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Phoenix dactylifera (Ajwa Date) Whole Fruit, Flesh and Powdered Seed Prevents Anti-Tuberculous Drug Induced Hepatotoxicity in Rabbits

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ABSTRACT

Introduction: Hepatotoxicity induced by anti-tuberculous medicine is known due to their oxidative stress. Ajwa dates may have a role to protect liver from oxidative stress
Aims & Objectives: To assess the preventive effect of Ajwa date on hepatotoxicity induced by anti-tuberculous drugs in rabbits.
Place and duration of study: Post Graduate Medical Institute, Lahore for three months, from May 2014 to July 2014.
Material & Methods: Thirty rabbits were distributed into five groups. Rabbits of Group A and of B were fed on normal diet in form of pellets. Group C, D and E were provided diet containing one whole Ajwa date, flesh of one Ajwa date and powdered seed of one Ajwa date respectively in each 100 grams of diet throughout study. Group B, C, D and E were administered 50mg/kg isoniazid and 100mg/kg rifampicin orally for 14 days. Serum levels of liver enzymes Alanine transaminase (ALT), Aspartate transaminase (AST) and Alkaline phosphatase (ALP) and bilirubin were estimated on day 0 and 14. One way ANOVA followed by post hoc Tukey's test and t-test were applied for statistical analysis using SPSS 20.
Results: Baseline LFTs were normal in all groups. Significant hepatotoxicity was observed after 2weeks of INH and rifampicin administration in disease control group B (ALT 200.2±19.3 & ALP 231.0±21.3 IU/L, AST 139.0±22 & bilirubin 0.48±0.046mg/dl, (p value < 0.001) as compared to healthy control group A (ALT47.2 ± 6.7 & ALP78.2 ±5.0 IU/L, AST 43.0 ± 9.7, bilirubin 0.10± 0.00mg/dl). (p value < 0.001). Concomitant Ajwa intake during the same period resulted in an equipotent significantly similar improvement in LFTs in Groups C (whole date) ALT55.7 ± 4.7&ALP 91.5 ±5.0IU/L, AST, 59.0 ± 15.3 &bilirubin 0.09 ±0.02 mg/dl): D (flesh) ALT89.8 ± 6.3 & ALP111.3 ±9.4 IU/L, AST73.7 ± 8.3 & bilirubin0.12± 0.04 mg/dl & E (seed powder) ALT85.8 ± 8.6 IU/L &ALP 92.8 ±11.4 IU/L, AST57.5 ± 5.3 & bilirubin 0.12 ±0.04 mg/dl) versus group B (p value < 0.001). and near normalization of liver function close to that of healthy control group
Conclusion: Co-administration of Ajwa date whole fruit, flesh and seed powder are equipotent and effective in preventing isoniazid and rifampicin induced hepatotoxicity

Key words: Ajwa date, Hepatoprotective, Rifampicin, Isoniazid.

INTRODUCTION

Tuberculosis has remained the prime culprit behind mortality among people owing to a single infectious agent,[1] with over 10 million people getting infected along with 1.5 million deaths occurring in 2018.[2] In 2019, Pakistan alone contributed 5.7% of cases to the global burden of TB.[3] For the treatment of drug susceptible pulmonary tuberculosis, isoniazid (INH), rifampicin (RIF), pyrazinamide (PZA) and ethambutol (EMB) are prescribed during the intensive phase while isoniazid and rifampicin are continued into the continuation phase of anti-tubercular treatment (ATT) for 8 weeks and 18 weeks, respectively.[4]
Liver is the prime organ involved in metabolism, activation and detoxification of almost all compounds reaching it through circulation. Many natural and synthetic compounds metabolised by liver may produce acute and chronic heterogeneous responses, the responses being outlined as drug-induced hepatotoxicity or drug-induced liver injury (DILI),[5] where DILI being strictly defined by AST levels greater than 5 times the upper normal limit.[6] The significance of DILI in respect of treatment is that of it being the sole adverse indication for halting an ongoing treatment or withdrawal of a drug from consumer market. For this reason,

prevention of some diseases, including tuberculosis, has been problematic.[7] Anti-tubercular drugs i.e., isoniazid and rifampicin, along with pyrazinamide, are known to have adverse side effects including drug-induced hepatotoxicity.[8] It has been demonstrated that a prolonged isoniazid and rifampicin combination therapy can induce hepatic stellate cells and fibrotic changes through oxidative stress.[9]

To ensure the progress of treatment avoiding hepatotoxicity, reduction in drug-induced oxidative stress can prove beneficial. A study illustrated how administration of antioxidants like Vit E and Vit C can reduce the levels of ATT induced oxidative stress by elevating levels of anti-oxidant enzymes.[10]

Phoenix dactylifera is a flowering plant belonging to palm family, *Arecaceae*. The fruit of which is an edible date, Ajwa being the best variety. Ajwa date has been used for its several benefits which include hepatoprotective,[11] nephroprotective,[12] anti-cancer[13] and antioxidant and anti-inflammatory effects[14] among many others. Ajwa date has shown to possess bioactive compounds that halt lipid peroxidation and inhibit COX1 and COX2.[14]. Our literature review has given us insight into hepatoprotective effects of ADE. We aim to explore this effect against anti-tuberculous drugs in a rabbit model using natural product instead of extract.

MATERIAL AND METHODS

This randomized control study was conducted at Post Graduate Medical Institute (PGMI), Lahore after approval of the Institutional Ethical Committee for Basic Sciences from May 2014 to July 2014. Sample size of 6 rabbits in each group was calculated using 90% power of study and 5% level of significance.[15] Adult healthy male rabbits, 30 in number were selected according to inclusion criteria of 1.2 to 1.5 Kg. They were bought from the local market kept in cages for 15 days for acclimatization and randomly divided into five groups (A, B, C, D and E).

One group (A) was administered distilled water in a volume equivalent to drug volume administered to the rest of the four groups. The other four groups (B, C, D and E) were administered isoniazid 50 mg/kg (SchazooZaka Pharmaceuticals, Pakistan Ltd) and rifampicin 100 mg/kg (SchazooZaka Pharmaceuticals, Pakistan Ltd).[16] Both drugs were dissolved in distilled water and kept in dark bottles at 4°C. They were administered as a single daily dose in the morning for a period of fourteen days.

Group A and B rabbits were given a preparation of diet pellets constituted of split chickpeas 500g, dry fodder 500g and jawar 500g. Plain flour 300g was used with water to bind all these ingredients in the form of pellets, which were then air dried and refrigerated. Diets for group C, D and E were prepared by mixing flesh and seed powder (group C), flesh only (group D) and seed powder only (group E) of one Ajwa date in 100gm of above-mentioned diet. Diet pellets and tap water was provided to all groups *ad libitum*.

Blood samples of about 2 ml were collected from the marginal ear vein while the animal was kept in a restraint cage, by using a 3 ml disposable (BD, Pakistan) syringe with a 23-gauge needle. Samples were collected on day 0 and 14. Blood was collected and put in a clot activator vacutainer (Biovac, Pakistan). After half an hour it was centrifuged at 2500 revolutions per minute for 10 minutes and serum was separated and stored at -20°C.

Serum samples were analyzed for estimation of alanine aminotransferase (AST), aspartate aminotransferase (ALT), alkaline phosphatase (ALP) and bilirubin. On day of estimation, stored samples were thawed to room temperature and kept in incubator (DIN-12880-K1, Germany) for 5 to 10 minutes at 37°C. Serum alanine aminotransferase (AST), aspartate aminotransferase (ALT) and alkaline phosphatase were estimated using diagnostic kits (DiaSys, Germany) and serum bilirubin was estimated using diagnostic kit (Human, Germany) using spectrophotometer (SLIM by SEAC, Italy). Significant rise above baseline and normal control (p value <0.05) was considered induction of hepatotoxicity.

Statistical analysis:

Data was analyzed by SPSS 20. The variables were expressed as mean ± SD after confirmation of normal distribution at baseline. One way ANOVA followed by post hoc Tukey's test was applied for comparison among groups of rabbits. Paired sample t test was used for comparison between day 0 and 14 in each group. P value<0.05 being considered significant.

RESULTS

Thirty adult healthy male rabbits weighing 1200 - 1500 grams were used in this study. They were divided into five groups (A, B, C, D and E). Each group contained 6 animals. Hepatotoxicity was induced by isoniazid and rifampicin. They were followed for 14 days with no mortality in any group. Body weight was measured weekly to adjust the dose of the drugs. Effect of Ajwa date on serum ALT, AST, ALP and bilirubin levels is presented as figure 1,2 3, and 4 respectively. Table 1 shows mean

difference in values between day 0 and 14 with p-value of all the groups. At baseline values were remarkably similar in all groups. After 14 days values were very significantly higher in ATT group (group B) as compared to all other groups.

Difference among the treatment groups themselves as well as with the normal control group was non-significant.

When we compared the rise in ALT level at the end of the study from baseline it was significant in groups B, D and E. The rise was four times higher in group B and less than twice to the baseline in groups D and E. When we compared the rise in AST and ALP levels at the end of the study from baseline, they were significant in groups B, C, D and E. The rise was three times higher in group B and less than one and half times in group C, D and E and numerically lowest in group C receiving whole Ajwa date. When we compared the rise in serum bilirubin levels at the end of the study from baseline it was significant in group B only.

In group A (Normal Control) the percentage increase in serum ALT was 0.4%, serum AST was 1.2%, serum ALP was 0.4% and serum bilirubin was 0%. In group B (Anti-tuberculous drugs) the percentage increase in serum ALT was 295%, serum AST was 217%, serum ALP was 184.4% and serum bilirubin was 380%. In group C (ATT + Whole Ajwa Date) the percentage increase in serum ALT was 10.3%, serum AST was 23.7%, serum ALP was 14% and serum bilirubin was 25%. In group D (ATT + Ajwa Date Flesh) the percentage increase in serum ALT was 87%, serum AST was 47.4%, serum ALP was 35% and serum bilirubin was 0%. In group E (ATT + Ajwa Date Seed Powder) the percentage increase in serum ALT was 75%, serum AST was 39.6%, serum ALP was 22.1% and serum bilirubin was 7.6%.

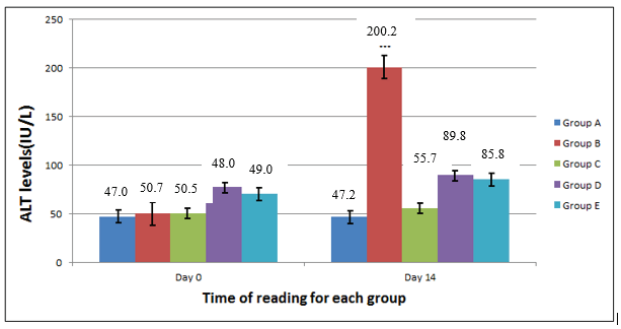

Fig-1: Effect of Ajwa date on ALT (mean ± SD) of ATT induced hepatotoxic rabbits (n=6)

Group A: Normal Control, Group B: Anti-tuberculous drugs (ATT), Group C: ATT + Whole Ajwa Date, Group D: ATT + Ajwa Date Flesh, Group E: ATT + Ajwa Date Seed Powder

***p value < 0.001 vs All other groups

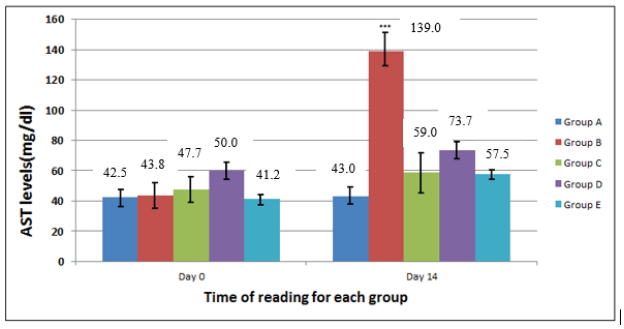

Fig-2: Effect of Ajwa date on AST (mean ± SD) of ATT induced hepatotoxic rabbits (n=6)

Group A: Normal Control, Group B: Anti-tuberculous drugs (ATT), Group C: ATT + Whole Ajwa Date, Group D: ATT + Ajwa Date Flesh, Group E: ATT + Ajwa Date Seed Powder

***p value < 0.001 vs All other groups

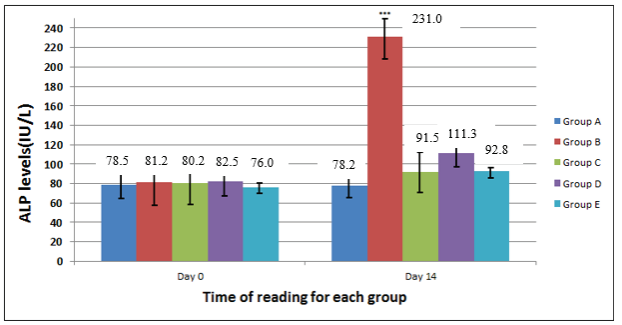

Fig-3: Effect of Ajwa date on ALP (mean ± SD) of ATT induced hepatotoxic rabbits (n=6)

Group A: Normal Control, Group B: Anti-tuberculous drugs (ATT), Group C: ATT + Whole Ajwa Date, Group D: ATT + Ajwa Date Flesh, Group E: ATT + Ajwa Date Seed Powder

***p value < 0.001 vs All other groups

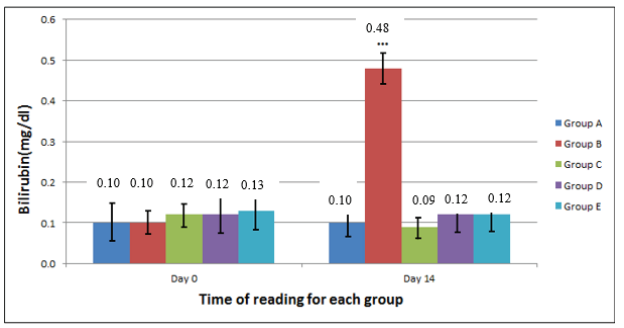

Fig-4: Effect of Ajwa date on serum bilirubin level (mean ± SD) of ATT induced hepatotoxic rabbits (n=6)

Group A: Normal Control, Group B: Anti-tuberculous drugs (ATT), Group C: ATT + Whole Ajwa Date, Group D: ATT + Ajwa Date Flesh, Group E: ATT + Ajwa Date Seed Powder

***p value < 0.001 vs All other groups

Groups	Serum ALT		Serum AST		Serum ALP		Serum bilirubin	
	Difference (mean ± SD)	P value (t- test)	Difference (mean ± SD)	P value (t- test)	Difference (mean ± SD)	P value (t- test)	Difference (mean±SD)	P value (t- test)
Group A	0.2 ± 0.7	0.611	0.5 ± 0.8	<0.203	0.3±0.2	0.363	0.00	1.000
Group B	149.5 ± 20.7	0.001	95.2 ± 18.7	< 0.001	149.8±25.5	< 0.001	0.38±0.02	< 0.001
Group C	5.2 ± 5.8	0.083	11.3 ± 3.4	< 0.001	11.3±3.5	0.003	0.03±0.00	0.203
Group D	41.8 ± 4.9	0.002	23.7 ± 6.1	<0.003	28.8±4.8	0.002	0.00	1.000
Group E	36.8 ± 6.3	0.002	16.3 ± 4.4	<0.001	16.8±3.8	0.010	0.01	0.363

Table-1: Mean ± SD of difference in Serum ALT, AST, ALP (IU/L) and bilirubin (mg/dl) level between day 0 and day 14 (n=6) Group A: Normal Control, Group B: Anti-tuberculous drugs (ATT), Group C: ATT + Whole Ajwa Date, Group D: ATT + Ajwa Date Flesh, Group E: ATT + Ajwa Date Seed Powder

DISCUSSION

Anti-tubercular therapy is notorious for its hepatotoxic side effects. Hepatotoxic effects remain the main reason for cessation of ATT and many complications that arise in result. It is known that anti-tubercular drugs like rifampicin and isoniazid damage the functional and structural integrity of liver by inducing oxidative stress, as indicated by increased levels of AST, ALT, ALP and bilirubin. A study suggested that rifampicin causes liver injury by inducing oxidative stress, elevated toxic metabolites caused by CYP induction and upregulation of PPAR gamma pathway.[17] Studies have also shown that metabolism of rifampicin and isoniazid dangerously deplete the glutathione reservoirs in liver and thus pave the path for lipid peroxidation, which ultimately leads to DILI.[18] Taking this finding into consideration, it was hypothesized that Ajwa date can be put to use as a hepatoprotective agent on part of its antioxidant properties based on high phenolic and flavonoid content and DPPH scavenging activity, as compared to other varieties of dates[19].

To observe the hepatoprotective effect of Ajwa date fruit, an experimental study was designed based on rabbit model. Thirty rabbits were divided into 5 groups (A, B, C, D and E) of 6 rabbits randomly assorted into each group. Administration of isoniazid and rifampicin raised level of liver enzymes and bilirubin three to four times above baseline (p value < 0.001). Similar results were seen in an identical study on rabbits using same dosage of INH and rifampicin.[16] The results of this experiment manifested that Ajwa date (as whole, flesh and seed powder) was able to significantly bring down the elevated levels of AST, ALT and ALP induced by anti-tubercular therapy in groups C, D and E receiving Ajwa date. Whole date, flesh and seed have shown statistically similar benefit. These results are in line with our hypothesis that Ajwa date fruit is able to counter the ATT induced hepatotoxicity.

The hepatoprotective effect of Ajwa date against various hepatotoxic compounds in different animal models has been demonstrated in several studies. Shamim et al. found that Ajwa date fruit extract (ADFE) is protective against monosodium glutamate induced oxidative stress.[20] Other studies also found hepatoprotective effects of ADFE against liver toxicity induced by CCL_4,[21] Ochratoxin A[22] and diclofenac.[23] One of these studies estimated the levels of liver enzymes as well as found higher hepatic reduced glutathione content and raised serum levels of superoxide dismutase, catalase and glutathione-S-transferase, after Ajwa extract treatment of hepatotoxic rats, indicating antioxidant potential of Ajwa date extracts[22]. The extract of Ajwa date fruit was used in previous studies, our results build on existing evidence that the whole date as well as the flesh and seed powder are also able to exhibit the same nature of results as ADFE.

The hepatotoxicity that emerges as a result of anti-tubercular drug intake severely hampers the goals of treatment as treatment needs to be stopped in order to avoid further complication. Our findings signify that this adverse effect can be countered by adding naturally occurring Ajwa date to the diets of patients receiving the treatment without adding any other drug to the regimen, which may overwhelm the liver already striving drug induced oxidative stress. As Ajwa seed powder has also shown promising results, it may be safely used in diabetics, being low in sugar content as compared to flesh.[24]

Findings of this study were limited by estimation of liver enzymes only. Estimation of markers of oxidative stress would have put light on targets of action, as antioxidant property of Ajwa date was basis of hypothesis of this study. Also, histopathological studies alongside biochemical tests, would have given a deeper insight and meaning to the results. The practical implication of this study faces a difficulty in adoption as Ajwa date being exotic to Pakistan, is an expensive fruit which is affordable to a few in the community. However, alternative date fruits of the same family of palms

more affordable to the general population can be studied for the same hepatoprotective effects.

CONCLUSION

Ajwa date flesh and seed powder are effective in preventing the rise in liver enzymes induced by isoniazid and rifampicin administration to rabbits. Whole fruit being only numerically superior to flesh or seed powder.

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REFERENCES

1. Global Tuberculosis Report 2019. WHO/CDS/TB/ 2019.15. [Internet]. Who. 2019. p. 2020–2. Available from: www.who.int/tb/data
2. MacNeil A, Glaziou P, Sismanidis C, Date A, Maloney S, Floyd K. Global Epidemiology of Tuberculosis and Progress Toward Meeting Global Targets — Worldwide, 2018. MMWR Morb Mortal Wkly Rep. 2020; 69(11):281-285.
3. Chakaya J, Khan M, Ntoumi F, Aklillu E, Fatima R, Mwaba P et al. Global Tuberculosis Report 2020 – Reflections on the Global TB burden, treatment and prevention efforts. Int J of Infect Dis. 2021; doi: 10.1016/j.ijid.2021.02.107.
4. Nahid P, Dorman S, Alipanah N, Barry P, Brozek J, Cattamanchi A et al. Executive Summary: Official American Thoracic Society/Centers for Disease Control and Prevention/Infectious Diseases Society of America Clinical Practice Guidelines: Treatment of Drug-Susceptible Tuberculosis. ClinInfect Dis. 2016; 63(7):853-867.
5. Fisher K, Vuppalanchi R, Saxena R. Drug-Induced Liver Injury. Arch Pathol& Lab Med. 2015; 139(7): 876-887.
6. Wang N, Huang Y, Lin M, Huang B, Perng C, Lin H. Chronic hepatitis B infection and risk of antituberculosis drug-induced liver injury: Systematic review and meta-analysis. J Chin Med Associat. 2016; 79(7):368-374.
7. Holt M, Ju C. Drug-induced liver injury. Handb Exp Pharmacol. 2010; (196):3-27.
8. El Hamdouni M, Ahid S, Bourkadi J, Benamor J, Hassar M, Cherrah Y. Incidence of adverse reactions caused by first-line anti-tuberculosis drugs and treatment outcome of pulmonary tuberculosis patients in Morocco. Infection. 2019; 48(1):43-50.
9. Biswas A, Santra S, Bishnu D, Dhali G, Chowdhury A, Santra A. Isoniazid and Rifampicin Produce Hepatic Fibrosis through an Oxidative Stress-Dependent Mechanism. IntJ Hepatol.2020;2020:1-12
10. Wardhan H, Singh S, Singh V. A study of the oxidative stress and the role of antioxidants in ATT induced hepatotoxicity in tuberculosis patients. Indian J Public Heal Res Dev. 2016; 7(2):243–249.
11. Sheikh BY, Elsaed WM, Samman AH, Sheikh BY, Bin Ladin A-MMA. Ajwa Dates As a Protective Agent Against Liver Toxicity in Rat. 2014; 3(2): 358-368.
12. Ali A, Abdu S. Antioxidant Protection against Pathological Mycotoxins Alterations on Proximal Tubules in Rat Kidney. Func Food Health Dis. 2011; 1(4):118.
13. Khan F, Ahmed F, Pushparaj P, Abuzenadah A, Kumosani T, Barbour E, et al. Ajwa Date (Phoenix dactylifera L.) extract inhibits human breast adenocarcinoma (MCF7) cells in vitro by inducing apoptosis and cell cycle arrest. PLOS ONE. 2016; 11(7): e0158963.
14. Zhang C, Aldosari S, Vidyasagar P, Nair K, Nair M. Antioxidant and Anti-inflammatory Assays Confirm Bioactive Compounds in Ajwa Date Fruit. J Agric Food Chem. 2013; 61(24):5834-5840.
15. Abdu S. B. The protective role of Ajwa date against the hepatotoxicity induced by ochratoxin A. Egyp J Natural Toxins, 2011; 8(1,2): 1-15.
16. Kalra BS, Aggarwal S, Khurana N, Gupta U. Effect of cimetidine on hepatotoxicity induced by isoniazid-rifampicin combination in rabbits. Indian J Gastroenterol. 2007 Jan-Feb; 26(1):18-21.
17. Kim J, Nam W, Kim S, Kwon O, Seung E, Jo J, et al. Mechanism investigation of rifampicin-induced liver injury using comparative toxicoproteomics in mice. Int J Mol Sci. 2017; 18(7):1417.
18. Pan Y, Tang P, Cao J, Song Q, Zhu L, Ma S, et al. Lipid peroxidation aggravates anti-tuberculosis drug-induced liver injury: Evidence of ferroptosis induction. Biochem Biophys Res Commun. 2020; 533(4):1512-1518.
19. Khalid S, Ahmad A, Kaleem M. Antioxidant activity and phenolic contents of Ajwa date and their effect on lipo-protein profile. Funct Foods Heal Dis. 2017; 7(6):396-410.
20. Shamim R, Iqbal Z, Tariq A, Zulfiqar S, Ara N, Khan F. Protective Effects of Phoenix Dactylifera on Monosodium Glutamate Induced Histomorphological Changes in Hepatocytes of Adult Male Albino Rats: A Light Microscopic Study. Med Forum Mon. 2018; 31(1):87-90.
21. Elsadek B, El-Sayed ES, Mansour A, Elazab A. Abrogation of carbon tetrachloride-induced hepatotoxicity in Sprague-Dawley rats by Ajwa date fruit extract through ameliorating oxidative stress and apoptosis. Pak J Pharm Sci. 2017;30(6):2183-91.
22. Al-Khafaf A, Jwad S. Study for the preventive effects of ajwadates alcoholic extract on thehepatic tissue functions and lipid profileregression induced by ochratoxin A in male albino rats. Int J Pharm Res. 2018; 10(3):391-398.
23. Aljuhani N, Elkablawy M, Elbadawy H, Alahmadi A, Aloufi A, Farsi S, et al. Protective effects of Ajwa date extract against tissue damage induced by acute diclofenac toxicity. J Taibah Univ Med Sci. 2019; 14(6):553-559.

24. Khalid S, Ahmad A, Masud T, Asad MJ, Sandhu M. Nutritional assessment of ajwa date flesh and pits in comparison to local varieties. J Anim Plant Sci. 2016; 26(4):1072-1080.

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