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In Vitro Antioxidant Activity of Aqueous Extract of Saffron and Prevents the Elevation of Oxidative Stress in the Kidney of Streptozotocin-Induced Diabetic Female Rats

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Keywords

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Abstract

In this work, the substance of hepatic non-enzymatic antioxidants in STZ-diabetic rats was analyzed according to the impacts of an aqueous saffron extract and vigorous activity. Utilizing a randomization interaction, 41 male rats (age: 12 weeks; weight: 31426.7 gr) were isolated into five gatherings: solid control (HC; n = 6); diabetic-control; diabetic-vigorous preparation; diabetic-saffron; and diabetic-high-impact preparing saffron (DATS; n = 5). The DS and DATS bunches ran on the treadmill for a long time, five straight meetings, at a speed of 12 m/min and a slant of 0% for 30 min. They likewise consumed 25 mg/kg of saffron aqueous extract every day for a long time. The rats were made it lights-out time prior to being killed. The livers were then taken out to gauge the degrees of malondialdehyde (MDA), glutathione (GSH), and generally speaking antioxidant limit. The DATS and DC groups, respectively, saw the greatest and smallest increases in TAC concentration (P=0.006). It would seem that using saffron extract in conjunction with aerobic exercise would be a suitable strategy for boosting the hepatic non-enzymatic antioxidant system in diabetic rats.

1. Introduction

The Global Diabetes Organization assesses that 246 million individuals overall have diabetes, which is the most serious metabolic condition. However, a recent prediction states that by 2025, the number would rise to 380 million. For its control, both pharmacological and non-pharmaceutical approaches (changing one's lifestyle) have been proposed. [1] Various tissues in diabetes make reactive oxygen species (ROS) because of different cycles, including non-enzymatic glycosylation occasions, the mitochondrial electron transport chain, and film bound NADPH oxidase. A few bits of proof have proposed a cozy connection between oxidative stress and the improvement of diabetes, and they likewise recommend that the oxidative stress welcomed on by hyperglycemia and hyperlipidemia happens before the beginning of diabetes' terminal clinical side effects, which is alluded to as the infection's essential gamble factor.

Currently, insulin and hypoglycemic medications are the primary and most successful treatments for diabetes mellitus; nevertheless, these combinations have a number of unfavorable side effects. Despite the fact that medicinal plants and their derivatives have long been used to treat diabetes mellitus and its side

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effects, no conclusive study has been discovered to support this. The lily family includes the perennial tiny plant known as saffron. It is a culinary flavour that is widely consumed and has a wide range of pharmacologic effects. According to research, even little doses of saffron—100 mg or 30 mg of its hydroalcoholic extract—can have a significant pharmacologic impact on people.

Recent research has shown that saffron extract has anti-tumor, anti-mutant, and inhibitory effects on the production of nucleic acids and cancer cells. Alpha crocetins, water-soluble carotenoids, crocin, including di, tri, and picrocrocin, and safranal are only a few of the numerous substances found in the saffron extract. Carotenoids' anti-cancer properties are well understood. However, it is still uncertain what physiological impact saffron has on conditions like diabetes. Consuming saffron enhancements might assist with lessening oxidative stress and forestall the beginning of type 1 diabetes since crocin, crocetin, and safranal in saffron have hostile to free revolutionary and antioxidant qualities. Additionally, the findings of the research have shown a correlation between physical activity and an increase in the production of free radicals, which is mostly due to an increase in oxygen consumption by active tissues.[2]

The results of a study in this area have shown that exercise, whether chronic or acute, which is accompanied by tissue degradation, causes an increase in the number of free radicals in biological tissues. According to the researchers, consistent exercise modifies the antioxidant capacity and shields cells from the damaging effects of oxidative stress, preventing the death of cells.

2. Literature Review

Rajaei, Z., Hadjzadeh, (2013). In this work, streptozotocin-induced diabetic rats were utilized as guineas pigs to decide the antihyperglycemic and defensive capability of crocin, a pharmacologically dynamic part of Crocus sativus L. [3] Crocin was given intraperitoneally to rats for a very long time at measurements of 15, 30, and 60 mg/kg body weight. As per our exploration, crocin might be useful in the treatment of diabetic patients since it shows hypoglycemia and antioxidative impacts in streptozotocin-induced diabetes.

Elgazar, A. F., Rezq, A. A., & Bukhari, H. M. (2013). The point of present review was to explore the impact of given oral organization of saffron water extract with various measurements on alloxan-induced diabetic rats. 35 male pale skinned person rats of the Sprague-Dawley strain weighing 200 ± 5 g were partitioned into five gatherings of equivalent number and weight. All in all organization of saffron extract decreased blood glucose level and the occurrence of various confusions as consequences of hyperglycemia. Saffron enjoy a benefit because of the presence of related bioactive mixtures with antioxidant properties which might apply further wellbeing advancing impacts.

Boskabady, M. H., &Farkhondeh, T. (2016) Numerous research published recently indicated that these therapeutic characteristics may be mediated via antioxidant, inflammatory, and immunomodulatory actions. The antioxidant capacity is increased by C. sativus, which also scavenges free radicals. Because of its antioxidant, mitigating, and immunomodulatory properties, C. sativus and its essential parts, including safranal, crocins, and crocetin, might be helpful against various issues.

Shaterzadeh-Yazdi, H., Noorbakhsh, (2018). The kidneys eliminate waste from the blood as important organs. Additionally, they could be involved in blood pressure control, red blood cell genesis, and electrolyte homeostasis. Diabetes, nephrotoxic substances, and ischemia/reperfusion damage are only a few of the possible causes of kidney illnesses. Logical information bases were inspected utilizing search queries such TQ, antioxidant, renal ischemia-reperfusion, diabetic nephropathy, and nephrotoxic specialist. In creature and in vitro models of various kidney diseases welcomed on by aggravation and oxidative stress, TQ showed mitigating and antioxidant impacts.

Hassani, F. V., Mehri,. (2017) The current study's objective is to examine the hepatoprotective properties of crocin, a saffron component, against BPA-induced liver damage. We demonstrated that administration of 0.5 mg/kg BPA to male Wistar rats for 30 days caused a rise in 8-isoprostane levels, a reduction in reduced glutathione levels, an increase in aspartate aminotransferase, lactate dehydrogenase, triglyceride, and glucose levels in the serum, and an inflamed periportal area. [4]

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Butnariu, M., Quispe, (2022) The sole species of crocus that is grown for food is Crocus sativus L. (saffron), which is mostly found in North Africa, Southern and Central Europe, and Western Asia as attractive plants in gardens and parks. Saffron's usage dates back thousands of years, and in addition to its use as a spice, it has long been valued for its therapeutic and decorative properties. It is used as a spice because of its unique taste and color, and because of its antibacterial and antioxidant action, which confers food preservation function.

3. Materials and Methods

3.1. Subjects

41 male rats (age: 12 weeks; weight: 31426.7 gm) were examined for this article. They were moved to the animal room at the Staff of Actual Training of Islamic Azad College, Tehran Focal Branch, where the climate's temperature was set at 222 and the light was directed (12-hour pattern of daintiness/dimness). They remained there for the necessary 8 days of similarity. The creatures had unlimited admittance to food and water. Streptozotocin (50 mg/kg of body weight) from Sigma Chemical Company was given intraperitoneally on the seventh day, after a one-night fast. [5] For the healthy control group, the same volume of citrate buffer solution was injected. Blood samples were taken from the animals' tails four days following the injection and tested using a Glucometer to determine the animals' blood sugar levels. Rats with glucose levels in excess of 300 mg/dl were decided to participate in the review. The rats utilized in this study were arbitrarily relegated to one of five gatherings: sound controls (n=6), diabetic controls (n=10), diabetics who went through high-impact work out (n=10), diabetics who got saffron (n=10), and diabetics who went through diabetes practice with saffron (n=5). Rats in the sound benchmark group got citrate cradle and were all healthy. In the diabetic oxygen consuming preparation bunch, diabetic rats went through a short activity routine for quite some time. The diabetic-saffron bunch got 25 mg/kg of aqueous saffron extract consistently for a long time, while the diabetic-saffron-high-impact preparing bunch got 25 mg/kg of aqueous saffron extract alongside gentle activity. The degrees of hepatic non-enzymatic antioxidants in each of the five gatherings were surveyed following fourteen days of preparing and extra saffron consumption by the exploratory gatherings. Malondialdehyde (MDA), a marker of oxidative stress and lipid peroxidation, glutation, a marker of the body's antioxidant limit with a high focus in the liver, and complete antioxidant limit (TAC) of plasma decided utilizing the ferric decreasing antioxidants power (FRAP) strategy were among the elements estimated.

3.2. Method of Producing Aqueous Extract of Saffron

To make an orange-shaded extract, 9.2 grams of powdered saffron (new shame) were broken down in 1000 cc of distillated water and kept at 50oC for 16 hours. A Whatman filter paper was used to filter the final product. Up until usage, the transparent dissolution was kept at 4 oC. It was administered to the rats orally using a unique gavage procedure.

Training Procedures The animals use the rodent treadmill to exercise. The animals practiced on the treadmill for four days before to the real experiment. They use the treadmill once a day for five minutes at a pace of ten meters per second throughout this time. The experiment excluded the animals that refused training. [6] After this time, the primary training program got underway. The animals run for 10 minutes, five days in a row, at an intensity of 10 meters per minute throughout the first week. In the second week, this was extended to 30 minutes, with the intensity reaching 10 m/min in the first 10 minutes and 12 m/min in the last 20 minutes. The animals were shocked electrically to get them moving.

Making Homogenized Hepatic Tissue and Biochemical Tests To make the homogenized hepatic tissue, the liver was gauged, ground with careful scissors, and put in a 50 cc falcone containing 10 cc of phosphate support 10 mM, pH=7.4, 0.2 molar EDTA, and 0.1 molar PMSF (proteinase inhibitor). The homogenization was done utilizing an electrical homogenizer, and 5 cc of the previously mentioned cushion was then added to the liver. The supernatant was taken after the arrangement was centrifuged at 4000 rpm at 4 oC for further exploration. \

3.3. Measurement of Antioxidants

3.3.1. Malondialdehyde (MDA)

Hepatic tissue from the aforementioned segment that had been homogenized for the experiment was centrifuged at 3000 rpm for 5 minutes. A tube was

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filled with one milliliter of the supernatant before being filled with two milliliters of the TBA reagent (15 weight/volume percent of trichloroacetic acid, 0.375 weight/volume percent of TBA, and 2.08 milliliters of HCl 37.7%) and vortexed. After sealing the tubes, they were placed in a bath of boiling water for 15 minutes before being allowed to cool. [7] They were centrifuged for 10 min. at room temperature at a speed of 1000 rpm, and a spectrophotometer was utilized to gauge the absorbance of the supernatant in the clear at 535 nm. The phosphate buffer, from which the homogenate was generated, was utilized in the blank in place of homogenized hepatic tissue.

The molar extinction coefficient of malondialdehyde (1.56*105 cm/mmol) was used to determine the concentration. (Where "A" rises to optical thickness, "E" rises to molar termination coefficient, "C" approaches fixation, and "L" approaches cuvett length.)

3.3.2. Reduced glutation (GSH)

Based briefly on Seldak and Lindsay's approach (1968), the decreased glutation was determined using Ellman's reagent. Five milliliters of homogenate were combined with four milliliters of distillate water, one millilitre of a 50% TCA solution (25 grams of TCA in about 50 milliliters of distillate water), and vortexed for 15 minutes. The resulting mixture was centrifuged for 15 min. at ambient temperature and 3000 RPM to thoroughly precipitate the tissue proteins. Two milliliters of the supernatant were combined with four milliliters of 4 M tris buffer that contained 0.2 M EDTA (pH = 8.9), one milliliter of 0.1 M DTNB in methanol (12 mgr DNTB was dissolved in five milliliters of methanol), and two milliliters of 0.1 M tris buffer. Since this solution is light-sensitive, it should be stored at 4 oC in the dark. Immediately, a spectrophotometer operating at a wavelength of 412 nm read the amount of light absorption.

The newly made GSH arrangement was utilized as a kind of perspective for drawing the schematic (0.01844 gr of glutation was broken down in 10 ml of water, and 9 ml of distillate water was then added to 1 ml of this answer for make the standard working arrangement with centralizations of 6 mol/L). The samples' absorption was identified, and the concentration was computed using the standard curve diagrams.

3.3.3. Total Antioxidant Capacity (TAC) of Plasma (FRAP Test):

Using the standard curve, the quantity of FRAP in the unidentified samples was determined (Table 1).

Table 1. Standard curve for estimating FRAP amounts.

	black	1	2	3	4
Iron solution (ul)	1	128	265	600	2000
Distillated water (ul)	2000	826	765	600	1
Concentration (ul)	1	126	254	600	2000

A dim holder was loaded up with 1.5 ml of the FRAP working arrangement (5 ml of 40 mM FeCl3 arrangement with the TPTZ reagent (15.5 mgr TPTZ), 5 ml of 40 mM HCl, and 50 ml of acetic acid derivation cradle. This arrangement (which was made consistently) was delicate to light and should have been put away in a dim compartment. [8] It was put into a test cylinder, and 50 microliters of the reference test and the unidentified example were added. All test tubes were checked for light ingestion at 593 nm subsequent to being vortexed at 37 oC for 10 min.

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3.4. Statistical Methods

The means and standard deviations of each piece of information were introduced. One-way examination of difference was performed to look at the between-bunch information. The Scheffe post hoc test was utilized to decide whether there was a huge contrast between the gatherings.

The SPSS Software, Version 16, was used to do all statistical analyses, taking into account the /0 05 significance level. [9]

4. Results and Discussion-

The consequences of the Kolmogorov-Smirnov test uncovered that the information on malondialdehyde, glutation, and TAC across the gatherings were regularly appropriated (P>0.06). Malondialdehyde showed a significant distinction following two weeks (F 4, 36= 24.431, P 0.001). [10] The discoveries exhibited that, in contrast with the diabetic-control bunch, the centralization of malondialdehyde in the saffron-vigorous preparation (P= 0.001), diabetic-oxygen consuming preparation (P= 0.001), and diabetic-saffron bunches generally impressively diminished following fourteen days of preparing (Figure 1).

Table 2: Malondialdehyde levels in six groups (*) demonstrate a significant departure from the diabetes control group (P 0.06).

Groups	
HC	2
DC	7
DAT	4.2
DS	3
DATS	2

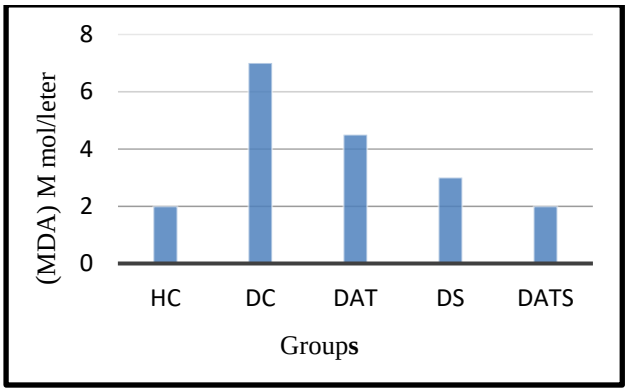

Figure 1: Malondialdehyde levels in 6 groups are shown graphically, and the asterisk (*) denotes the significant difference from the diabetes control group (P 0.06)

Following fourteen days of therapy, there was a tremendous distinction in glutation focus between the gatherings (F4, 36=21.028, P=0.001), with the saffron-vigorous preparation bunch having significantly higher glutation fixations than the diabetic-control bunch (P=0.012). In Figure 2.

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Table 3: In each of the five groups' glutamate concentrations, an asterisk (*) signals a significant divergence from the diabetic-control group (P = 0.06)

Groups	
HC	12
DC	5
DAT	5.2
DS	5.5
DATS	10

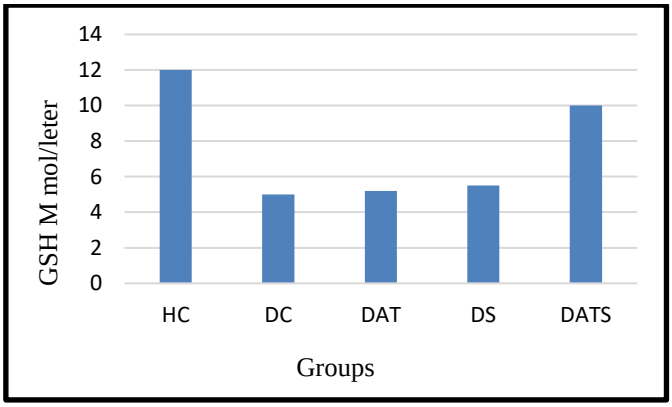

Figure 2: The centralization of glutation in the five gatherings, * means a huge takeoff from the gathering of diabetic controls (P 0.06).

The convergence of TAC (F4,36= 9.712, P= 0.001) was likewise researched between the gatherings such that it altogether expanded in the saffron-oxygen consuming preparation bunch contrasted and the diabetic-control bunch (P≤ 0.006) (Figure 3).

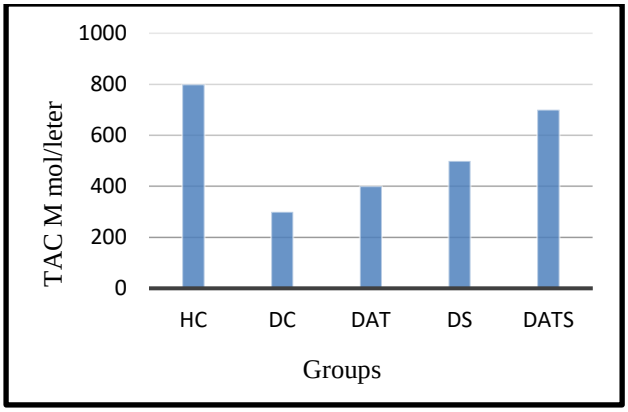

Figure 3: Total antioxidant capacity (TAC) in five groups is shown with an asterisk (*), which denotes a significant departure from the diabetic-control group (P 0.06).

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The ongoing review's goal was to figure out what influence high-impact practice and aqueous saffron extract had on the substance of non-enzymatic antioxidants in the liver of STZ-diabetic rats.[11]

The study's findings revealed that the STZ-diabetic rats' hepatic non-enzymatic antioxidant concentration significantly decreased; however, after saffron was administered orally, an aerobic exercise program was implemented, and saffron administration and exercise were also combined, the diabetic rats' antioxidant capacity significantly increased. This increase was more pronounced in the group that combined saffron intake and exercise, and their hepatic non-enzymatic antioxidant concentration significantly increased.[12] It seems that various illnesses that cause disruptions in the electron transport pathway coexist with diabetes.

Finally, it lowers the level of blood antioxidants and raises the number of free radicals produced as a consequence of oxidation pressure. Furthermore, some exploration' discoveries have shown that delayed openness to high measures of glucose, free unsaturated fats, or a blend of the two might obstruct the capability of beta cells. Since beta cells have low degrees of free extreme antioxidant compounds such as catalase, glutathione peroxide, and superoxide dismutase, they are especially helpless against reactive oxygen species.

The findings of the current study were consistent with those of the earlier ones and demonstrated that as diabetes progresses and problems with insulin hormone production and secretion affect other systems, the body's antioxidant capacity significantly declines.[13]

5. Conclusion

The findings of the current investigation demonstrated that oral saffron extract supplementation combined with aerobic exercise had greater benefits on the enhancement of total antioxidant capacity in diabetic rats. [14] This is explained by the combination of the aforementioned factors pertaining to the impact of cardiovascular exercise and saffron consumption on the body's antioxidant capacity. It is therefore suggested to use the saffron supplement as an effective factor to improve the results of aerobic exercise, particularly in cases aiming at improving antioxidant capacity and, as a result, prevention from autoimmune diseases and prevention from the destruction of tissue cells caused by the high oxidation pressure which is induced by the incomplete combustion of oxygen in the mitochondria cycle.[15]

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