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Study of the protective role of the alcoholic extract of Cordia myxa against tartrazine hepatotoxicity

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***Abstract*— The current study aims to know the protective effect of the alcoholic extract of the fruits of the plant Cordia myxa on the liver tissue in white rats treated with tartrazine. The current study included 24 laboratory rats, their weights ranged between (250-200) grams, and their ages between (11-12) weeks. The animals were divided into four groups, with six animals for each group. The first group was considered a negative control group, given water and fodder freely. The second group was dosed with the aqueous solution of tartrazine at a dose of 400) mg/kg of body weight, while the third group was dosed with the alcoholic extract of the fruits of the plant Cordia myxa at a concentration of (1000) mg/kg of body weight. The fourth group was considered a protective group and was dosed with the alcoholic extract of the fruits of Cordia myxa at a dose of 1000) mg/kg of body weight. After 3 hours, it was dosed with tartrazine. After the 30-day experimental period, the animals were anesthetized with chloroform, dissected, and liver samples were taken to prepare histological sections. The results of examining the histological sections showed histological changes in the liver tissue of the animals treated with tartrazine, caused by oxidative stress resulting from the release of free radicals into the blood. The results of examining the prepared sections showed no histological changes in the liver tissue of the animals treated with the alcoholic extract. The results of examining the liver tissue of the animals treated with the extract of the bambur plant as a preventative substance showed that they were less affected by tartrazine toxicity when compared to the samples of the rat group treated with tartrazine, as the tissue appeared closer to normal, with a normal central vein and strong regularity of the hepatic cords. We conclude that the alcoholic extract of the pomegranate fruit can be used in medical treatments as it has a preventive effect against the toxicity caused by tartrazine dye by improving the liver structure..**

***Keywords* — Tartrazine, Punica granatum fruit extract, Liver.**

INTRODUCTION

Medicinal plants are One of God's divine gifts, medicinal plants come handy as a source of food and medicine. Medicinal plants were used in treating many diseases by ancient people. The mode of getting medicinal plants is varied, some of them being from natural sources in the environment, others home grown from farms and gardens, while others from the foreign markets and shops. As a result, the global interest among researchers and scientists in employing these plants in traditional medicine, the exploration of their constituents to discover their therapeutic properties for controlling a wide spectrum of diseases, is a significant phenomenon (1).

Medicinal plants remain popular by virtue of their safety and effectiveness in treatment of diseases, in absence of easily observable toxicity, and cost-effectiveness relative to the chemical alternatives. Therefore, medicinal plants have gained popularity among people today (2).

The Cordia myxa been known to have great medicinal and curative attributes when used as a medicinal plant. It is a diuretic, antifungal, antibacterial, analgesic, antitussive and blood pressure decrescent. Among its many benefits, are chest and respiratory concerns, liver cirrhosis and bone strength along with skin conditions (3).

On the other hand, the study of food has singled out food dyes as highly dominant among food and pharmaceutical additives, since today there are more than 2500 kinds of food dyes in application. These dyes are added in order to enhance its consumer appeal and form a part of the food colour component used in ice cream, sweets, premium bakery products, confectionery, fruit juices, snacks, soft drinks and alcoholic drinks (4)

Categorization of food colorings is divided into natural and synthetic. The majority, about 95%, of the dyes used in the production of food are synthetic due to their ease and low cost of manufacture. Tartrazine emerges among these dyes as the most applied. This separately bonded powder dye, which supplies a yellowish-orange tone, is found in many food

products. It is water soluble and can be used to out shine the colors of such foods as colored drinks, ready-to-eat foods, cakes, pickles, ice cream, jam, yogurt, honey products, butter and cheese. Tartrazine also exists in some cosmetics and personal-care products (5).

Due to the marked similarities between dyed and canned foods, the changing lifestyles as well as indiscriminate use of same products, this study was commenced with the aim of: The study was to consider whether the alcoholic extract of Bamber plant can be used to counter the negative effects of high dosages of one such coloring agent – tartrazine in albino rats under lab conditions.

MATERIALS AND METHODS

Produced from orchards in the holy Karbala Governorate, the Bamber fruit plant was cleaned with water from tap to remove dust, and left for 20 days to dry at room temperature before processing into a coarse powder for extraction purposes. Thirty grams of dried Cordia myxa powder was used and placed in 300 ml ethanol solution prepared on mixing 70 ml of absolute ethanol and 30 ml distilled water prior to extraction at the Soxhlet apparatus over a period of twenty-four hours at a range of 45-40 degrees Celsius. The filtrate was then used to be placed on a rotary evaporator at 45-40 Celsius until it hardened to a gummy material. Then, the sample was sealed in the electric oven at the temperature between 45 and 40 degrees Celsius. It was later stored in the refrigerator before it was required for testing (6).

EXPERIMENT ANIMALS

For the study, in use were (24) male Rattus norvegicus from an albino breed, weighing (200-250) grams, aged (11-12) weeks. Special plastic cages for the animals had metal lids and inside was lined with wood shavings on the bottom. Food and water were unlimited for the animals. Regular cleaning of the cages and (20-25) degrees Celsius temperature conditions were achieved. The animals were subjected to hours of light equaling twelve a day. Following the acclimatization, the animals were divided into four different groups. The first group was used as control and only provided with access to food and water. The second group was used as a positive control and administered an aqueous solution of tartrazine at the dose of 400 mg/kg of body weight (7). The third group received a third group received alcoholic extract of the fruit of bamber fruit at a concentration of 1000 mg/kg of body weight (6), after that the fourth group received the same extract at the same concentration after initiating placebo after three hours. Once the animals completed the 30-day experiment, they were chloroformed in a sealed chamber, after which liver samples were excited. The specimens were placed in 10% of formalin solution for 48 hours before they were switched to 70 % ethyl alcohol, and remained in storage till they were ready for use in a microscope.

PREPARING SAMPLES FOR HISTOLOGICAL EXAMINATION

The processes indicated by Spencer et al. (8) were implemented so that the tissue would be suitable for carrying out histological examination in the lab.

FIXATION OF SAMPLES

After the animal was dissected, the liver tissue slices were put into a 10% formalin solution containing 10 ml of formalin and 90 ml of tap water, and left in the solution for 48 hours.

THE ANKAZ

Each tissue slice was exposed to ethanol solutions of varying concentrations, for two hours, in turn: The water was gradually removed from the tissue in 70% v; 80% v; 90% v; and two times at 100% v.

PROMOTING

This was followed by samples treated in xylene for 5–7 minutes, processed to enhance transparency and clean them of staining solution.

IMPREGNATION

After settling the samples were taken and deposited in glass beakers containing 1:1 mixture of molten paraffin wax and xylene, and then incubated in an electric oven adjusted to 59-60 degrees Celsius. Then the samples were placed into new bottles filled with melting paraffin wax. Two more wax replaces were made to ensure complete absorption, which took (2-1.5) hours at each replacement.

BURIAL WITH WAX

The samples were placed in iron molds containing paraffine wax, and bubbles over the content of the sample were removed using a heated needle over a flame. After the sample had hardened firmly at laboratory temperature, it was carefully extracted from its mould and kept until the right time to cut it.

TRIMMING AND CUTTING

After the wax molds were trimmed using a sharp scalpel, they were readied for cutting in the Rotary Microtome, and then sectioned at 5 micrometers in thickness. Steps proceeded after the strips were immersing in a 45-degree Celsius water bath for achieving uniform flattening of the tissue. Following this, the tissue samples were laid on sterile glass slide carriers. The prepared slides were set on a hot plate at (37) degrees Celsius for an hour to dry and then left to dry at laboratory temperature for a night.

STAINING

Subsequent to the samples were subjected to a sequential staining process with ethyl alcohol (100%, 100%, 90%, 80%, 70%) and subsequently submerged in hematoxylin for seven minutes. Subsequently, they were rinsed with tap water for ten minutes, followed by a brief wash with eosin stain lasting four minutes. They were then immersed in distilled water, processed through a sequential series of ethyl alcohol solutions (70%, 80%, 90%, 100%, 100%), transferred to xylene, and mounted with a cover slip prior to microscopic examination.

RESULT AND DISCUSSION

Histological examination of liver tissue samples from the negative control group, shown in Figure (1), revealed a normal structure, with the central vein aligned to the hepatic cord, and typical hepatic sinusoids, hepatocytes, and nuclei. Consistent with the liver sample slides from the positive control groups, as shown in Figure (2), liver sections from animals exposed to a

400 mg/kg aqueous tartrazine solution showed congestion and dilatation of the central vein, as well as congestion and enlargement of the hepatic sinusoids, and marked deformities of the hepatic cords. Upon histological analysis of the alcoholic extract of the bamber plant at a concentration of 1000 mg per kg of body weight from liver sections, it was found that the tissues displayed a normal appearance without any modification in tissue composition. This clearly demonstrates that these liver sections contain a regular central vein and hepatic cords without pathological changes, as shown in Figure (3), indicating that the alcoholic extract is safe and free of harmful effects on the liver. Histological examination of liver samples exposed to 1000 mg/kg body weight of the alcoholic extract of the bamber plant and then treated with 400 mg/kg body weight of aqueous tartrazine revealed that, on the other hand, tissue sections treated with the bamber plant extract appeared less toxic than those exposed to tartrazine, and had a more normal structure with distinct veins as a center, well-organized hepatic cords, hepatocytes, nuclei, and sinusoids, as shown in Figure (4).

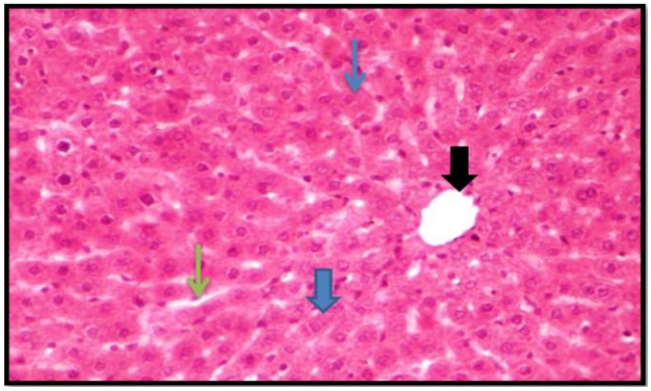

Figure 1. A cross-section of the rat liver tissue from the control group illustrating the existence of the central vein (Black arrow) and the regularity of the hepatic cords (Dark blue arrow) with the presence of sinusoids (Green arrow) and hepatocytes and their nuclei (Light blue arrow) (H & E 200X).

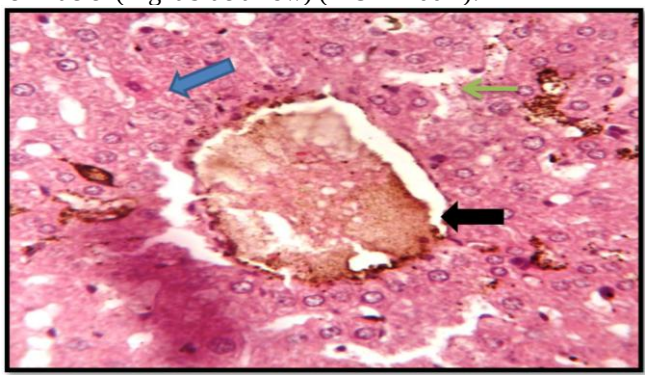

Figure 2. A cross-section of rat liver tissue from the group administered tartrazine at a dose of 400 mg/kg body weight, exhibiting congestion and dilatation of the central vein (Black arrow) are observed, along with congestion and dilation of the sinusoids (Dark blue arrow) and severe irregularity in the hepatic cords (Green arrow) (H & E 200X).

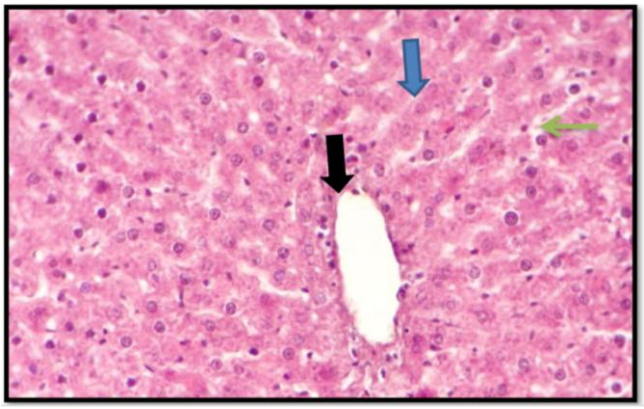

Figure 3. A cross-section of rat liver tissue from the group administered an alcoholic extract of Cordia myxa fruit at a dose of 1000 mg/kg body weight, demonstrating the presence of a normal central vein(Black arrow), regularity of the hepatic cords (Dark blue arrow), and the presence of sinusoids (Green arrow) (H&E 200X).

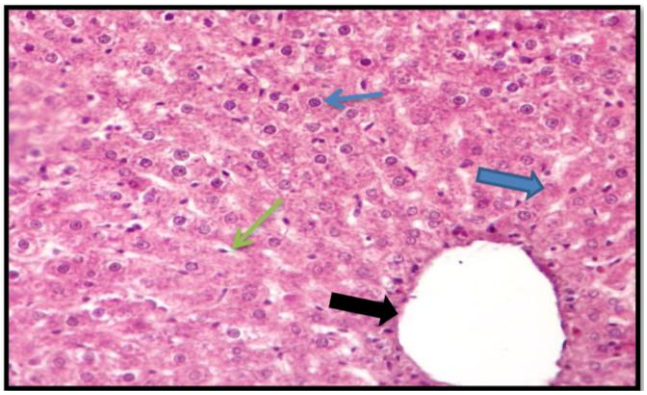

Figure 4. A cross-section of rat liver tissue in the preventative group treated with an alcoholic extract of Cordia myxa fruit at a dosage of 1000 mg/kg, alongside tartrazine at 400 mg/kg of body weight, indicates that the tissue resembles normal morphology, with a typical central vein (Black arrow), regularity of the hepatic cords (Dark blue arrow), liver cells and their nuclei (Light blue arrow) with sinusoids (Green arrow) (H&E 200X).

Our study results showed that tartrazine exposure led to severe liver damage in treated animals, which triggered changes in liver enzyme concentrations, mainly because of the broken liver tissue. The results follow those reported by Ali et al. (9) with respect to the effect of tartrazine on liver tissue. The research emphasized that the tartrazine dye toxicity resulted in auto-oxidation of liver cells due to increased generation of reactive oxygen species or free radicals, causing the cells and mitochondrial membranes injury, buildup of lipids, MDA levels rise, concentrations of liver enzymes (AST, ALT, ALP) to increase as well as the reduced activity of antioxidants enzymes.

Our study replicates the findings reported by the Zand et al. (10) research, i.e., a tartrazine dye induces congestion and even dilation of the central vein and congestion of the blood in the sinuses.

Likewise, research done by Abd-Elhakim *et al.* (11) pointed out the liver toxicity of tartrazine dye where it said it had a detrimental effect in liver tissue; this included increase and congestion of central vein, expansion of sinusoids, liver cell degeneration, and cellular infiltration, as well as impaired blood vessel functions. The discoveries of our investigation showed that the exposure to alcoholic extract of Bamber fruit did not cause any damage to the liver in rats. This discovery corresponds with the data given by (12). This is because Bamber fruit contains essential active substances that are important for the health and integrity of functions, structures, and cells of liver cells, as well as its phenols having a protective effect against liver injury (13).

The results of our investigation match those of Shamkhy & Abbas (14), indicating that the alcoholic extract of the fruit of pomegranate does not have influences on liver histology, as the effectiveness of the active constituents of the extract are well-tolerant to the liver pulses, and do not promote the onset of oxidative stress or create free radicals. Alcoholic extracts extracted from the pomegranate fruit contain phenols, alkaloids, saponins and vitamins that serve as antioxidants reducing cholesterol and regulating liver fat metabolism to help the liver act as a protective antioxidant.

The results of our present research are consistent with various studies that point to the protective properties of burdock alcohol extracts from different pathogens, due to their plentiful mineral and amino acid, vitamin content. The extract has been proven to have anti-ulcer, anti-cancer, anti-inflammatory, cough suppressing, urinary tract infection fighting, anti-arthritic as well as detoxifying properties due to the presence of large numbers of bioactive compounds and minerals. The alcoholic extract of burdock used was found to have an important role in minimizing oxidative stress since free radicals that damage cellular membranes were neutralized. Therefore, it is rich in substantial phenolic compounds which include phenols and flavonoids (15,16).

A study by Shamkhy & Abbas, (14), demonstrated the protective role of the alcoholic extract of the pomegranate fruit in preventing many diseases, as it contains many substances with beneficial medicinal properties, including enzymatic and non-enzymatic antioxidants and vitamins that provide antioxidant activity, thus scavenging the effect of free radicals that damage liver cells and tissues.

In the same vein, researchers (17) indicated in their study on the protective role of the squill fruit plant in improving liver function in mice exposed to oxidative stress with melatonin. Giving the extract contributed to an effective reduction in liver weight, as well as decreased urea and creatinine concentrations when compared to the melatonin group. Additionally, there was an increase in antioxidant concentrations, including GSH and CAT, with an improvement in liver pathological tissues when compared to the negative control group. The study recommendations indicated that the use of squill extract is safe and does not cause any pathological symptoms. It can be used as an antioxidant in the body. This stems from the squill plant's content of many active substances, including vitamins and phenols.

CONCLUSION

Through our study we found that tartrazine dye caused toxic and structural changes on liver tissues, i.e central vein congestion, sinusoidal dilation, marked irregularities on the hepatic cords. Our current study confirms that alcoholic extract of Bamber fruit does not do harm to liver tissues and it is safe. It is possible to estimate the range of opportunities of this extract at certain doses with regard to enhancing the function of bodily organs. The results showed that alcoholic extracts of Bamber fruit help protect liver tissue from tartrazine dye exposure through enhanced structural support to its tissue.

REFERENCES

1) Bennur, P. L., O'Brien, M., Fernando, S. C., & Doblin, M. S. (2025). Improving transformation and regeneration efficiency in medicinal plants: insights from other recalcitrant species. *Journal of Experimental Botany*, 76(1), 52–75.

2) Buddhadev, S. S., Hirawala, J. H., Buddhadev, S. G., Baghel, S. S. K. S., Paun, J. S., & Rahamathulla, M. (2025). Sustainable Solutions for Ayurvedic Pharma Waste Management: Harnessing Nature for Responsible Disposal and Reuse. In Solid Waste Management for Rural Regions. *IGI Global Scientific Publishing*. pp. 275–298

3) Mohtasham, N., Zarei Ahmadi, A., Alisamir, M., Shakiba Maram, N., & Larki, A. (2022). Preparation of Sepestan Anti-cough Syrup and Comparison of its Effectiveness with Diphenhydramine Syrup in Treating Cough in Children with Upper Respiratory Tract Infection: A Double-blind Randomized Clinical Trial. *Jundishapur Scientific Medical Journal*, 21(4), 574–585.

4) Pangtu, S., Sharma, P., Dhiman, S. R., Dhiman, M. R., Kashyap, B., & Chhabra, R. (2025). Evaluating the effectiveness of tinting with various food dyes for enhancing the value and quality of ornamental kale genotypes. *Journal of Applied Horticulture*, 27(1), 82–88.

5) Haridevamuthu, B., Murugan, R., Seenivasan, B., Meenatchi, R., Pachaiappan, R., Almutairi, B. O., Arokiyaraj, S., & Arockiaraj, J. (2024). Synthetic azo-dye, Tartrazine induces neurodevelopmental toxicity via mitochondria-mediated apoptosis in zebrafish embryos. *Journal of Hazardous Materials*, 461, 132524.

6) Al-Hamdani, H. M. S., Alfraji, A. S. A., Muhammed, A. I., & Mosa, R. J. (2019). Exploration the effect of higher dose of aqueous and alcoholic extracts of Bamber fruit (Cordia myxa) of some histological parameter and symptom in mice. *The Iraqi Journal of Agricultural Science*, 50(4), 1405–1415.

7) Al-Daamy, A. M. Z., & Al-Zubiady, N. M. H. (2020). Study of the toxic effect of tartrazine dye

on some biochemical parameters in male albino rats. *Scientific Journal of Medical Research*, 4(16), 111–117.

8) Spencer, L., Bancroft, J., Bancroft, J., & Gamble, M. (2012). Tissue processing. Bancroft's Theory and Practice of Histological Techniques. 7nd Ed. Netherlands, *Amsterdam: Elsevier Health Sciences*, 105–123.

9) Ali, F. A., Abdelgayed, S. A. S., EL-Tawil, S. O., & Bakeer, M. A. (2016). Toxicological and histopathological studies on the effect of tartrazine in male albino rats. *International Journal of Pharmacological and Pharmaceutical Sciences*, 10(8), 527–532.

10) Zand, A., Macharia, J. M., Szabó, I., Gerencsér, G., Molnár, Á., Raposa, B. L., & Varjas, T. (2025). The Impact of Tartrazine on DNA Methylation, Histone Deacetylation, and Genomic Stability in Human Cell Lines. *Nutrients*, 17(5), 913.

11) Abd-Elhakim, Y. M., Moustafa, G. G., Hashem, M. M., Ali, H. A., Abo-EL-Sooud, K., & El-Metwally, A. E. (2019). Influence of the long-term exposure to tartrazine and chlorophyll on the fibrogenic signalling pathway in liver and kidney of rats: the expression patterns of collagen 1-α, TGFβ-1, fibronectin, and caspase-3 genes. *Environmental Science and Pollution Research*, 26, 12368–12378.

12) Al-Muhana, W. H. Y., & Alaridhi, J. A. M. (2023). Studying Protective Role of Nano-Extract of Fruits of Cardi Myxa Plant Against Effects of Indomethacin Drug on Kidneys of Male White Rats. *Journal of Chemical Health Risks*, 13(4), 158–165.

13) Afzal, M., Obuekwe, C., Khan, A. R., & Barakat, H. (2009). Influence of Cordia myxa on chemically induced oxidative stress. *Nutrition & Food Science*, 39(1), 6–15.

14) Shamkhy, B. M., & Abbas, Y. K. (2021). The Biological Activity of Alcoholic Extracts of Cordia myxa Plant Against Klebsiella. Isolated from Infected Patients. *Indian Journal of Forensic Medicine & Toxicology*, 15(4).

15) Abdel Kawy, M. A. (2023). An Overview on Botanical Characteristics, Phytochemical Constituents and Pharmacological Effects of CordiaDichotoma (G. Forst.) and Cordia Sebestena (L.)(Boraginaceae). *Egyptian Journal of Chemistry*, 66(13), 437–459.

16) Sun, S., Batool, S., Saba, I., Ahmed, I., Ahmad, S., Tariq, T., Khan, I., Mustafa, G., & Hasan, M. (2025). Biological entities of green factories for nano-grafting zinc oxide: a comparative antibacterial mechanism. *New Journal of Chemistry*, 49(4), 1459–1477.

17) Tak, Y., Samota, M. K., Meena, N. K., Kaur, G., Jain, M. C., Kumar, R., Kaur, M., & Amarowicz, R. (2024). Underutilized fruit lasoda (Cordia myxa L.): Review on bioactive compounds, antioxidant potentiality and applications in health bioactivities and food. *Fitoterapia*, 105898.