body, then 2.5% glutaraldehyde in 0.1 mol/L cacodylate buffer (pH 7.3) was used for adrenal partial fixation [11]. Then, the heart and adrenal gland, which had been fixed in 10 percent neutral buffered formalin for forty-eight hours, were removed during laparotomy. After a brief rinse in distilled water, the fixed samples were dried in ethyl alcohol of progressively higher concentrations for an hour each, followed by an hour in absolute alcohol. After two hours of xylene clearing, the specimens were embedded in soft paraffin and then hard paraffin. Using a rotatory microtome (Leica RM 2125 UK), 5 μm-thick sections were produced for staining. These sections were stained with Haematoxylin & Eosin (H&E), then a light microscope (Primo star, ZEISS, China) was used to examine the slides [6].

2.4 Statistical Methods:

One-way analysis of variance (ANOVA) was used to analyse the data and was performed using the Statistical Package for the Social Sciences (SPSS) program, version 25. The Bonferroni test was used as a method to compare the significance between groups. We utilized the IBM SPSS software package edition 20.0 to analyze the information that had been entered into the computer. IBM Corp., New York, "Armonk" Counts & percentages have been employed to describe qualitative as well as quantitative information. The test known as the Kolmogorov-Smirnov was used to determine if the pattern of distribution was normal. Quantitative data were represented using variation (which includes minimum as well as MAX), SD, mean, median, and interquartile range (IQR). The results were

deemed significant at the level of five percent, according to [12]. The tests used were the One-way ANOVA test. To assess more than one group for quantitative factors with normally distributed distributions.

3. Results

Documented study results explain that, when rosemary and tartrazine were administered together, the body weight of the rosemary group significantly decreased, but in contrast to the tartrazine group, body weight significantly increased (**Table 1**). Present research has shown that tartrazine significantly raised the Cortisol level (p <0.05). However, in the group given tartrazine with rosemary, the level of cortisol was significantly reduced (p <0.05) (**Table 2**)

According to the LDH level, the tartrazine group showed a considerably higher level (p <0.05) than the control group. The LDH level was considerably reduced (p <0.05) after the administration of Rosemary with tartrazine, as shown in **Table 3**. Tartrazine significantly raised cTnI levels when compared to the control group. But the rosemary plus tartrazine group had a considerably decreased cTnI level (**Table 4**, **Figures 1-6**).

Table 1: Effects of studied groups on rats' body weight**.**

Experimental groups	Body wt. (g)
Control group	215.00±4.47
Tartrazine-treated group	263.00±2.53
Rosemary group	223.33±8.76
Tartrazine + Rosemary group	245.17±2.79
Test	15.325
P-value	0.001*

Table 2: Comparison of the research groups based on adult albino rats' cortisol levels**.**

Experimental groups	Cortisol level
Control group	5.5±2.1
Tartrazine-treated group	20.1± 4.51
Rosemary group	1.5 ± 0.5
Tartrazine + Rosemary group	11.6 ±5.21

Test	12.365
P-value	0.001*

Table 3. Effect of studied groups on the LDH level of adult albino rats.

Experimental groups	**LDH (U/L)**
Control group	244.83±4.79
Tartrazine-treated group	1015.50±23.71
Rosemary group	241.50±7.50
Tartrazine + Rosemary group	500.67±15.78
Test	17.65
P-value	0.001*

** significant at p < 0.05.*

Table 4: Effect of studied groups on cardiac troponin (cTnI) level in adult albino rats.

Experimental groups	**cTnI (ng/mL)**
Control group	0.9±0.12
Tartrazine-treated group	2.5 ± 0.51
Rosemary group	1.1 ± 0.19
Tartrazine + Rosemary group	1.6 ±0.21
Test	10.284
P-value	0.001*

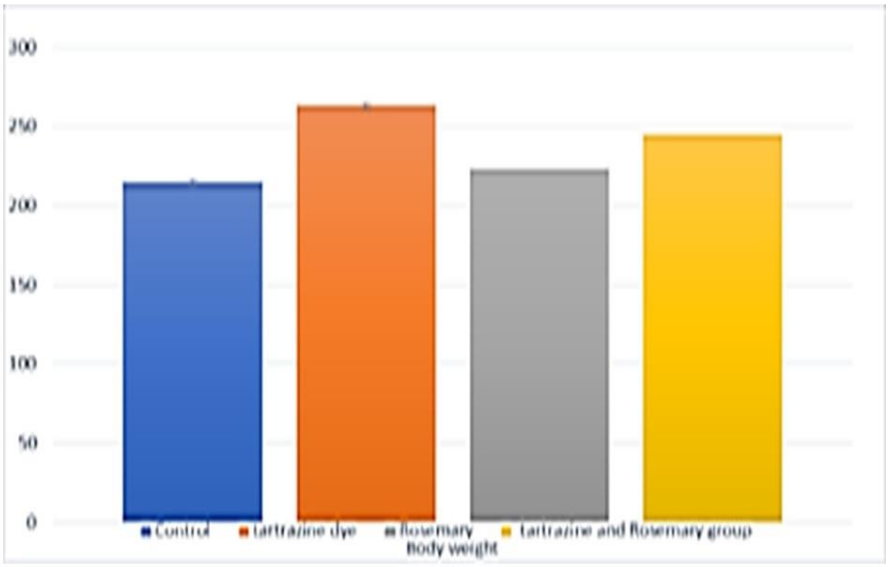

Figure 1: Effect of studied groups on the body weight of adult albino rats.

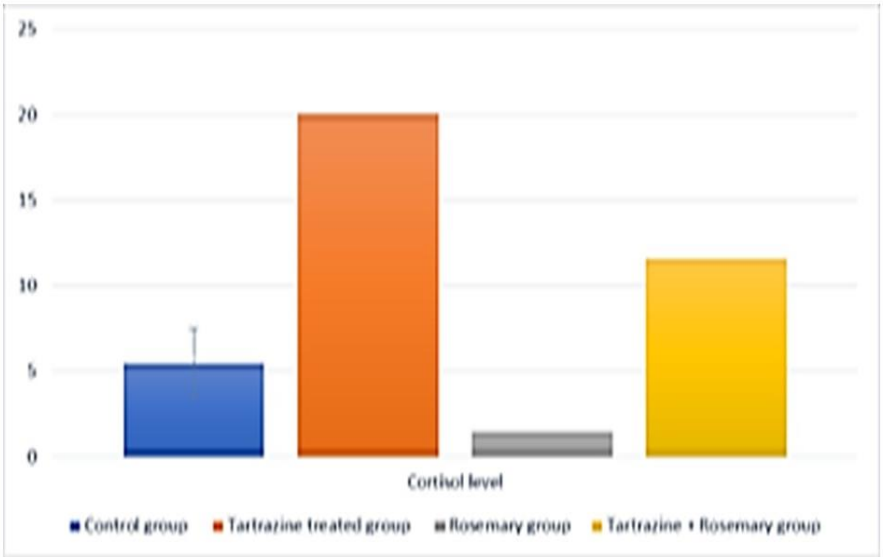

Figure 2: Cortisol levels of adult albino rats tested in groups treated with tartrazine dye, rosemary, or a combination of the two.

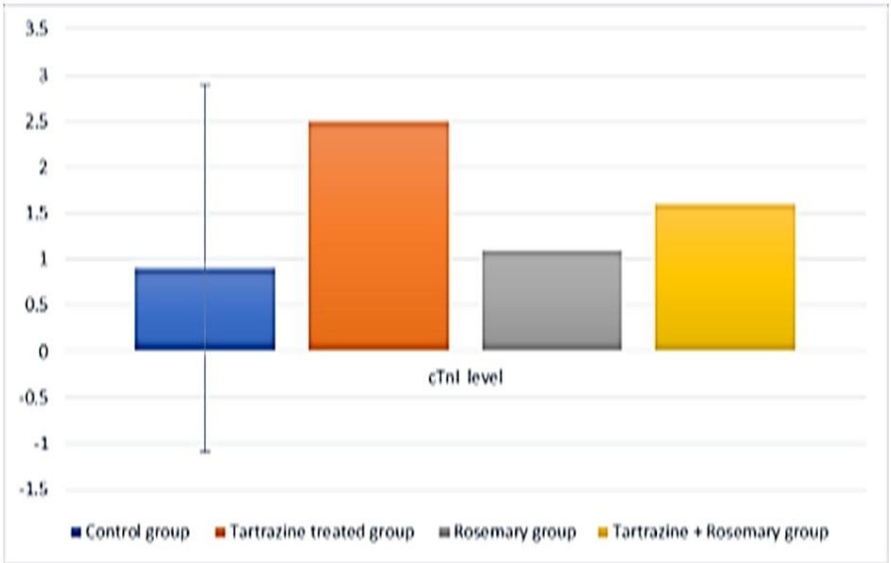

Figure 3: Efficacy of Rosemary and tartrazine dye on the LDH level in the adult albino rat group under study.

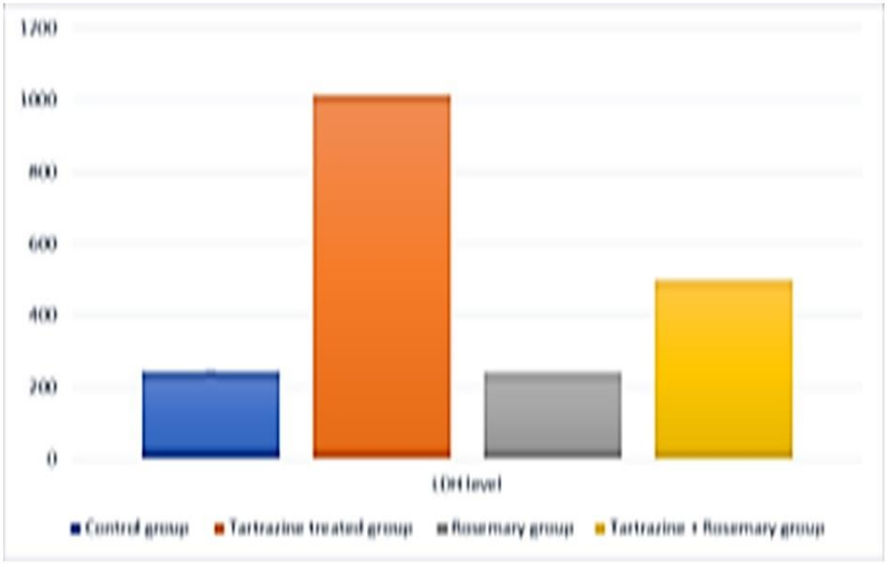

Figure 4: Effect of studied groups on cardiac troponin (cTnI) level in adult albino rats.

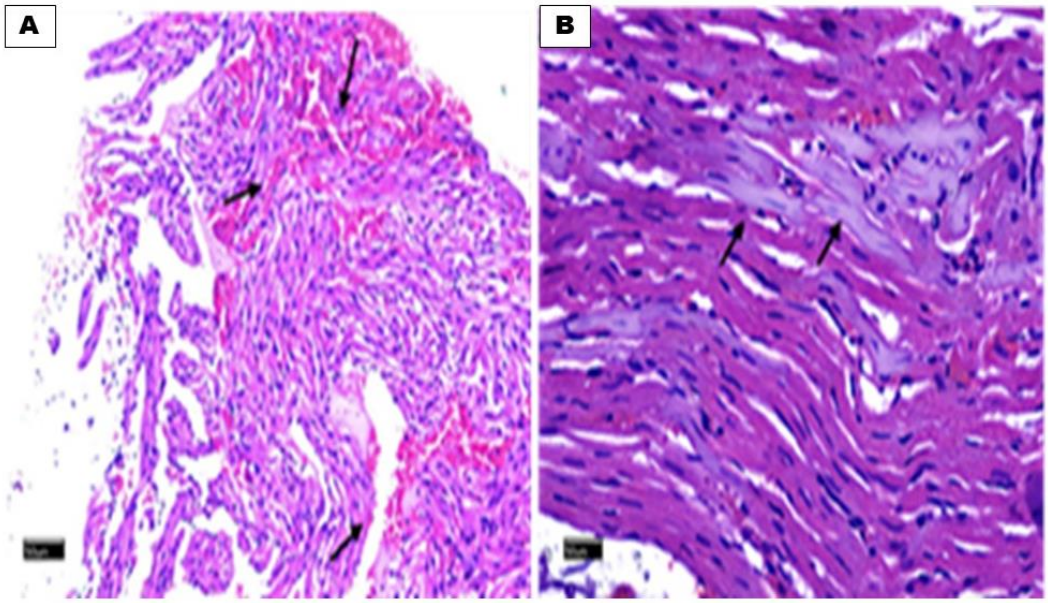

Figure 5: Section in cardiac muscle fibers in the tartrazine group. A) A section from the cardiac muscle of a rat from group 2 showed marked congestion and extravasation of RBCs [arrows]. B) A section from the cardiac muscle of a rat from group 2 showed marked hydropic degeneration of the cardiac cells, where the affected cells are enlarged, showing pale cytoplasm and eccentric nuclei [arrows] (H&E40x).

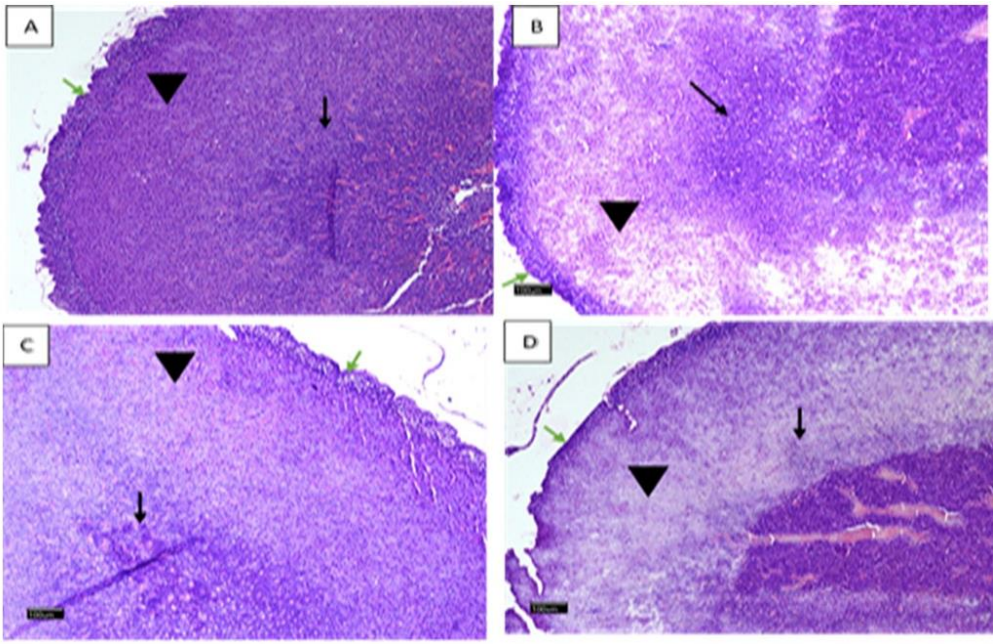

Figure 6: Section in the adrenal gland in the control and tartrazine-treated group**. S**erial sections of the suprarenal glands of rats were involved in the different groups. A) control group, Adrenal cortex has 3 zones that can be appreciated at the microscopic level. Zona glomeruloza (just beneath the connective tissue capsule, green arrow). Zona fasciculata (Middle / intermediate layer of cortex, black arrow head). Zona reticularis (immediately adjacent to adrenal medulla, black arrow). B) Tartrazine group: Zona fasciculata (black arrow head) shows marked hydropic degeneration with enlarged pale vacuolated cells. C) Rosemary group: the adrenal cortex layers are merely normal as described above. D) Rosemary and tartrazine group: Zona fasciculata (black arrow head) shows mild hydropic changes, and the cells show pale cytoplasm.

4. Discussion

One of the several artificial food coloring substances, Trisodium 5-hydroxy-1-(4-sulfonatophenyl), is referred to as Phenylazo-4-(4-sulfonato) Tartrazine, sometimes referred to as Chemical E 102, FD & C yellow number 5, is H-pyrazol-3-carboxylate. This chemical is derived from coal tar and is an azo compound. One of its distinguishing features is the presence of an azo functional group with conjugated aromatic rings (N=N). According to [8]. The azo link is responsible for the carcinogenic and cytotoxic effects of these compounds via direct action or biotransformation reduction processes.

Rosemary is known for its many uses in cooking, but it is also used medicinally, is

an ingredient in many foods, and contains powerful antioxidants. Several components, such as diterpenes and flavones, have been extracted from rosemary. *Rhodiola rosea* leaves include the antioxidant phenolic diterpenes carnosic acid and carnosol as their principal bioactive components [12].

The anti-inflammatory and antioxidant properties of Rosmarinus acid are well-documented. The results of further research showed that chemicals derived from rosemary reduced levels of IL-β and TNF-α while simultaneously raising glutathione peroxidase (GSH-Px) and superoxide dismutase (SOD) activity in several investigations. According to a previous report, rosemary also had a positive effect on the oxidative stress state in the hearts of elderly rats [5].

A previous study showed that sub-chronic tartrazine poisoning leads to a significant increase in body weight relative to the control group, which is in line with our findings [9]. While a previous study discovered that tartrazine substantially raised the average weight of rats [7], another discovered that the proportion of weight of the brain was somewhat elevated in rats treated with the drug [8]. Rosemary may have an antioxidant effect that reduces tartrazine-induced kidney damage in rats, according to research by Tochitani et al (2019), lending credence to our findings [7].

In line with these findings, a previous study found that rats given a high dose of tartrazine (500 mg/kg bw) had significantly higher serum cTnI activities compared to control rats, and that rats given a low dose of tartrazine (15 mg/kg bw) had significantly higher serum lactate dehydrogenase activity compared to control rats [6]. Also, Tochitani et al. (2019) demonstrated that after 30 days of consuming chemicals used as artificial food colorants regularly, cardiac function was affected [7]. Necrosis, infiltration, and vacuolation were among the histopathological alterations brought about by tartrazine treatment.

In this study, we found that regarding Aldosterone level, Tartrazine caused a significant increase in Aldosterone level, in comparison with the control group. Administration of Rosemary with tartrazine produced a significant decrease in Aldosterone level compared with the tartrazine group. A previous study found that administration of Rosemary evoked a significant decrease in serum aldosterone levels and a significant increase in serum

renin when compared to the control group [5]. Rosemary with tartrazine-treated rats induced a highly significant decrease in serum aldosterone levels and a significant increase in serum renin compared to the control group. Another study administered Rosemary as a protective in a rat tartrazine-induced hepatotoxicity model [1]. They determined a decrease in Aldosterone levels in the Tartrazine-administered group when compared with all the other groups. They reported improvements in both biochemical and histological findings with Rosemary administration when compared to the tartrazine administration group. In this study, we found that examination of H and E-stained heart sections, the control group had the typical histological architecture of the myocardium, with longitudinally striated branching and anastomosing muscle fibers with centrally located oval vesicular nuclei and acidophilic sarcoplasm they are separated by narrow intercellular spaces. Fibroblast nuclei were detected in the interstitial tissue between myocytes. The cardiac muscle fibers appeared polygonal or polyhedral in transverse sections, with central rounded nuclei. The Rosemary-treated group's histological appearance was identical to that of the control group. However, there were several cardiac changes were observed in the tartrazine group, including hypertrophy of cardiac muscle fibers, destruction of some cardiomyocytes, and marked hydropic degeneration of the cardiac cells, where the affected cells are enlarged, showing pale cytoplasm and eccentric nuclei. Also, marked venous congestion could be detected. Wide intercellular spaces separated these cardiomyocytes from each other. Between the cardiac muscle fibers, there was a large area of hemorrhage and mononuclear cellular infiltration. The combination of Rosemary and tartrazine alleviated all the adverse effects seen in the tetrazine group.

These findings are correlated with a previous study, which found that the histomicrograph of the hearts of rats administered the dyes shows significant histological distortions in their normal appearance and architecture [5]. The cardiac tissues were characterized by deformed nuclei, disarray of myofibrils, and connective tissue deposits, which might probably due to degeneration of the structural protein in mitochondria of the cytoplasm. Rats that received the dye combination were found to possess severe histological changes in the cardiac tissues compared to the control.

A previous study agreed with these results, where they found that, as regards histological results, tartrazine resulted in alteration in the normal histological structure of the heart in the form of hypertrophy of cardiac muscle fibers [13]. Destruction and vacuolization of some cardiomyocytes, as some cardiomyocytes were discontinuous, interrupted, and separated from each other by wide intercellular spaces. There was marked deposition of collagen fibers between the cardiomyocytes and around the blood vessels. Strong nuclear and cytoplasmic immunoreactions for nuclear factor Kappa ß in the cardiomyocytes by immunohistochemical stain were noticed.

5. Conclusion

Because of its antioxidant properties, rosemary may mitigate tartrazine's harmful effects; in such a situation, it would be prudent to provide rosemary to people. The adrenal glands and heart are particularly vulnerable to tartrazine's harmful effects. More research is needed to determine whether tartazine hurts other body organs and its possible molecular mechanisms of toxicity on other organs. Other studies should be conducted to evaluate the effects of rheas' colors on other organs. Also, we elucidated the promising protective role of rosemary as an antioxidant to lessen the noxious effects triggered by tartrazine. Other protective agents should be studied to mitigate tartrazine toxicity. So, the result of this study showed that rosemary, by its antioxidant activity, can prevent tartrazine’s toxicity, and it should be provided to humans in case of need to protect them against tartrazine's hazardous effects. Because tartrazine has hazardous effects on the heart and adrenal glands.

Ethical approval and consent to participate: During the period of October and November 2023, we performed the present research at Fayoum University's Faculty of Science. The research ethics board number (M 646) developed the experimental research ethical standards. On 12 February 2023, at Fayoum University's school of medicine were followed while dealing with animals.

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AI declaration: Not applicable

Author contributions: HKM, Data management, manuscript management,

manuscript writing /editing**.** **GMA:** Data analysis, manuscript writing, and editing**.** **MAM:** protocol & project development, manuscript writing. **EMM:** Data management, manuscript editing**.** All authors have read and approved the manuscript.

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