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Effect of Gemzar drug and plant extract of Crocus sativus L on some antioxidants in male rats

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Abstract

The current study was designed to evaluate the antioxidant effects of the chemical drug "Gemzar". In addition to study the role of the aqueous extract of the stigma of plant saffron to decrease the toxicity of Gemzar. In this study, 30 white adult rats aged 9-12 weeks were grouped into five groups (6 rats in each group). The first group was negative control which received normal saline for four weeks. The second group was positive control received Gemzar in a dosage 15 mg/kg of body weight for four weeks. The third group was orally received a plant extract at a dosage of (100) mg/kg of body weight before medication for two weeks, then received drug for two weeks. The fourth group was received both plant extract (100) mg/kg and the drug for four weeks. The fifth group was received plant extract two weeks after medication.

The experiment was maintained for four weeks, then the rats were killed, and the blood was drawn to study the pathological effects in the examined parameters. The statistical analysis showed a considerable decrease in catalase, albumin, with increasing of lipid peroxide levels in the group treated with drug in comparison with control group. However, the treatment with both plant extract 100 mg/kg and the drug was administered in three treatments with a plant extract (before, during, and after), the drug receiving. The current study showed a significant improvement in some cases of negative control. It may revealed that the exposure to Gemzar caused alterations in biochemical indicators of rats blood. The current study improved that that the uptake of the plant extract simultaneously with the drug or (before and after) the drug has a significant role to increasing the harm consequences caused by the drug.

Keywords: Extract Of Saffron, Gemzar, Oxidative Stress, Antioxidant

Introduction

Since the early decades, the natural products are plant-based, especially the secondary metabolic compounds used in the treatment of various chronic diseases, including cancer. In the past, in Asian nations and Greece, plants or their components were used as remedies, and they were developed as an impressive modern medicines (Kharb *et al.*, 2012).

Herbal medicine played an important role in preventing the side effect of cancer drugs by activating enzymes and hormones, as well as promoting the DNA repair mechanism. The protective enzymes, stimulating antioxidants and improving immunity play role against cancer effect (Thakore *et al.,* 2012). Therefore, plant extracts were widely studied, and the current study focuses on the role of pharmaceutical plants including stigma the a of saffron plant (Crocus Sativus

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Stigma) against the side effect of chemotherapy, which is one of the most frequently used in cancer treatment, the cytotoxic consequence of chemotherapy is not selective for cancer cells as it affects the normal tissues as well. The severity of damage is based on the type and duration of treatment (Minami *et al*.,2010;Liu ,2009).

Crocus Sativus

Crocus Sativus is the most expensive spice in the world. It contains the dried stigmas known as saffron. It belongs to the family Iridaceous, which is a perennial plant that is commonly cultivated around the world, especially in Iran (Sanchez-Vioque *et al* .,2012), its presence is limited to Eastern Europe and middle Asia, where it stretches from the Mediterranean Sea through Iran to India. The distribution of this plant over a large distance need human, as its flowers can't produce seeds to distribute naturally, thus, its growth rate is very slow (Schmidt *et al.,* 2007). Crocus Sativus is an ancient spice that is important in cooking, cosmetics, perfumes, the economy, and medicine (Dog , 2006).

Gemzar

Its traditional name is Gemcitabine Hydrochloride and the molecular weight is 299.66. The pharmaceutical description is 2',2'-Difluorodeoxycytidine monohydrochloride (β-isomer) and the chemical composition is C9H11F2N3O4.HCl. It is a white substance that is water soluble and slightly soluble in methanol, while it is insoluble in ethanol and organic polar solvents (Eli and Company ,2011), it is a drug that destroys cancer cells, and its structure is based on the structure of the nucleoside dCyd (Deoxycytidine), but it contains two fluorine atoms attached to the 2nd carbon atom of the deoxyribose sugar, thus, it is analogue of this nucleoside. it stimulate S phase and inhibit DNA synthesis. It used against many solid tumors including: Pancreatic, lung, ovarian, colon, bladder, and breast cancer (Chitkara *et al*., 2013 ; Martin *et al.,* 2013).

MATERIALS AND METHODS

Experimental animals

This experiment was accomplished at the animal laboratory in the College of Education/University of Al-Qadisiyah at standard temperature (22-28°C), aeration, and lighting duration (14 hrs of light and 10 hrs of darkness.), animals received and water free diet and water throughout the experiment.

Design of Experiment

The experimental animals were chosen randomly and grouped into five groups (six rats in each group), the total number (30) rats was divided as follows:

1-Negative control: they received Normal saline only and the animals were killed after four weeks of administration.

2- Positive control: They received Gemzar at a dosage of 15 ml/kg of body weight, and the animals were killed at the end of administration.

3- The third group: was received the plant extract two weeks before the drug at a dose of (100) mg/kg of body weight , then the drug was given for two weeks and the animals were killed at the end of administration.

4- The fourth group: They received both the plant extract (100) mg/kg body weight and the drug uptake at the same time for four weeks.

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5- The fifth group: They received the plant extract (100) mg/kg two weeks after drug uptake and for four weeks, the animals were autopsied and blood was extracted from them, then some antioxidants were assessed.

Preparation of aqueous extract of Crocus Sativus

The aqueous extract of Crocus sativus L. (stigma) was Prepared according to (Salami , 1998; Al-Mansour,1995) .

Preparation of the stock solution for the Gemzar drug

The solution was prepared by dissolving 1000 mg of chamomile powder (Eli Lilly and Company, 2003) in 333 ml of 0.9% NaCl and kept in the refrigerator (4°C) (Veltkamp *et al.*, 2008).

Study criteria

Determination of Malondialdehyde in Serum

The level of malondialdehyde in the serum was measured by a procedure modified by the researchers (Guidet & Shah, 1989).

Assessment of the catalase activity

The procedure of (Aebi, 1974) was used to measure the activity of catalase in serum. This method based on the degradation of hydrogen peroxide (H_2O_2) into two water molecules ($2H_2O$) and one Oxygen molecule (O_2).

Assessment of Serum albumin concentration

The kit of serum albumin assessment from Randox Company was used, and it followed the Company instruction (Rodkey, 1965).

statistical analysis:

SPSS V.25 was used for statistical analysis after data collection and tabulation. The data was statistically evaluated using the one-way ANOVA test, and the trial group averages were compared when the differences between them were significant Using the Least Significant Difference (LSD) test with a significance threshold of 0.05 (Daniel and Cross, 2018).

Results and discussion

Changes in Catalase, Albumin and Malondialdehyde Levels

The current study revealed a substantial increase ($p< 0.05$) in the MDA level of rats male treated with Gemzar at a concentration of 15 mg/kg in comparison with negative control, and this agreed with the study of (Tousson et al., 2014). The increase of the synthesis of free radicals and the decrease of the antioxidants, as a result of the increasing of lipid peroxidation in serum causing an increasing of the MDA level produced in the renal serum and renal tissue, as treatment with the chemotherapy shows an increase in the activity of synthesis of nitrite-independent calcium oxide (NOS) in the kidney and liver, which leads to the formation of improved nitrite oxide which react with free superoxide forming Peroxynitrite which is a more effective oxidizing agent which react with sulfhydryl residues in the cell membrane that induce lipid peroxidation or react with DNA causing cytotoxicity. These actions may increase MDA levels in rat serum and renal tissue (Srivastara *et al.*, 1996; Radi *et al.,* 1991). The findings also showed a statistically significant decrease ($p< 0.05$) in MDA level of male rats treated with the plant extract of saffron stigma at a concentration of (100) mg/kg of body weight (before, after) the drug uptake in comparison with the positive control. On the other hand, the treatment with the plant extract (before and after) the

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drug uptake showed a statistically significant increase in MDA levels ($p< 0.05$) in comparison to negative control. Whereas, the comparison among the groups that treated with the aqueous extract of saffron stigma showed a statistically significant increase ($p< 0.05$) in MDA levels of the aqueous extract group (after the drug) in comparison with the aqueous extract group (with the drug) (as showed in table-1), the increasing of MDA level in the aqueous extract group (after) the drug than the other groups may belong to the effect of the drug on the stimulation of Fatty-acyl-CoA enzyme and the oxidization of fatty acids, which increases the endogenous H_2O_2 production, thus contributes to the production of fat peroxidation (Basha and Sovers, 1996; Osumi and Hashimoto, 1978).

The current study also shows a statistically significant decrease ($p<0.05$) in catalase level for male rats treated with Gemzar in comparison with the negative control, this may resulted from the decrease of kidney to accomplish lipid peroxidation (Halliwell and Gutteridge, 2007) or because ROS free radicals removed by antioxidants such as CAT, GSH-PX. The decrease of these enzymes in the cellular structure of active mitochondria as a result of the toxic effects of chemotherapy in the renal convoluted tubules, which made it difficult to produce sufficient antioxidants to remove free radicals, thus, a reduction in level of these enzymes in serum (Ji et al., 2014; Zhao et al., 2014). In addition, the result shows a statically significant increase ($p<0.05$) of catalase level in male rats treated with a plant extract of saffron stigmas at a concentration (100) mg/kg of body weight (before, with and after) the drug uptake in comparison with the positive control. While the findings showed a statistically significant decrease ($p<0.05$) in catalase level for male rats treated with the plant extract of saffron stigmas at a concentration of (100) mg/kg of Body weight (before, with, and after) the drug in comparison to the negative control. When the aqueous extract groups compared with each other, a statistically considerable decrease ($p < 0.05$) in catalase levels in the aqueous extract group (after) the drug in comparison with the other groups. Additionally, a statistically considerable increase ($p < 0.05$) in the aqueous extract group (with the drug) in comparison with the group (before the drug) because the extract contains carotenoids, which have antioxidant properties against oxidative stress (Goli *et al.*, 2012; Karimi *et al.*, 2010). These compounds reduce lipid peroxidation and increase antioxidants to free radicals (Hosseinzadeh and Nassiri, 2013). In addition, phagocytes can also protect cells from antioxidant damage and improve the response of T and B lymphocyte proliferation (Bendich, 1989).

The present study revealed a statically considerable decrease in albumin level ($p<0.05$) for male rats treated with Gemzar in comparison with the negative group because of the changes in protein and free amino acid and their composition in the infected hepatocytes and the increasing in protein damage (EL-Maraghy *et al.* al.,2009). It is also due to the increase in oxidative stress by the drug, especially since albumin is an endogenous (non-enzymatic) antioxidant that play role in preventing the formation of free radicals by breakdown the chain of reactions and reforming the biomolecules damaged by oxidative stress and thus decrease its level in the blood (Halliwell and Gutteridge, 1999).

The effect of the drug causes damage to hepatocytes, which in turn affects the membranes permeability which causes seeping of amino acids, which are the building blocks of protein to the outside with a weakness in cellular ability to produce proteins, as well affects the re-absorption of proteins in the urinary tubules. (Ahn *et al.,* 2000; Silbergeld *et al.,* 2000).

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Additionally, this study revealed a statically substantial increase ($p < 0.05$) in the albumin level of male rats given the plant extract of saffron stigma at a concentration of (100 mg/kg) of body weight (before, with and after) the drug in comparison with the positive control. This might be belong to the fact that the aqueous extract containing vitamin E, which breaks down the chains of reactions that form the free radicals, prevent lipid peroxidation, and maintain the integrity of the membranes of hepatocytes, which enables it to carry out its essential functions, including proteins production (Upasani and Balaraman, 2001). That is because the aqueous extract contain flavonoids that act as antioxidants and protect against oxidative stress and remove the free radicals. This study agrees with the study of (Hassan *et al.*, 2000).

The results of this study also revealed a statically significant decrease ($p < 0.05$) in the albumin level of male rats treated with the plant extract of saffron stigmas at a concentration of 100 mg/kg of body weight (before and after) the drug uptake in comparison with the negative control. There were no significant differences in male rats treated with plant extract of saffron stigma at a dose of (100) mg/kg of body weight (with the drug) in comparison to negative control. Whereas, when comparing the groups of aqueous extract of saffron stigma among themselves, it showed a statically significant decrease ($p<0.05$) in the albumin level of aqueous extract group (after the drug) than the other groups .The outcomes are also showed a statically significant decrease ($p<0.05$) for the group (before the drug) in comparison with the group (with the drug). That may be due to the strength of the chemotherapy and its effect on hepatocytes and the occurrence of severe bleeding as well as the seeping of amino acids. (Table-1).

Table 1: The Influence of chamomile drug and plant extract on the level of catalase, lipid peroxidation and albumin of white male rats in three interactions.

Albumin (mg/dl)	Catalase (U/mL)	MDA (µmol/L)	Standards totals
3.65 ± 0.04a	1.02±0.35^{a}	0.04^{a}± 1.18	Negative control
1.98 ± 0.03b	0.28±0.03b	3.38 ± 0.03^{b}	Positive control
3.15 ± 0.02^{c}	0.80 ± 0.01^{c}	1.47 ± 0.02c	Aqueous extract before drug
3.54 ± 0.03^{a}	0.92 ± 0.02^{d}	1.25 ± 0.01^{a}	Aqueous extract with drug
2.45 ± 0.02^{d}	0.65 ± 0.03^{e}	2.37 ± 0.02^{d}	Aqueous extract after the drug
L.S.D= 0.122	L.S.D=0.079	L.S.D= 0.092	L.S.D

*Different letters indicate significant differences between the treatments ($P<0.05$) *Similar *letters indicate that there are no statistically significant differences between the treatments ($P<0.05$).

References

1. **Ahn, H., Hwang, K., Hong, S., Yang, D., Lee, B., Todd, A.(2000)**. Chronic lead nephropathy with excessive body lead burden. *J Occup Health*. 2:260–262.

Czech
Journal of Multidisciplinary Innovations
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Website: www.peerianjournal.com Email: editor@peerianjournal.com

2. **Al-Mansour, Nasser Abd Ali. (1999).** Evaluation of the efficiency of some plant extracts in affecting the hatching and mortality of mosquitoes *Culex quinquefasciatns* Say (Diptera: culicidae). Basra Journal of Agricultural Sciences 12(2).183-193.
3. **Aebi, H. (1974)** " Methods in Enzymatic Analysis Bergmeyer ".(ed). Verlay chem. Wrinheim ; 673.
4. **Agramonte, M. D. A. R.; Gonçalves, C. A. G. and. Siniscalco, D.(2019). Selected Papers from CUBANNI-2017"The Fourth International Workshop of Neuroimmunologi" published in behavioral Science. Pp:53-54.** https://doi.org/10.3390/books978-3-03897-488-8
5. **Basha, B. and Sovers, I. (1996).** Atherosclerosis: an update. *Am. Heart. J.*, 131, 1192-1202. DOI: 10.1016/s0002-8703(96)90096-4
6. **Bendich, A. (1989).** Carotenoids and the immune response. *J. Nutr.* 119: 112-115. DOI: 10.1093/jn/119.1.112
7. Cusano E, Consonni R, Petrakis EA, Astraka K, Cagliani LR, Polissiou MG.Integrated analytical methodology to investigate bioactive compounds inCrocus sativus L. flowers. Phytochem Anal. 2018;29(5):476-86
8. (PDF) Effect of Afghan Saffron (Crocus sativus L.) Aqueous Extract on Withdrawal Signs in Morphine-Dependent Rats. Available from: https://www.researchgate.net/publication/345251562_Effect_of_Afghan_Saffron_Crocus_sativus_L_Aqueous_Extract_on_Withdrawal_Signs_in_Morphine-Dependent_Rats [accessed Feb 19 2023].
9. **Chitkara, D. , Anupama, M. ,Stephan, W., Behrman ,N. K., and RamI,M.(2013)**. Self-Assembling,Amphiphilic Polymer- Gemcitabine Conjugate Shows Enhanced Antitumor Efficacy against Human Pancreatic Adenocarcinoma. *Bioconjugate chemistry*24(7):1161-73. DOI: 10.1021/bc400032x
10. **Daniel, W. W. and Cross, C. L. (2018).** Biostatistics: a foundation for analysis in the health sciences.*Wiley*.19-750.
11. **Dog, L.W.(2006)**. Areason to season: the therapeutic benefits of spices and culinary herbs . Diet and Nutrition 2(5),446-449. DOI: 10.1016/j.explore.2006.06.010
12. **EL-Maragy**, **S. A, Rizk, S.A. and EL-Sawalhi, M.M.(2009).** Hepatoprotective potential of crocin and curcumine against iron overload- iduced biochemical alterations in rat. *Arf. J. Biochem. Res.*3(5): 215-221.
13. **Goli, S. A. Mokhtari, F. and Rahimmalek, M.(2012).** Phenolic Compounds and Antioxidant Activity from Saffron (Crocus sativus L) Petal. *J Agr Sci* 4:175-181.
14. **Halliwell, B.; Gutteridge, J.M.C.** (1999). "Free Radical in Biology and Medicine".3rd edn.Oxford, Oxford University Press, pp.146-163, 399-430.
15. **Hassan, S. M.; Al-Kennany, E. R.; Al-Hafez, H. A. (2000).** Hydrogen peroxide-induced atherosclerosis in chicken effect of vitamin C. Iraqi J. Vet., 13, 249-270.
16. **Hosseinzadeh, H. and Nassiri, A. M.(2013).** Avicenna's (Ibn Sina) the Canon of Medicine and Saffron (Crocus sativus): A Review. *Phytother Res* 27:475-483. DOI: 10.1002/ptr.4784

Czech
Journal of Multidisciplinary Innovations
Volume 21, September, 2023. ISSN (E): 2788-0389
Website: www.peerianjournal.com Email: editor@peerianjournal.com

17. **Ji, J., Liu, J., Liu, H., Wang, Y. (2014).** Effects of fermented mushroom of cordyceps sinensis, rich in selenium, on uterine cervix cancer. *Evid Based Complement Alternat Med*.1(2):1-12. DOI: 10.1155/2014/173180
18. **Karimi, E., Oskoueian, E., Hendra, R. and Jaafar, H.Z.(2010).** Evaluation of Crocus Sativus L. Stigma phenolic and flavonoid compounds and its antioxidant activity .*Molecules* 15:6244- 6256. DOI: 10.3390/molecules15096244
19. **Kharb, M.,Jat, R.K. ,Gupta, A (2012).** A review on medical plants used As a source of anticancer agents. Int J Drug Res Technol 2:177-183.
20. **Liu, F.S.(2009).** Mechanisms of chemotherapeutic drug resistance in Cancer therapy – aquick review . Taiwan .J.Obst.Gyn., 48(3):239-244. DOI: 10.1016/S1028-4559(09)60296-5
21. **Martin-B**, **Lucia,E.D *et al*.(2013)**. Biocompatible –Based Nanomedicine Engineered by Flow Focusing Efficient Antitumor Activity . *International Journal of Pharmaceutics* 443(1-2):103-9. DOI: 10.1016/j.ijpharm.2012.12.048
22. **Minami, M., Mastsumoto, S., Horiuchi, H. (2010).** Cardiovascular side effects of Modern cancer therapy .Cric.J.74(9): 1779-1786. DOI: 10.1253/circj.cj-10-0632
23. **Osumi, J. and Hashimoto, T. (1978).** Acyl-CoA oxidase of rat liver: A new enzyme for fatty acid oxidation. *Biochem. Biophys. Res. Commun.* **83**, 479-485. DOI: 10.1016/0006-291x(78)91015-x
24. **Radi, R., Beckman, J. S., Bush, K. M. and Freeman, B. A. (1991)**. Peroxynitrite oxidation of sulfhydryls. The cytosolic potential of superoxide and nitric oxide. *J. Biol. Chem*. 266: 4244-4250. https://doi.org/10.1016/S0021-9258(20)64313-7
25. **Rodkey, F.L. (1965).** Directed spectrophotomeric determination of albumin human serum.Clin. Chem. 1: 478.
26. **Salami, Wajih Mazhar. (1998).** Effect of extracts of the plant *Convolvulus arvensis L.* and Hendale *lpomoae carrica (linn)* on the biological performance of wheat bug *Schizaphis graminu* PhD thesis, College of Science / University of Babylon 111 pages.
27. **Sanchez-Vioque, R., Rodrigues-Conde, M.F.,Reina-Urena, J.V., Escolano-Tercera, M. A.,Herraiz-Penalrer, D,Santana –Meridas, O** (2012). In vitro antioxidant and metal chelating Properties of corm , petal and leaf from saffron (Crocus sativus L.) Ind Crop Prod39:149- 153.
28. **Schmidt, M., Betti, G., Hensel, A. (2007):**Saffron in phytotherapy : pha- Rmacology and clinical use.Wien .Med.Wochenschr.,157:315- 319. DOI: 10.1007/s10354-007-0428-4
29. **Silbergeld E, Waalkes M, Rice J.(2000)**. Lead as a carcinogen:Experimental evidence and mechanisms of action. *Am J Indian Med*. 38:316- 323.DOI: 10.1002/1097-0274(200009)38:3<316::aid-ajim11>3.0.co;2-p
30. **Sudhakar, S., Seema, M., Rudra , D.T., Sanjay, D., Dharmendra, K.G.(1996).** Copper-induced oxidative stress and responses of antioxidants and phytochelatins in Hydrilla verticillata (L.f.) Royle. *Aquatic Toxicology*.80(4): 405-415. DOI: 10.1016/j.aquatox.2006.10.006
31. **Thakore, P., Mani, R.K., Singh, J., Kavitha, A.S.(2012)**. A brief review of plants Having anti-cancer property . *Int J Pharm Res Dev* 3:129-136.

Czech
Journal of Multidisciplinary Innovations
Volume 21, September, 2023. ISSN (E): 2788-0389
Website: www.peerianjournal.com Email: editor@peerianjournal.com

32. **Tousson, E., Zaki, T. Z., Walid, A. A., Hamada, H.(2014).** Methotrexate-induced Hepatic and Renal Toxicity: Role of L-carnitine in Treatment. *Biomedicine and Biotechnology, 2014, Vol. 2, No. 4, 85-92.*
33. **Upasani, C. and Balaraman, R.(2001).** Effect of vitamin E, vitamin C and spirulina on levels of membrane bound enzymes and lipids in some organs of rats exposed to lead. *Indian J Pharmacol.* 33:185-191.
34. **Veltkamp, S.A., Pluim, D., Van, T. O. Beijnen, J.H, Schellens, J.H.(2008)**. Extensive metabolism and hepatic accumulation of gemcitabine after multiple oral and intravenous administration in mice. *Drug Metab Dispos* .36:1606–15. DOI: 10.1124/dmd.108.021048
35. **Zhao, J.A. , Peng, L., Geng, C.Z. , *et al* .(2014).** Preventive effect of hydrazine curcumin on carcinogenesis of diethyl nitrosamine-induced hepatocarcinoma in male SD rats. *Asian Pac J Cancer Prev*, 15, 2115-21. DOI: 10.7314/apjcp.2014.15.5.2115