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Original Research

Effect of Saffron (*Crocus sativus* L.), as a Bioenhancer, on Pharmacokinetic of Acetaminophen: An Animal Study

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Abstract

In Traditional Persian Medicine (TPM) saffron is used as an accompaniment agent "*Mobadreq*" in polyherbal formulations. According to TPM texts, "*Mobadreq*" is a substance (or drug) which facilitates access of drugs or food to the whole body or specific organs. This study investigated the effect of oral co-administration of *Crocus sativus* L. (saffron) on the absorption and some pharmacokinetic parameters of acetaminophen in rats. Two groups of rats (n=6) were treated by 1: acetaminophen 10 mg/kg along with *Crocus sativus* 4 mg/kg (test group) and 2: 10 mg/kg acetaminophen (control). The plasma concentrations of acetaminophen after oral administration (at 0, 5, 10, 15, 20, 40, 60, 90, and 120 min) were monitored by an HPLC-UV method. Results indicated that the plasma concentration of acetaminophen in the test group was reached to the maximum concentration (C_{max}) faster than control group. As a result, at 5 to 40 minutes after drug gavage, the concentration of acetaminophen in both groups was significantly different. It was also found that co-administration of acetaminophen and saffron significantly increased acetaminophen's area under concentration curve (AUC0-60) in comparison to the acetaminophen alone ($p<0.025$). These results suggest that saffron could increase the absorption rate of acetaminophen. Consequently, saffron can be considered and introduced as an enhancer of absorption rate and efficacy of acetaminophen and other drugs at least by oral route and the drug interactions with this herb should be considered.

Keywords: Saffron; Acetaminophen; Pharmacokinetics; Traditional persian medicine

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Introduction

Two terms of drug delivery and drug targeting are used in modern pharmaceutical and researchers have introduced several methods in order to improve delivery and targeting of the drug [1]. Several studies have already been done to improve the bioavailability of drugs using herbs and their compounds as bioenhancer agents such as piperine, quercetin, genistein, naringin, sinomenine, and curcumin with various proposed mechanisms of actions [2,3].

The mechanisms of bioenhencers are reduction of hydrochloric acid secretion and increasing blood flow to the gastrointestinal tract, prevention of gastrointestinal transport, gastric emptying time and intestinal movements, changes in penetration of the epithelial cell membrane, cholagogue effect, thermogenic properties of bioenhancers and inhibition of the first metabolism, inhibition of the metabolizing enzymes and stimulation of the gamma glutamyl transpeptidase (GGT) enzyme activity [4].

In the view point of Traditional Persian Medicine (TPM), effectiveness, low side effects and acceptability of the drug are important factors in pharmacoterapy, that can be achieved through combination of drugs. In this regard, drug delivery and targeting with the aim of increasing efficiency of the drug has been one of the unique topics in TPM. For this purpose, medical scholars combined some excipients with the main drug to keep it from being digested, speed up the absorption and its distribution to an organ, sustaining its release, or targeting to a specified organ. In the review of TPM books, we encounter a word "*Mobadreq*" or convoy drug as an accompanier agents which are a group of modifiers for the effects of the main drugs. These agents have a good and rapid penetration to the entire body or a specific organ and can increase the speed of drug delivery to the site, thereby enhance the presence of drugs in particular organs [5,6].

Saffron (*Crocus sativus* L.) is a plant from the Iridaceae family, which has long been used as a medicinal plant, spice, color and food flavor. Also, it has been used as a convoy drug in TPM. Moreover, it has great anti-obstructive activity and effects on various diseases [7,8]. Saffron has beneficial effects in the treatment of neurological and psychiatric disorders such as Alzheimer's disease (AD) and depressive disorders [9], as well as treatment of metabolic syndrome and digestive conditions [10,11]. In addition, *Crocus sativus* exerts beneficial effects on age-related diseases such as cardiovascular, ocular and neurodegenerative diseases due to its anti-oxidant and anti-inflammatory properties [12]. The purpose of this study was to evaluate the effect of oral co-administration of saffron on the pharmacokinetic parameters of acetaminophen in an animal model.

Materials and Methods

Acetaminophen (Sigma-aldrich), Ketamine (Sigma-aldrich), xylazine (Sigma-aldrich), Methanol; HPLC grade (Merck) and Acetonitrile; HPLC grade (Merck) were prepared from related companies.

Preparation of Drugs

Saffron was purchased from Tehran herbal medicine market and was authenticated in the herbarium of faculty of pharmacy, Tehran University of Medical Sciences with the voucher number PMP-527.

Acetaminophen (10 mg/kg) was used as a base drug [13] as well as 4 mg/kg of saffron powder [5] were dispersed in water. The suspensions of both drugs were completely stirred before intake.

Animal study

Male Wistar rats weighing 245±10 g (8-10 weeks) were obtained from the Pasteur Institute, Iran. One week before the tests, the animals were kept in the animal house, to be acclimatized with animal house condition and were evaluated in terms of health. The animal house condition was as follows: temperature 24±1°C and cycle of 12 h of lighting and 12 h of darkness. Laboratory animal diets included compressed food and water for animals and animal consumption of food and water was not limited. This study was approved by the ethics committee of Tehran University of Medical Sciences (TUMS), Tehran, Iran (IR.TUMS.REC.1394.1263).

Animals were divided randomly into two groups (n=6); 1. Control group: Rats that received acetaminophen at a dose of 10 mg/kg (average 0.5 mL) by oral gavage, 2. Test group: Rats receiving acetaminophen suspension at a dose of 10 mg/kg (average 0.5 mL) and a solution containing saffron powder at a dose of 4 mg/kg (average 0.5 mL) simultaneously.

Sampling of blood and animal tissues

After gavage, the rats were anesthetized using ketamine + xylazine. Blood samples were taken from the heart of the animal at 0-5-10-15-20-40-60-90 and 120 minutes using insulin syringe (0.3 mL) and were transferred to heparinized tubes. After blood sampling in 120 minutes, the animal was sacrificed and the tissue samples (liver, kidney, spleen, lung, heart and brain) were separated. The plasma samples taken after centrifugation of blood samples along with tissue samples stored at -80 °C until further analysis.

HPLC analysis

Analyses of samples were conducted by High Performance Liquid Chromatography (HPLC) by a 600E Waters system (Waters, MA, USA) with an isocratic pump and an ultraviolet detector, using a C18 ODS3, 5 µm, 125 cm x 4.0 mm, MZ, Perfectsil Target at room

temperature. The mobile phase was phosphate buffer (0.02 M, pH 2.2) solution: acetonitrile (93:7 v/v) (isocratic). Injection volume, flow rate and λ absorbance were 100 µL, 1 mL/min and 254 nm, respectively. Detection and quantitative analysis of Acetaminophen was performed by comparison of the elution time and the integration of absorption peak area. The peak of acetaminophen was appeared at 3.3 minutes.

Calibration curve of acetaminophen

To prepare different concentrations of acetaminophen, 5 mL of acetaminophen solution at a concentration of 1000 µg/mL was taken and mixed with 5 mL of the plasma without the drug and completely mixed. Then different dilutions of 100, 50, 25, 10, 5, 2.5, 1 µg/mL were prepared from the stock solution. Analysis of acetaminophen concentration by HPLC was performed based on the previous reported methods [14]. In order to ensure reproducibility of results each sample was injected three times.

Plasma analysis

Preparation and analysis of samples and determination of acetaminophen concentration by HPLC were performed based on the previous reported methods [14]. Drug extraction from plasma samples were as follows: 100 µL of plasma was completely mixed with 250 µL of methanol and centrifuged at 10,000 rpm for 10 minutes. Then the supernatant was filtered through the 0.45 µm filter to be ready for HPLC analysis.

Preparation of tissue samples

To prepare the sample from animal tissues, each frozen tissue (liver, kidney, heart, spleen, lung and brain) was transferred to the appropriate tube. Then, as much as twice the weight of each tissue, normal saline was added and homogenised. The samples were then centrifuged at 10,000 rpm for 10 minutes. 100 mL of the supernatant was transferred to micro-tubes and mixed with 250 mL of methanol and stirred for 10 minutes. After centrifugation of the samples, the supernatant was used for injection into HPLC apparatus.

Table 1. Peak area of acetaminophen concentrations (µg/mL) spiked in the plasma (n=3)

Concentration (µg/mL)	1	2.5	5	10	25	50	100
1	42.86	78.96	172.11	258.73	588.46	1064.92	2370.84
2	58.41	70.63	163.44	285.49	521.22	1084.2	2318.42
3	50.12	90	143.88	248.36	576.26	1067.37	2209.62
average	50.63	79.86	159.81	264.19	561.98	1072.16	2299.63
SD	7.77	9.71	14.46	19.16	35.82	10.5	82.23
%CV	15.42	12.17	9.05	8.22	6.37	0.98	3.58

Statistical Analysis

The AUCs in test and control group were compared by unequal t-test by EXCEL2013.

Results

Calibration curve of acetaminophen spiked in the plasma

The results of peak area of acetaminophen spiked in the plasma with concentrations of 1-100 µg/mL are shown in table1. Injection of samples was performed at least three times. The line equation of the peak area against the concentration of acetaminophen was obtained as follows: $y= 22.412x+21.644$, $R^2=0.9981$. Chromatograms of acetaminophen concentrations (1-100 µg/mL) spiked in plasma were shown in figure 1.

Analysis of acetaminophen data

Analysis of samples, 5-120 min after receiving acetaminophen (10 mg/kg) alone and acetaminophen (10 mg/kg) with saffron (4 mg/kg) are shown in figures 2-4.

There was a significant difference between the plasma concentration of acetaminophen in the two groups 5, 10, 15, 20 and 40 minutes after drug gavage ($p<0.007$), ($p<0.01$), ($p<0.002$), ($p<0.03$), and ($p<0.009$), respectively. There was no significant difference in the plasma concentration of acetaminophen between the two groups, from 60 to 120 minutes after receiving the drug.

Table 2 shows the difference in AUC, p values, at different times after oral administration of drugs. AUC analysis at 0 to 60 minutes after drugs administration showed a significant difference between the two groups. AUC analysis at 60-90 and 90 minutes to 120 minutes after drugs administration showed no significant difference between the two groups. Analysis of AUC0-60 (µg*min/mL) in the two groups showed significant difference ($p<0.025$) but there was no significant differences in AUC between 0-90 and 0-120 aminutes after administration.

Acetaminophen concentrations in animal organs

Liver, kidney, heart, spleen, lung and brain tissues

were prepared for analysis using HPLC system 120 minutes after oral administration of drugs, in order to determine the tissue distribution of the drug. In analyzing of tissue samples from both groups, peak of acetaminophen was not detectable due to the low concentration of the drug in the tissues.

Discussion

The main purpose in pharmacy research is improving the effectiveness and reducing side effects of drugs by delivering the drug at the right time with the right amount to the right target site. For this reason a variety of drug delivery strategies have been developed [15]. In TPM some herbal drugs such as *Crocus sativus* (saffron), called as "*Mobadreq*", are accompanied with the main drugs to improve their efficacy. Many

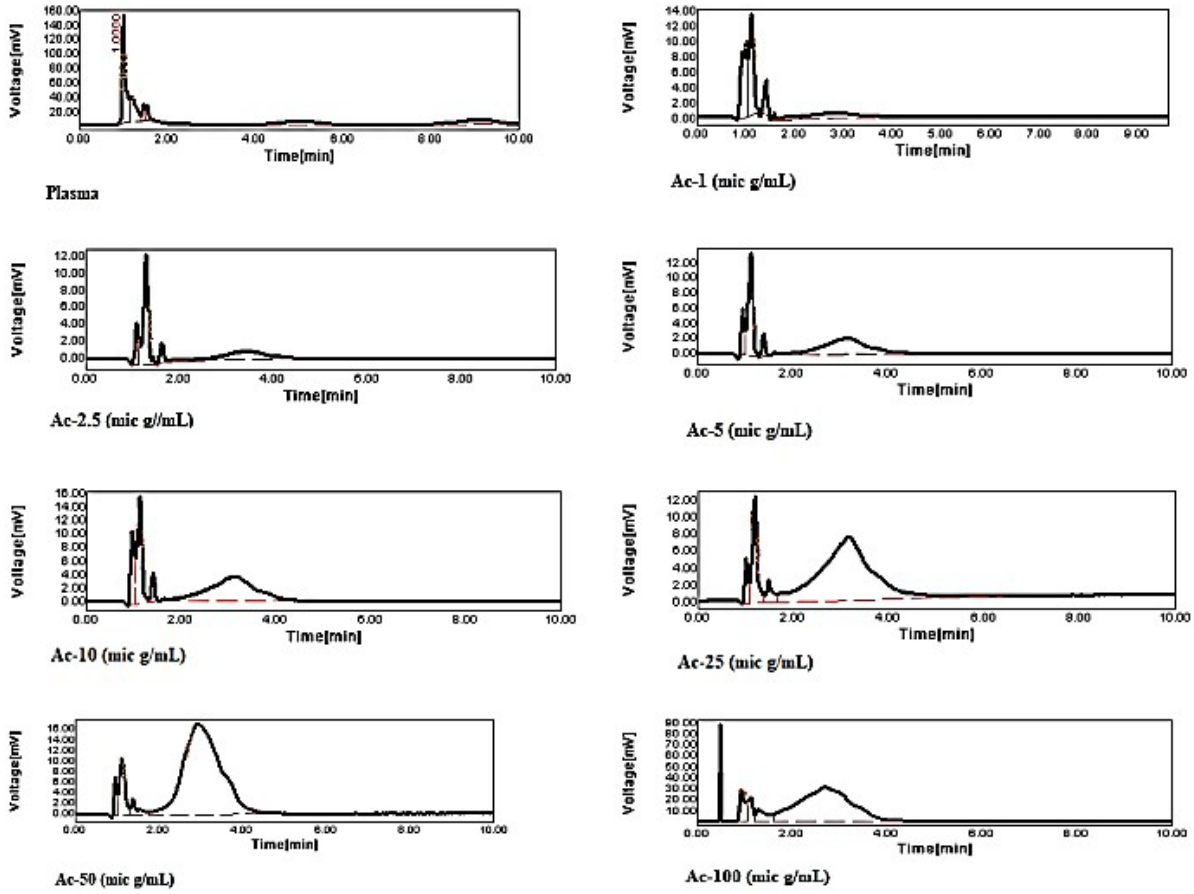

Figure 1. Chromatograms of acetaminophen concentrations (1-100 µg/mL, Retention time 3.3 min) spiked in plasma.

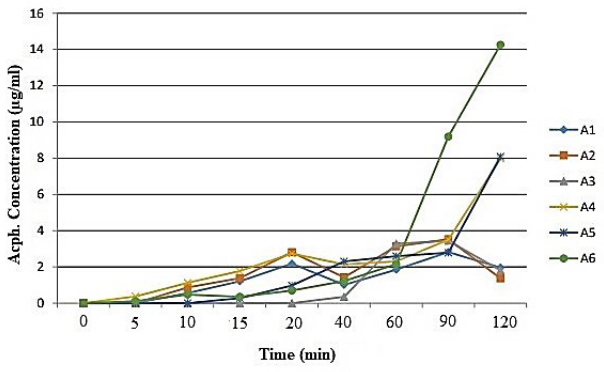

Figure 2. plasma concentrations of acetaminophen (Acph) (µg/mL), 5-120 min after receiving acetaminophen (10 mg/kg), A1-6.

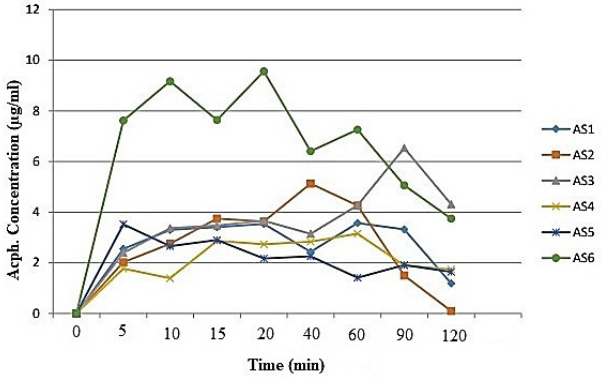

Figure 3. Plasma concentrations of acetaminophen (Acph) (µg/mL), 5-120 min after receiving acetaminophen (10 mg/kg)+saffron (4 mg/kg), AS1-6.

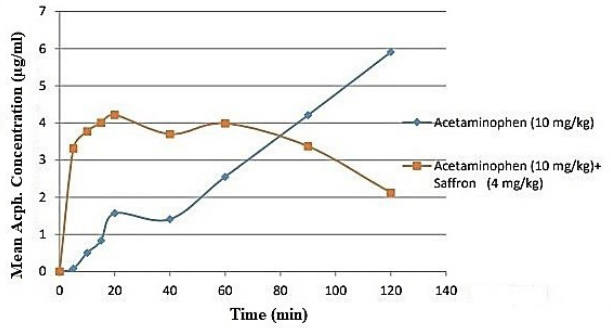

Figure 4. Mean acetaminophen (Acph) concentration, 5-120 min after receiving Acph (10 mg/kg) and Acph (10 mg/kg)+saffron (4 mg/kg), n=6.

group was 414.7 ± 194 µg*min/mL, which is not statistically significant, that may be due to small sample size of the study. In future studies, longer sampling times, e.g. till 240 minutes is suggested.

The results of previous studies have shown that gastric emptying is one of the effective factors in increasing the amount of acetaminophen concentration in the blood. In cases where gastric emptying speed is faster, peak concentration is higher while stomach emptying time is shorter [20]. According to the results of this study, the rate of acetaminophen absorption in the test group was higher than control group, so it may be due to increasing the speed of gastric emptying by saffron.

Table2. Analysis of AUC at different times (min) after oral administration of Acetaminophen (Acph) alone or with saffron (Saf). ($p<0.05$)=*

AUC	Acph (µg*min/mL)	Acph+Saf (µg*min/mL)	P value	
0-5	0.2	8.3	p<0.015	*
5-10	1.5	17.7	p<0.022	*
10-15	3.3	19.4	p<0.015	*
15-20	6.0	20.6	p<0.022	*
20-40	29.7	79.2	p<0.036	*
40-60	39.6	76.9	p<0.043	*
60-90	101.4	110.4	p<0.738	
90-120	151.8	82.4	p<0.201	
0-60	80.3	220.0	p<0.025	*
0-90	181.8	332.4	p<0.063	
0-120	333.6	414.8	p<0.424	

of the traditional uses of saffron have been studied and confirmed by modern investigations but according to our knowledge its potential effect on bioavailability and absorption enhancement of other drugs have not been considered yet [2,8].

Acetaminophen (N-acetyl-p-aminophenol, APAP), is an analgesic and antipyretic drug with high oral bioavailability (88%). The time to reach the maximum concentration in the blood is 90 minutes after its consumption [16]. APAP is not extensively bound to plasma proteins and has a plasma half-life of 1.5-2.5 hours at therapeutic doses. Moreover, APAP is mainly converted to the inactive glucuronide (APAP-gluc, 52-57% of urinary metabolites) and sulfate (APAP sulfate, 30-44%) conjugates by the liver, kidney, and intestine, while a minor amount of APAP is being oxidized to a reactive metabolite NAPQI (5–10%) [17,18].

The results of the present study revealed that acetaminophen concentration curve has two peaks (Figure 4). First peak was observed in both groups at 20 minutes, and the second peak appeared in the group receiving acetaminophen with saffron (test group) at 60 minutes after gavage. In the acetaminophen group alone, the concentration curve of acetaminophen in plasma was still increasing up to 120 minutes. Enterohepatic circulation is one of the reasons for the dual peak phenomenon. Animal studies indicated a dose-dependent increase in bile excretion of acetaminophen. Biliary secretion of glucuronide metabolite which is hydrolyzed to primary drug by intestinal flora and reabsorption is the reason of this fact [19].

Plasma concentration of acetaminophen in the test group was reached to the maximum concentration (C_{max}) faster and begun to decrease after 60 minutes of consumption, while in control group, acetaminophen concentration was slowly increased and concentration continued to rise until 120 minutes. At 5 to 40 minutes after drug gavage, the concentration of acetaminophen in both groups was significantly different. However, there was no significant difference in plasma concentrations of acetaminophen in two groups at 60 to 120 minutes.

According to the results, the area under the plasma concentration curves (AUC) of acetaminophen were significantly different in the two groups at 0-5, 5-10, 10-15, 15-20, 20-40, 40-60 minutes, and the AUC of acetaminophen was more in test group. AUC0-120 for control group was 333.5 ± 136 µg*min/mL and in test

In the TPM texts Saffron has hot and dry nature, so it can enhance stomach muscle strength [21]. Saffron has been noted as astringent (qabez) agent and gastric tonic in TPM literatures [8], therefore, it can accelerate the gastric emptying. Consequently, one of the reasons for increasing the rate of absorption of acetaminophen and also possibly other oral medications can be attributed to gastric emptying property of saffron, which requires further studies. In the other words, saffron can be used for drugs that need to have a higher and faster initial concentration. This idea, is in agreement with the conclusion reported by Almodo´ var et al.. According to the in vitro and clinical studies with a galenic preparation of saffron extract, they recommended that adding saffron can be useful for fast-acting formulations [22].

On the other hand, in TPM drugs functions are interrelated to each other. Saffron has been mentioned as a strong "*Mofatteh*" [23]. "*Mofatteh*" or anti-obstructive/opener drug is a drug which alters rheological properties of the obstructing material to eliminate it from the body. These types of drugs dilute the thick material and degrade the viscous material into small pieces [24]. Therefore, other drugs can pass easier through vessels and ducts by removing the accumulated materials. Most of the convoy drugs have this function which may be effective in improving bioavailability of their co-administered drug [6]. Direct anti-atherosclerotic effects of *Crocus sativus* have been reported by Christodoulou et al.. Saffron aqueous extract administration dose dependently reduces degree of arterial lumen stenosis and enhances plaque stability in $Apo^{E-/-}$ mice [25]. Atherosclerosis can be considered as a vessel obstruction which prevents blood from reaching organs [24]. Considering the properties of saffron and its bioactive compounds, further experiments can be designed to clarify various aspects of its potential absorption enhancer effect.

Conclusion

According to the results, the plasma concentration of acetaminophen in the simultaneous receiving of saffron was reached faster to maximum concentration. This increase in absorption speed can be related to the function of saffron in strengthening the stomach muscles and increasing the speed of its emptying. Therefore, we can conclude that, saffron can be used with drugs that need to pass faster from the stomach and be absorbed more rapidly, which requires further studies.

Conflicts of Interests

The authors declare that there is no conflict of interest.

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References

[1] Kshirsagar NA. Drug Delivery Systems. Indian J Pharmacol. 2000; 32: 54-61.

[2] Peterson B, Weyers M, Steenekamp JH, Steyn JD, Gouws C, et al. Drug bioavailability enhancing agents of natural origin (bioenhancers) that modulate drug membrane permeation and pre-systemic metabolism. Pharmaceutics 2019;11:33.

[3] Tatiraju DV, Bagade VB, Karambelkar PJ, Jadhav VM, Kadam V. Natural bioenhancers: an overview. J Pharmacogn Phytochem 2013;2:55-60

[4] Ajazuddin, Alexander A, Qureshi A, Kumari L, Vaishnav P, et al. Role of herbal bioactives as a potential bioavailability enhancer for Active Pharmaceutical Ingredients. Fitoterapia 2014; 97:1–14.

[5] Ebadi N, Masoomi F, Yakhchali M, Sadati Lamardi SN, Shams-Ardakani MR, et al. Convoy drugs in Traditional Persian Medicine: The historical concepts of bioavailability and targeting. Trad Integr Med 2016;1:18-27.

[6] Sadati Lamardi SN, Shams Ardekani MR, Ebadi N, Yakhchali M, Raees Dana A, et al. Review of scientific evidence of medicinal convoy plants in traditional Persian medicine. Pharmacogn Rev 2016;10:33-38.

[7] Hosseinzadeh H, Nassiri-Asl M. Avicenna's (Ibn Sina) the canon of medicine and saffron (crocus sativus): a review. Phytother Res 2013;27:475-483.

[8] Javadi B, Sahebkar A, Emami SA. A survey on saffron in major islamic traditional medicine books. Iran J Basic Med Sci 2013;16:1-11.

[9] Omidkhoda SF, Hosseinzadeh H. Saffron and its active ingredients against human disorders: A literature review on existing clinical evidence. Iran J Basic Med Sci 2022;25:913-933.

[10] Razavi BM, Hosseinzadeh H. Saffron: a promising natural medicine in the treatment of metabolic syndrome. J Sci Food Agric 2017;97:1679-1685.

[11] Rezaee Khorasany AR, Hosseinzadeh H. Therapeutic effects of saffron (Crocus sativus L.) in digestive disorders: a review. Iran J Basic Med Sci 2016;19:455-469.

[12] Ghaffari S, Roshanravan N. Saffron; An updated review on biological properties with special focus on cardiovascular effects. Biomed Pharmacother 2019;109:21-27.

[13] Neirinckx E, Vervaet C, De Boever S, Remon JP, Gommeren K, et al. Species comparison of oral bioavailability, first-pass metabolism and pharmacokinetics of acetaminophen. Res Vet Sci 2010;89:113-119.

[14] Tripathi YB, Tiwari OP, Nagwani S, Mishra B. Pharmacokinetic-interaction of Vitex negundo Linn. & paracetamol. Indian J Med Res 2009;130:479-483.

[15] Laffleur F, KeckeisV. Advances in drug delivery systems: Work in progress still needed? Int J Pharm 2020;590:119912.

[16] Mazaleuskaya LL, Sangkuhl K, Thorn CF, FitzGerald GA, Altman RB, et al. PharmGKB summary: pathways of acetaminophen metabolism at the therapeutic versus toxic doses. Pharmacogenet Genomics 2015;25:416-426.

[17] McGill MR, Jaeschke H. Metabolism and disposition of acetaminophen: recent advances in relation to hepatotoxicity and diagnosis. Pharm Res 2013;30:2174-2187.

[18] Bessems JG, Vermeulen NP. Paracetamol (acetaminophen)-induced toxicity: molecular and biochemical mechanisms, analogues and protective approaches. Crit Rev Toxicol 2001;31:55-138.

[19] Hendrickson RG, McKeown NJ, West PL, Burke CR. Bactrian ("Double Hump") acetaminophen pharmacokinetics: a case series and review of the literature. J Med Toxicol 2010;6:337-344.

[20] Heading RC, Nimmo J, Prescott LF, Tothill P. The dependence of paracetamol absorption on the rate of gastric emptying. Br J Pharmac 1973;47:415-421.

[21] Mousavi SZ, Bathaie SZ. Historical uses of saffron: Identifying potential new avenues for modern research. Avicenna J Phytomed 2011;1:57-66

[22] Almodo´ var P, Briskey D, Rao A, Prodanov M, Inarejos-Garcı´a AM. Bioaccessibility and Pharmacokinetics of a Commercial Saffron (Crocus sativus L.) Extract. Evid Based Complement Alternat Med 2020;1575730.

[23] Avicenna H. Al-Qanun fi al Tibb [The Canon of medicine]. Vol 1. Introduction and Scientific critical by Masoudi [In Arabic]. Al-Mai publications. Tehran 2013; p 85.

[24] Yakhchali M, Alizadeh Vaghasloo M, Mirabzadeh Ardakani M, Vazirian M, Sadrai S. Targeting Atherosclerotic Plaque in Avicenna's View. J Integr Cardiol. 2020;3.

[25] Christodoulou E, Kadoglou NPE, Stasinopoulou M, Konstandi OA, Kenoutis C, et al. Crocus sativus L. aqueous extract reduces atherogenesis, increases atherosclerotic plaque stability and improves glucose control in diabetic atherosclerotic animals. Atherosclerosis 2018;268:207-214.