Original Article

Effect of phoenix dactylifera l (Ajwa date) seed powder on non-alcoholic fatty liver disease in high fat fed rat

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Objective: To determine the effect of Ajwa date seed powder on non-alcoholic fatty liver disease (NAFLD) in high fat fed rats.

Methodology: We randomly divided 36 Sprague-Dawley female into 3 groups. Control group was given standard rat chow for period of 12 weeks. High fat diet (HFD) group received high fat diet, while experimental group (HFD + ADS) was fed with 2% Ajwa date seed powder added in high fat diet throughout the study duration. Rats were sacrificed after 12 weeks of study and livers were resected, weighed and formalin fixed for histological examination.

Results: The treated group showed reduction from grade 3 to grade 1 in steatosis, inflammation, fibrosis and necrosis of hepatocytes. ($p < 0.001$). The mean body weight also decreased significantly in comparison to the disease control group ($p < 0.05$).

Conclusion: Addition of Ajwa date seed powder to diet can prevent weight gain and high fat diet induced injury to the liver. It can be used as a nutraceutical agent to prevent central obesity and related diseases.

Keywords: Fatty liver, NAFLD, hepatic steatosis, obesity, lipogenesis.

INTRODUCTION

Non-alcoholic fatty liver disease (NAFLD) is one of the most common liver disorders worldwide.[1] Obesity is one of the main causes of NAFLD.[2] Non-alcoholic steatohepatits (NASH) is characterized by excessive accumulation of triglycerides (steatosis), inflammation, injury and apoptosis in the liver cells, which in extreme cases may lead to cirrhosis and liver cancer.[3,4] Hypercaloric and high-fat diets seem to increase intrahepatic fat content.[5] When the liver capacity to use, store and export free fatty acids as triglycerides (TGs) is surpassed, it results in hepatic lipotoxicity.[6] Primary step in weight reduction and prevention of NAFLD is dietary modification and adoption of active lifestyle.[7]

Drugs used for obesity are currently not approved for long term use due to their severe cardio toxic effects and psycho-stimulatory adverse effects.[8] Despite the high prevalence of NAFLD/NASH, there is currently no approved drug for its treatment even after years of intense research worldwide.[9] The use of herbs has been on the rise in the last few decades as these are easily available, cheap and have few side effects.[10] However, the studies regarding the effects of herbs on metabolic syndrome and associated NAFLD are scarce.

Phoenix dactylifera L (Ajwa date) is one of the emerging medicinal plants belonging to palm family Arecaceae. Ajwa date seed (ADS) is rich in micronutrient polyphenols and its subtypes.[11] Herbal medicines containing polyphenols are effective against obesity and fatty liver disease.[12] The objective of this study was to evaluate the effect of ADS powder on NAFLD in high fat fed rats.

METHODOLOGY

This experimental study was conducted at Post Graduate Medical Institute, Lahore (PGMI) after approval by Ethical Committee of the Institute. The sample size was calculated as 12 rats in each group.[13] We selected 36 Sprague-Dawley female rats at the age of 4 weeks weighing between 40-50g from breeding house of PGMI and divided them randomly into three equal groups by lottery method. Rats showing any signs of disease were excluded from the study. The animals had free access to normal food and water for one week for acclimatization. ADS powder was prepared by grinding the fully dried seed in electric grinder. High fat diet (HFD) was prepared by mixing 210 g of beef tallow oil with 780 g of normal rat chow and 10 g of sodium deoxycholate.[14] Normal control group was given standard rat chow for period of 12. HFD group received high fat diet while experimental group (HFD+ADS) was fed with 2% ADS powder added in HFD throughout the study duration.[15] Body weight of each rat was measured in grams (g) at base line and weekly for 12 weeks. Rats were sacrificed after 12 weeks of study duration under light chloroform anesthesia. Liver of each animal was resected and

weighed in grams. Hepatosomatic index was calculated by ratio of liver weight to body weight and then multiplying with hundred.[16]

A piece of liver specimen was resected from each animal and fixed into 10% formalin. Slides were prepared and stained with Hematoxylin and Eosin and analyzed for presence of steatosis under light microscope. Slides were examined for parenchymal involvement by steatosis, lobular inflammation, hepatic fibrosis and ballooning. Histopathological parameters were classified by using Non-Alcoholic Steato-Hepatitis, Clinical Research Network (NASH CRN) scoring system.[17]

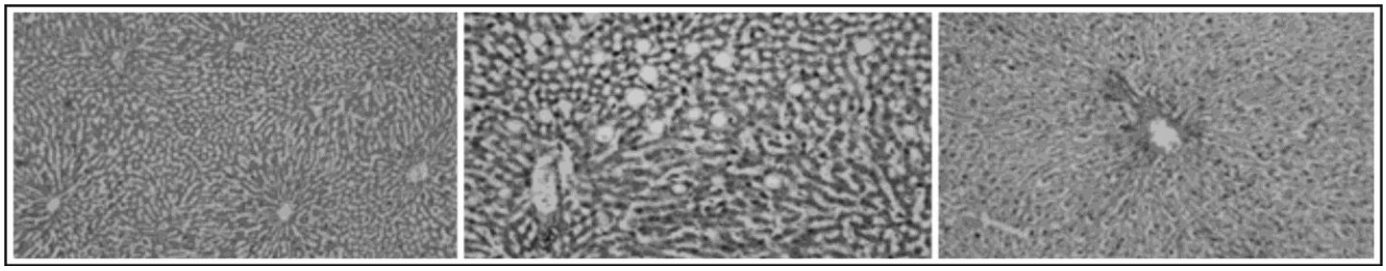
Statistical Analysis: Data were analyzed on SPSS version 20.

RESULTS

Table1 displays that body weights were almost similar at week zero in all the study groups. One way ANOVA was applied to compare the mean values of body weight in all three groups and a significant difference was observed ($p = 0.01$) at 12^{th} week. Hepatosomatic index showed a minor decrease in experimental group, however it was statistically insignificant.

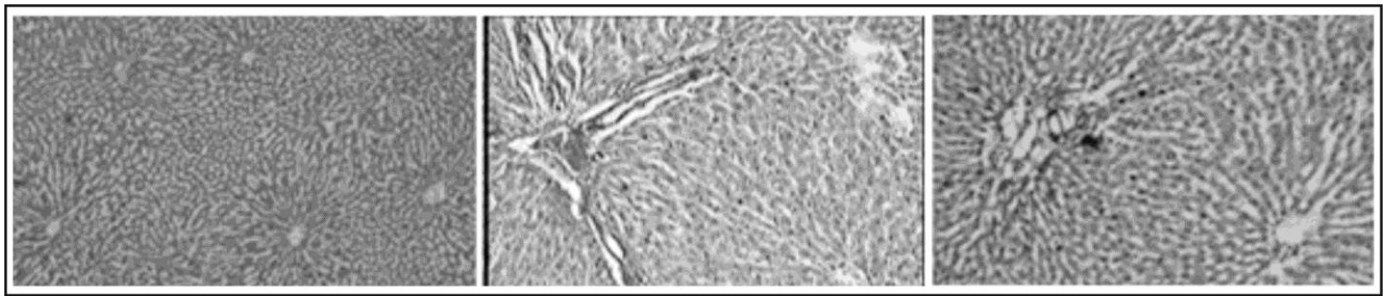
Table 1: Effect of *Phoenix dactylifera L* (ADS) powder on body weight, liver weight and Hepatosomatic index of study groups (n = 12).

Parameter	Normal Control Group Mean± SD	HFD Group Mean ± SD	HFD+ADS Group Mean ± SD	ANOVA p-value
Body weight (g) at week 0	56 ± 5.75	55 ± 3.98	57 ± 3.98	0.63
Body weight (g) at week 12	172 ± 14.38**	191 ± 18.98	173 ± 18.36*	**0.01***
Liver weight (g) at week 12	5.6 ± 0.75	5.7 ± 0.63	5.5 ± 0.87	0.87
Hepatosomatic index	3 ± 0.64	3.27 ± 0.27	3.16 ± 0.23	0.22



(A) (B) (C)

Fig. 1: Percentage of fatty changes in liver parenchyma (hepatic steatosis) under light microscope in groups: (A) = normal liver parenchyma in normal control group, (B) = grade III steatosis in HFD group and (C) = grade I steatosis in HFD+ADS group. (H&E 10X).



(A) (B) (C)

Fig. 2: The lobular inflammation of liver parenchyma under light microscope in groups: (A) = normal liver parenchyma in normal control group, (B) = congested blood vessels in portal area and sinusoidal lymphocytes infiltration in HFD group and (C) = liver parenchyma with reduced portal vessels congestion in HFD+ADS group. (H&E 10X).

Table 2: Pair wise comparison of histopathological changes among groups using Mann Whitney U test.

Group(I)	Groups (J)	Hepatic Steatosis	Lobular Inflammation	Hepatic Fibrosis	Hepatic Ballooning
Normal Control Group	HFD Group	< 0.001***	< 0.001***	< 0.001***	< 0.001***
	HFD+ADS Group	< 0.001***	<. 001***	0.002**	0.148
HFD Group	HFD+ADS Group	**< 0.001*** **	**< 0.001*** **	**0.023***	**< 0.001*** **

Histopathology of Liver: No hepatic steatosis, lobular inflammation, fibrosis or ballooning were observed in all animals of control group. HFD group showed grade 3 steatosis, inflammatory cell infiltration in sinusoidal spaces along with macrophages and portal to portal bridging while HFD + ADS group showed grade 1 steatosis, a reduction in number of inflammatory foci and congestion of blood vessels in portal areas and comparatively lesser or no perisinusoidal/ periportal fibrosis (Table 2 and Fig. 1 and 2).

The statistical analysis revealed highly significant differences among groups for fatty changes, lobular inflammation, fibrotic changes and hepatic ballooning ($p < 0.001$ each). The groups were compared independently with each other by using Mann Whitney U test (Table 2).

DISCUSSION

Diet is the major contributor in the development of obesity associated disorders. Different plants have been employed in different scientific studies for the prevention of adiposity and eventually reduction of complications.[18] ADS was considered in this study because of its phytochemical profile and its use in various studies.[11] Parameters included body weight, liver weight, hepatosomatic index and histopathology of liver. Co-administration of ADS powder with HFD caused markedly less weight gain. Diet intake of study groups was not exactly measured but it was noticed that HFD group ate less amount as compared to HFD+ADS group indicating less weight gain was not due to reduction in calorie intake.

These findings are consistent with the results of a study by Sultan et al.[19] However, there was no significant difference in mean values of liver weight and hepatosomatic index of all the groups. Histopathology revealed a lot of fatty changes the HFD group. When this hepatic steatosis was graded according to NASH scale,[14] it was found that the HFD+ADS group had significantly less steatosis in the liver parenchyma as compared to HFD group, however the normal lobular structure was not restored.

In the current study, ADS powder not only reduced steatosis but also reduced the high fat diet induced inflammation of liver parenchyma. The treated group showed reduction in steatosis, inflammation, fibrosis and necrosis of hepatocytes. It was observed that ADS powder had the ability to prevent adiposity and high fat diet induced NAFLD. Similar anti-inflammatory results were observed in a study in which acute diclofenac induced hepatotoxicity was reversed.[20]

ADS used in the present study has considerable number of polyphenols and their subtypes; that might be responsible for hepatoprotective action against steatosis and inflammatory response. Polyphenols have multiple features, mainly protective in nature, which include amelioration of NAFLD by modulating oxidative stress and hepatocellular damage. Furthermore, it down-regulates the expression of pro-inflammatory cytokines like IL-6, IL-10 and TNFα together with apoptotic markers like caspase-3 and Bax. It also restores GSH and transforming growth factor (TGF) levels and normalizes hepatic stellate cells (HSC) activation and proliferation leading to decreased fibrogenesis.[12]

This is the first study to convey usefulness of ADS, for prevention of obesity and associated liver damage, when given as nutraceutical at an early age. The limitation of this study is that phytochemical analysis was not performed. Determination of cellular mechanism of action was not possible due to limited resources. ADS powder may be used as a nutraceutical to prevent central obesity and its associated health risks. Further studies on humans should be conducted to observe the effect on various obesity predictors and risk.

CONCLUSION

This study showed that addition of Ajwa date seed powder to diet can prevent weight gain and high fat diet induced injury to the liver.

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Conception and design: Farhana Yasmeen, Sadia Chiragh.,
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Analysis and interpretation of data: Farhana Yasmeen Bhatti, Rukhsana Kausar, Mahwash Malik, Amer Hassan Siddiqui, Sadia Chiragh.
Drafting of the article: Farhana Yasmeen, Rukhsana Kausar.
Critical revision of article for important intellectual content: Amer Hassan Siddiqui, Sadia.
Statistical expertise: Amer Hassan Siddiqui.
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REFERENCES

1. Lonardo A, Mantovani A, Lugari S, Targher G. Epidemiology and pathophysiology of the association between NAFLD and metabolically healthy or metabolically unhealthy obesity. Ann Hepatol. 2020; 19: 359-66.
2. Pang Q, Zhang JY, Song SD, Qu K, Xu XS, Liu SS, et al. Central obesity and nonalcoholic fatty liver disease risk after adjusting for body mass index. World J Gastroenterol. 2015; 21: 1650-62.
3. Neuschwander-Tetri BA. Non-alcoholic fatty liver disease. BMC Med. 2017; 15: 45-9.
4. Pierantonelli I, Svegliati-Baroni G. Nonalcoholic fatty liver disease: basic pathogenetic mechanisms in the progression from NAFLD to NASH. Transplantation, 2019; 103: 1-13.
5. Pafili K, Roden M. Nonalcoholic fatty liver disease (NAFLD) from pathogenesis to treatment concepts in humans. Mol Metab. 2020: 101122.
6. Rada P, González-Rodríguez Á, García-Monzón C, Valverde ÁM. Understanding lipotoxicity in NAFLD pathogenesis: is CD36 a key driver? Cell Death Dis. 2020; 11: 802-16.
7. Dhurandhar NV, Schoeller D, Brown AW, Heymsfield SB, Thomas D, Sørensen TI, et al. Energy Balance Measurement Working Group. Energy balance measurement: when something is not better than nothing. Int J Obes. 2015; 39: 1109-13.
8. Yanovski SZ, Yanovski JA. Long-term drug treatment for obesity: a systematic and clinical review. JAMA. 2014; 311: 74-86.
9. Raza S, Rajak S, Upadhyay A, Tewari A, Sinha RA. Current treatment paradigms and emerging therapies for NAFLD/NASH. Front Biosci (Landmark Ed). 2021; 26: 206-37.
10. Firenzuoli F, Gori L. Herbal medicine today: clinical and research issue. Evid Based Complement Alternat Med. 2007; 4: 37-40
11. Khalid S, Khalid N, Khan RS, Ahmed H, Ahmad A. A review on chemistry and pharmacology of Ajwa date fruit and pit. Trends Food Sci Technol. 2017; 63: 60-69.
12. Simón J, Casado-Andrés M, Goikoetxea-Usandizaga N, Serrano-Maciá M, Martínez-Chantar ML. Nutraceutical properties of polyphenols against liver diseases. Nutrients, 2020; 12: 3517-35.
13. An MH, Park YS, Lee KD, Kim RJ, Lee WS. Antiobesity and lipid lowering effects of Bifidobacterium spp. in high fat diet-induced obese rats. Lipids Health Dis. 2011; 10: 116-27.
14. Hariri N, Thibault L. High-fat diet-induced obesity in animal models. Nutr Res Rev. 2010; 23: 270-99.
15. Mushtaq Z, Kausar N, Kausar S, Chiragh, S. Effect of Ajwa date seed on lipid profile of hyperlipidemic rabbits. KMUJ. 2017; 9: 135-39.
16. Yu J, Yang J, Li M, Yang X, Wang P, Xu J. Protective effects of Chinese Fenggang zinc selenium tea on metabolic syndrome in high-sucrose-high-fat diet-induced obese rats. Sci Rep. 2018; 8: 1-1.
17. Brunt EM, Kleiner DE, Wilson LA, Belt P, Neuschwander-Tetri BA. NASH Clinical Research Network (CRN). Nonalcoholic fatty liver disease (NAFLD) activity score and the histopathologic diagnosis in NAFLD: distinct clinic pathologic meanings. Hepatol. 2011; 53: 810-20.
18. Didi A, Meziane RK, Amamou F, Benhmidat A, Sari DC. Metabolic and antioxidant effects of Nigella Sativa oil on prevention of obesity development in Rats fed high-fat diet. Eur Chem Bull. 2014; 3: 888-96.
19. Sultan F, Anwar S, Alam SS, Farooq H, Afzal A, Ashraf M. Anti-obesity and hypolipidemic effects of ajwa date seed compared to Simvastatin in butter fed dyslipidemic rats. Proceedings SZPGMI. 2019; 33: 6-12.
20. Aljuhani N, Elkablawy MA, Elbadawy HM, Alahmadi AM, Aloufi AM, Farsi SH, et al. Protective effects of Ajwa date extract against tissue damage induced by acute diclofenac toxicity. J Taibah Univ Sci. 2019; 14: 553-59.