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The preventive and therapeutic role of hexane extract of the roots of Capprise spinosa in reducing oxidative stress induced by tatrazine dye E102 in male rats

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***Abstract*---**This study was designed with the aim of verifying the effect of hexane extract of the roots of the plant Capprise spinosa through daily dosing on the level of oxidative stress induced by the food dye tatrazine (E102) through several parameters such as the antioxidant defense system in the body as well as its effect on kidney function in laboratory rats and liver enzymes. Capprise spinosa was collected from different areas in the city of Al-Diwaniyah, washed, and the leaves, stems and roots were isolated and dried, then they were ground and placed in a hexane solvent until the extract was obtained by soaking method, where the extract was filtered and dried. An experiment was then conducted for the interaction of the extract with DPPH This is to find out the highest inhibition rates for plant parts, and the highest scavenging rate was for the roots extract. The study was conducted on thirty rats (120-180) gm divided into five groups: The control group G1, the second group G2 that dosed hexane extract of the root of the plant Capprise spinosa daily for three weeks from the beginning of the experiment, the third group G3 that dosed the food dye E102 alone for three weeks, the fourth group G4 that dosed the hexane extract of the roots for three weeks and then the dye E102 after that for another three weeks And the fifth group G5, which dosed the food dye tatrazine E102 for three weeks, then dosed the hexane extract of the root of the plant Capprise spinosa. At the end of the experiment, blood serum samples were collected from rats under anesthesia to determine the biochemical changes. The animals were sacrificed to extract kidneys and livers and examined microscopically in the different groups. The oxidative state of the groups was

evaluated by measuring the activity of superoxide dismutase SOD, Catalase CAT, Glutathione transferase GST, and Malone Dialdehyde MDA. Also, renal indicators such as Urea concentration and Creatinine level were measured. Liver enzymes such as Alanine amino transferase ALT, Aspartate amino transferase AST and Alkaline phosphatase ALP in the blood were also measured. At the end of the study, a significant decrease ($p<0.05$) in body weight and the effectiveness of the antioxidant system was observed, as well as a significant increase ($p>0.05$) in the concentration of Malone Dialdehyde, urea and creatinine, and a significant increase ($p>0.05$) in the level of alkaline phosphatase enzymes (ALT, AST, ALP) for the third group that was given the food dye only. For the third group that was dosed food dye only. The results also showed that hexane extract of the roots of Capprise spinosa had a significant effect in reducing oxidative stress MDA, Urea, Creatinine And a significant increase in the effectiveness of the antioxidant system ($p>0.05$) increased the level of SOD, GST, CAT Improving the histological structure of the kidney and liver. It can be concluded that the plant extract has antioxidant activity and reduces the level of oxidative stress, thus protecting the liver and kidneys from complications that may be caused by high oxidative stress.

***Keywords*---**Capparis spinosa, Food dyes, Oxidative stress, Renal indicators, Liver enzymes.

Introduction

Many studies dealt with the therapeutic roles of many plant extracts, especially plants with medicinal qualities, which were previously studied and known by many ancient peoples, which encouraged the opening of horizons to a lot of medical and pharmacological research for various types of plants, also known as herbal science [(1)].

Capprise spinosa it is a perennial spiny with many evergreen branches. Its leaves are thick and smooth, like thorns. It has beautiful white flowers that bloom in the morning and then turn dark red in the afternoon. The plant bears a pear-shaped fruit. The Capprise Spinosa plant has many advantages that made it the target of many studies, and it is still under research because of its great medical importance.

The Capprise Spinosa plant has many advantages that made it the target of many studies, and it is still under research because of its great medical importance. Capprise spinosa is found in East Asian countries, Mediterranean countries and many other regions around the world [(2)]. Capprise spinosa has anti-bacterial properties. Studies have indicated that it has anti-bacterial properties. It is worth mentioning that part of the plant. Whether it is a fruit, roots, leaves or stems, it has a greater effect on a particular type of bacteria than the other [(3)].

Capprise spinosa also has an effective anti-inflammatory activity, as studies confirm that the extract of the capers plant leaves has anti-inflammatory activity. This activity appears remarkably by its inhibition of inflammatory cytokines. The extract of the fruit treats arthritis and reduces pain [4]. People use Capprise spinosa for diabetes, fungal infections, liver scarring, gas, arthritis, fatty buildup in blood vessels, breast congestion, intestinal worms, and a skin disease caused by parasites called leishmaniasis. Capprise spinosa is also used as a tonic. Some people apply shafalfa directly to the skin for dry skin and other skin disorders, to protect from the sun, and to improve blood flow near the surface of the skin. It also has anti-inflammatory, anti-viral and anti-diarrheal properties that can be treated, and the Capprise spinosa plant contains glucosinolates, glucopyrin, glucocaber, sinigrin, gluclomine and glucocabagin [5].

Capprise spinosa contains phenols, vitamin E, glucosides such as rutin, myronase enzyme, capric and pectic acid, quaternary alkaloids such as stacadrine, and a group of sugars and volatile oils that smell like garlic because of their sulfur, soapy materials, gels, sterols, organic and fatty acids, coumarins [6]. The antioxidant activity of Capprise spinosa was determined by the interaction of the plant extract Capprise spinosa with DPPH 2,2-diphenyl-1-picrylhydrazyl [7]. This is for all parts of the plant from leaves, stems and roots in hexane solvent. Studies indicated that the highest scavenging percentage was for the roots extract compared to the extracts for the rest of the parts. As for the added food dyes and preservatives, they have spread recently and in a very wide manner because of their impact on attracting the consumer by changing or adding taste and colors that make food items with a distinctive shape and taste.

Among the most important types of food dyes added are tatrazine, carmoisin and many other dyes, these dyes are distinguished by containing the azo group (-N=N). Recent studies have largely focused on the biological effects of food dyes and their toxic and carcinogenic effects [8]. There are many studies that included studies on metabolic disorders and toxicity that occur due to these artificial dyes on rats and some other types of milk. In addition, many compounds Azo has a toxic or carcinogenic effect on laboratory animals [9].

The industrial colorings dissolved in water constitute the majority of the dyes that are permitted to be used in foodstuffs, while the use of industrial colorants dissolved in fats is prohibited [10]. The reason is that the dyes dissolved in fats can be stored in the fatty tissue of the human body after ingestion, and this results in very serious health damages. While dyes dissolved in water are excreted directly from the human body after consumption [11]. However, doctors and specialists warn against the use and accumulation of artificial food dyes in the body, because of their effects on public health, and the allergic diseases they cause (skin and respiratory), especially in children. It also causes acute or chronic infections in the stomach and leads to stomach ulcers, and the most dangerous of all is that the large accumulation of its use and demand eventually leads to cancer [12].

Damage can occur as a result of food dyes interacting with the packaging, forming substances with a toxic effect. The accumulation of industrial dyes and the demand for foods containing large quantities of them eventually lead to cancer,

because some dyes form effective receptors that have carcinogenic potential, and the most important of these dyes belong to the mono-azo or di-azo group [13].
Several studies have indicated the therapeutic nature of the extracts of the Capprese spinosa plant against the oxidative stress that is induced by these dyes, and many experiments have been conducted in this regard. Such as measuring the level of creatinine in the blood, measuring the level of urea, as well as measuring liver enzymes such as alanine aminotransferase(ALT), alkalin phosphatase (ALP), aspartate aminotransferase (AST) and enzymes related to oxidative stress such as superoxide dismutase (SOD) and glutathione transferase (GST), and measuring the oxidative state, which is the oxidation of super lipids such as Malone Dialdehyde (MDA) [14].

Aim of the study

1- Study of oxidative stress that food dyes such as tatrazine can cause on kidney function and liver enzymes in male rats.
2- A study of the extract of the plant capprise spinosa as an effective antioxidant by evaluating the level of oxidative state enzymes, renal indices and liver enzymes in male rats.
3- Predicting the therapeutic and preventive role of the extract of the capprise spinosa plant

Material and methods

Chemicals

All chemicals were of analytical grade and were purchased from Sigma-Aldrich Co.DPPH, nHexane 99% and Capprise Spinosa plant1-chloro-2,4-dinitrobenzen(CDNB), Dipotassium hydrogen phosphate, Disodiumethylendiamintetraacetic acid (Na2EDTA), Epinephrine, Glutathione (GSH), Hydrogen peroxide, Potassium dihydrogen phosphate, Sodium hydroxid, Potassium dihydrogen phosphate, Sodium carbonate, Tartrazine (E102), Thiobarbituric acid-TBA, Alanine Aminotranaseferase kit, Creatinine kit, Aspartate Aminotransferase kit, phosphatase Alkaline kit, Urea kit, ethanol alcohol, Trichloro acetic acid-TCA.

Preparation of Extraction

After the process of collecting and grinding the plant parts, the extraction process was carried out using soaking with the usual solvent hexane with a concentration of 99%, and then the filtration and drying process, where the extract was obtained in powder form [15].

Determenation of the Scavenging Percentag

The process of determining the percentages of inhibition was done by the interaction of the extract of the capris spinosa plant with (2,2-diphenyl-1-picrylhydrazyl) DPPH, which is a source of free radicals, and thus the inhibitory percentages of the plant parts were compared among themselves, and the highest percentage was for the roots and it was adopted in the study [16].

Experiment animals

Healthy male Wistar rats 30 with an average mass of (120-180)g were obtained from the animal house in the College of Science, University of Al-Qadisiyah, where the study was conducted.

The animals were housed in a temperature-controlled room at 21–28°C. The animals were randomly divided into five groups:

1- G1 control group
2- G2 that dosed hexane extract of the root of the plant Capprise spinosa daily for three weeks.
3- G3 that dosed tatrasine dye (E102) daily for three weeks.
4- G4 that dosed the hexane extract of the roots for three weeks and then the dye E102 after that for another three weeks.
5- G5 which dosed the food dye tatrazine E102 for three weeks, then dosed the hexane extract of the root of the plant Capprise spinosa.

Superoxide dismutase assay

The liver SOD activity was measured according to the method of (Marklund and Marklund, 1974) [16].

Catalase assay

CAT activity was assayed according to the method of Beers and Sizer with hydrogen peroxide (30 mM) as the substrate (Beers and Sizer, 1952) [17] .

Glutathione-S-Transferase assay

The level of GST was estimated as per the method of Habig (Habig 1974) [18] .

Lipid peroxidation assay

The measurement of MDA is based on its reaction with thiobarbituric acid (TBA), then it is detected by spectrophotometric method [19] .

ALT, AST, ALP, Creatinine and Urea assays

The activities of above mentioned biochemical parameters were measured by the use of specific kits from Biosystems – Spain [20].

Statistical Analysis

Total data were read statistically by means of SPSS software, version 26 software (2019). Test methods include one-way ANOVA for comparisons between groups followed by least significant difference (LSD) test for comparison between five groups. Probability values ($P<0.05$) are prepared to score statistical significance. All data are expressed as mean ±S.D standard deviation.

Results

Superoxide dismutase assay SOD

The results in table (1) show a significant decrease ($P<0.05$) in SOD enzyme activity in the group of animals treated with E102 dye alone (G3E102) and the group of post-treated animals (G5) when compared to the control group, while it was not recorded. When comparing the level of enzyme activity in the CSE (G2)

alone group to the control group, there is a significant change (P>0.05) (G1). SOD is a powerful antioxidant that protects cells from oxidative stress damage. It's also recognized as an antioxidant that protects against oxidative stress caused by free radicals produced by the metabolism of E102 dye. SOD is an enzymatic antioxidant that works to convert superoxide radical $O_2^{-\cdot}$ and neutralizes free radicals. The enzyme catalase transforms H_2O_2 into water and oxygen, which can then be disposed[21].

Glutathione –S-transferase assay GST

From the results in table (1)When compared to the group of control animals, the activity of GST enzyme was significantly reduced (P<0.05) in each of the groups of animals treated with E102 dye alone ($G3_{E102}$) and the group of animals administered CSE after delivering the dye (G5) post-treatment (G1).

Catalas assay

The results of the table(1) group ($G3_{E102}$) that was given E102 alone revealed a substantial decrease in CAT activity in blood when compared to the control group (G1) (p<0.05) as indicated in table (2). There were no significant differences in CAT activity in the treated intact group (G2) compared to the G1, but pre-treated (G4) and post-treated (G5) exhibited a significant improvement in CAT activity (p<0.05) in comparison to G2, although it remained low compared to G1, G2as in figure (3). Catalase is an important antioxidant enzyme that helps to reduce oxidative stress by eliminating cellular hydrogen peroxide and producing water and oxygen. Catalase deficiency or malfunction has been linked to the development of numerous degenerative illnesses.

Malondialdehyde (MDA) assay

Table (1) shows that the level of MDA in the group of animals treated with E102 dye (G3E102) increased significantly (P<0.05) when compared to both the control group and the group of animals given CSE alone (G2). There was a significant decrease (P<0.05) in the level of MDA in the group of G2 animals that were given CSE alone compared to the control group compared to the group G3E102, but it remained significant (P<0.05) compared to each of the groups (control G1, G2), while there was no significant difference (P<0.05) in the level of MDA in the group of G2 animals that were given CSE alone compared to the control group as shown in figure (4).

Urea assay

Table (2) shows that the level of urea in the group of animals treated with E102 dye (G3E102) increased significantly (P<0.05) when compared to both the control group (G1) and the group of animals given CSE alone (G2), while there was no significant difference (P>0.05) in the level of urea in the group of animals given CSE alone (G2) when compared to the control group. At the same time, the study's findings revealed a substantial drop (P<0.05) in the number of animals administered CSE before being dyed (G4) pre-treatment and (G5) post-treatment. It showed a significant drop (P<0.05) when compared to the control group

($G3E_{102}$), but it remained significant ($P<0.05$) when compared to the control group ($G3_{E102}$) figure (5).

Creatinine assay

Table (2) shows that the level of creatinine in the group of animals treated with E102 (G3E102) dye increased significantly ($P<0.05$) when compared to each of the control animals and the group of animals given CSE alone (G2), while there was no significant difference ($P>0.05$) in creatinine level in the group of animals given CSE (G2) alone compared to the control group (G1).
The two groups of animals that were given CSE before dyeing (G4) pre-treatment and the group (G5) post-treatment that were dosed CSE after dye recorded a significant decrease ($P<0.05$) compared to the group $G3_{E102}$, but it remained significantly high ($P<0.05$) compared to Each of the groups of animals that were given CSE before dyeing (G4) pre-treatment and the group (G5) post-treatment that were dosed CSE after dye recorded a significant decrease ($P<0.05$).

Aspartate aminotransferase (AST) enzyme assay

From the results In table (3) each of the groups of animals treated with E102 dye alone (G3E102), the group of animals pre-treated G4 with extract, and the group G5 post-treated, there was a significant increase ($P<0.05$) in the activity of (AST) enzyme compared to the control group, while there was no significant difference ($P>0.05$) in the level of enzyme activity in the group of animals given the extract G2 alone compared to the control group.
In the group of rats given the extract (G2), however, the activity of the AST enzyme was much lower than in the control group.
There was a significant reduction ($P<0.05$) in the post-treated group G5 of animals compared to the control group ($G3_{E102}$).

Alanine aminotransferase (ALT) assay

The activity of liver enzymes (ALT) was significantly increased ($P<0.05$) in each group of animals treated with E102 dye alone (G3 E102) compared to the control group animals, as shown in Table (3). The study outcomes were also recorded at the same time. When the G4 group was given the plant extract CSE before the dye pre-treatment, there were no significant variations in the level of ALT enzyme compared to the control group.
In the group of rats administered CSE after receiving the dye post-treatment G5, the level of the enzyme was significantly lower ($P<0.05$) than in the control group ($G3_{E102}$).

Alkaline phosphatase (ALP) assay

The results shown in Table (3) indicate a significant increase ($P<0.05$) in the activity of the enzyme (ALP) in both the group of animals treated with E102 dye alone ($G3_{E102}$) and the group of animals given CSE after giving the dye (G5) post-treatment compared with the group of control animals (G1) (G1). The study also found that when the G2 group was given just CSE, there was no significant difference ($P<0.05$) between them and the control group.

While the study's findings revealed a substantial drop (P>0.05) in enzyme activity in the G2 group of mice as compared to the G1 group (G3, G5).
As shown in figure (10), the pre-treatment group (G4) had a substantial unambiguous drop (P>0.05) compared to the control group (G1 and G2), although it remained high compared to the control group (G1 and G2).

Table (1)

Group	SOD		MDA		GST		CAT	
	Mean	S.E	Mean	S.E	Mean	S.E	Mean	S.E
1	1.98	± 0.07	1.3683	± 0.06896	7.4633	± 0.82041	2.9382	± 0.1214
2	2.07	± 0.02	1.3070	± 0.15441	8.7570	± 1.29354	2.7513	± 0.20306
3	1.00	± 0.01	3.6378	± 0.19252	1.3763	± 0.30574	0.3683	± 0.04373
4	1.91	± 0.01	2.5595	± 0.17221	4.8305	± 0.44012	1.3725	± 0.16474
5	1.82	± 0.01	2.8755	± 0.27652	3.1043	± 0.41809	1.3647	± 0.19977

Table (2)

G	Urea		Creatinine	
	Mean	S.E	Mean	S.E
1	16.6690	± 1.62879	0.5535	± 0.07378
2	15.3455	± 1.63308	0.5981	± 0.09406
3	38.8823	± 4.19965	2.4060	± 0.13724
4	29.5167	± 1.48670	1.2881	± 0.12422
5	31.1450	± 1.74183	1.3020	± 0.27715

Table (3)

Group	AST		ALT		ALP	
	Mean	S.E	Mean	S.E	Mean	S.E
1	1.1753	± 0.29721	0.9400	± 0.47639	1.180	± 0.58803
2	0.5998	± 0.06659	0.8900	± 0.32907	1.4020	± 0.49486
3	8.6967	± 0.38960	3.6000	± 0.13843	7.0515	± 0.0259
4	2.4337	± 0.26719	1.0010	± 0.72887	1.8445	± 0.5907
5	3.0002	± 0.13730	2.5000	± 0.50911	2.8363	± 0.9331

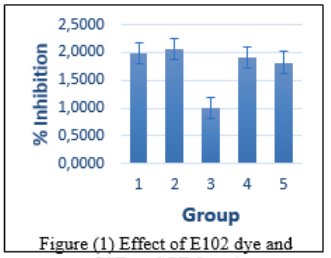

Figure (1) Effect of E102 dye and CSE on SOD Level

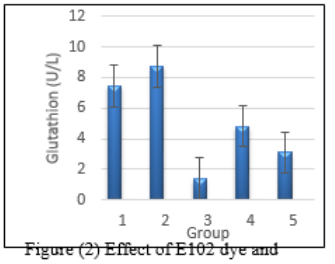

Figure (2) Effect of E102 dye and CSE on Glutathione

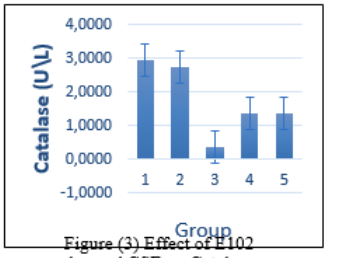

Figure (3) Effect of E102 dye and CSE on Catalas

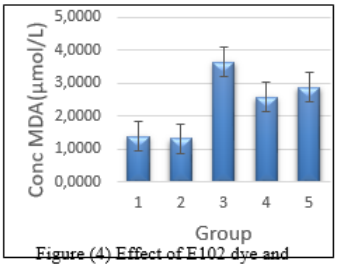

Figure (4) Effect of E102 dye and CSE on MDA

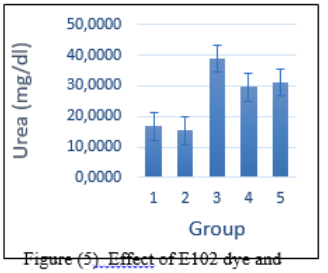

Figure (5) Effect of E102 dye and CSE on Urea

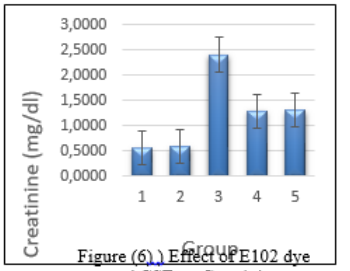

Figure (6)) Effect of E102 dye and CSE on Creatinine

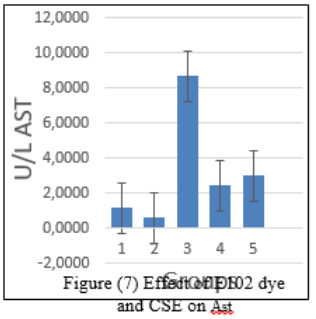

Figure (7) Effect of E102 dye and CSE on Ast

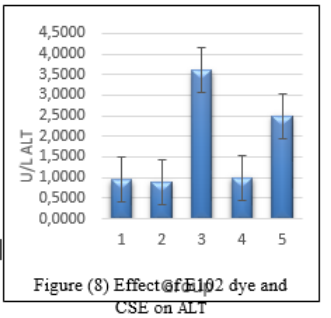

Figure (8) Effect of E102 dye and CSE on ALT

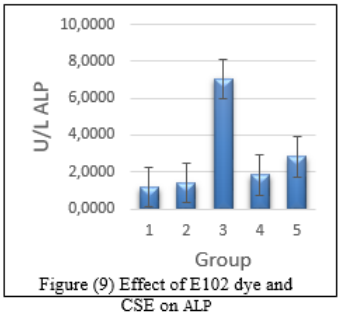

Figure (9) Effect of E102 dye and CSE on ALP

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