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Ajwa date (*Phoenix dactylifera* L.) is hepatoprotective against toxicity by antituberculosis drugs - an experimental study

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ABSTRACT

Background and Objective: Hepatotoxicity induced by antituberculosis drugs is quite common and often linked with oxidative stress. Ajwa dates are rich in antioxidants and flavonoids; therefore, these may be protective against the oxidative stress to liver. This study was designed to determine the hepatoprotective effects of Ajwa dates on hepatotoxicity induced by antituberculous drugs in an experimental model.

Methods: This experimental study was conducted at the Post Graduate Medical Institute, Lahore, Pakistan. A total of 30 male rabbits were divided into 5 groups, with 6 animals in each group. Group A and B were fed on normal diet. Group C, D, and E were fed on a diet supplemented with whole Ajwa dates, flesh, and seed powder, respectively. Group B, C, D, and E were given isoniazid 50 mg/kg and rifampicin 100 mg/kg orally for 14 days. After the rabbits were sacrificed, hepatotoxic changes were examined histologically in all groups according to standard criteria.

Results: Liver to body weight ratio was higher in disease group (B) as compared to the healthy control group A (p -value = 0.03), Ajwa flesh group D (p -value = 0.02) and Ajwa seed powder group E (p -value = 0.07). Differences between experimental groups were not statistically significant for both liver weight, and liver weight to body weight ratio. On histological examination, degeneration, necrosis, steatosis, triaditis, and fibrosis were seen in the disease group B while no such changes were observed in group C, D, and E.

Conclusion: Ajwa dates (*Phoenix dactylifera* L.) has a protective role against isoniazid and rifampicin-induced hepatocellular injury and fibrosis.

Keywords: Ajwa dates, anti-tuberculosis drugs, hepatotoxicity, histology, antioxidant.

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Introduction

Tuberculosis (TB) is one of the most common infectious diseases, affecting millions throughout the world per annum. Drugs used for the treatment of TB include isoniazid, rifampicin, ethambutol, and pyrazinamide. These drugs are used in combination for maximum efficacy and prevention of the emergence of resistant strains. Although effective, all of these drugs are notorious for causing serious adverse effects such as hepatotoxicity and neuropathy.¹

The reported incidence of hepatic damage caused by co-administration of isoniazid and rifampicin is 2.0%.² Drug-induced liver injury (DILI) can manifest as an acute hepatocellular insult, cholestatic injury, or mixed liver injury. Among DILI, hepatocellular and cholestatic forms of liver injury are the most prevalent.³ Metabolic oxidation of acetyl

hydrazine leads to the formation of oxidative species that bind covalently to proteins and cause hepatic damage and shows a strong correlation between oxidative stress and hepatic insult.⁴

Isoniazid acts by inhibiting the synthesis of mycolic acid in *Mycobacterium tuberculosis*. It is metabolized in the liver by acetylation to acetyl-isoniazid which is further metabolized to mono-acetyl hydrazine, diacetyl hydrazine, and a few other metabolites. Free radicals produced from the metabolism of mono-acetyl hydrazine and covalent bonding of acetyl hydrazine to macromolecules of the liver cause hepatocytes injury.⁵

Rifampicin is used in combination with isoniazid for TB treatment. It hinders the growth of *M. tuberculosis*

via inhibition of the mycobacterial DNA-dependent RNA polymerase.⁶ Rifampicin induces the function of hepatic enzymes. It also induces oxidative stress and impairs the antioxidant defense in liver cells.⁷ Pyrazinamide is metabolized to pyrazinoic acid, which is hepatotoxic. It also alters nicotinamide acetyl dehydrogenase levels in the liver, leading to the production of free radicals that cause liver injury.⁸

Ajwa date (*Phoenix dactylifera* L.) is among one of the most ancient fruit crops. It is commonly grown in the Middle East, North Africa, and the Arabian Peninsula. The flesh and seeds of Ajwa dates are rich in alkaloids, protein, carbohydrates, fatty acids, vitamins, minerals, polyphenolic compounds, flavonoids, and tannins.^{9,10} Ajwa dates are also known to possess strong antioxidant activity due to their high content of polyphenols and flavonoids.¹¹ Ajwa dates have shown the protective effect of seeds against carbon tetrachloride (CCl₄) and paracetamol-induced liver lesions.^{12,13}

This study is therefore designed to observe the antioxidant and protective effects of Ajwa dates on hepatotoxicity induced by anti-TB drugs.

Methods

This experimental study was conducted at the Post Graduate Medical Institute, Lahore, Pakistan from November 2016 to March 2017 after getting approval by the Institutional Ethical Committee. A sample size of 30 was calculated by applying 90% power of study and 5% level of significance.¹⁴ Healthy adult albino male rabbits, approximately 4 months old, weighing 1.2-1.5 kg, were purchased from the local market. The animals were categorized into five groups, with six animals in each group. They were kept for 1 week for acclimatization, under standard laboratory conditions, before starting the study.¹⁵

The rabbits were fed recommended diet and water. Diet pellets were prepared with split chickpeas, dry fodder, jawar, and plain flour.¹⁵ All ingredients were mixed with water and pellets were formed. For whole fruit supplementation, flesh and seeds of Ajwa dates were mixed with rabbit diet.

Thirty rabbits were divided into five groups. Group A and B were fed on a normal diet. Group C, D and E were fed on diet supplemented with whole Ajwa dates, flesh and seed powder, respectively (one date/100 g). Hepatotoxicity was induced in Group B, C, D, and E by the administration of single daily dose of isoniazid 50 mg/kg and rifampicin 100 mg/kg orally for 14 days.¹⁵ Both drugs were obtained from Schazoo Zaka Pharmaceuticals, Pakistan Ltd.

For histological examination of the liver, rabbits were sacrificed after 14 days. Liver was dissected out and weighed. The liver to body weight ratio was estimated. Tissue sections from the liver were fixed in 10% formalin. After processing,

approximately 3-5 mm thick sections of tissue were cut by rotary microtome and stained with hematoxylin and eosin (H&E). Pathological changes were observed under high power field. Whole slide was thoroughly studied for presence or absence of degeneration, steatosis, necrosis, fibrosis, and degeneration.¹⁶

Statistical analysis

Statistical Package for the Social Sciences version. 20 was used for analyzing the data. One way analysis of variance was used for comparison of liver weight and liver to body weight ratio. To find the difference in mean between the groups, *post hoc* Tukey's test was applied. The significance of the difference in time in all groups was calculated by a paired *t*-test. Fischer's exact test was applied for comparison of qualitative parameters (degeneration, necrosis, fibrosis, steatosis, and triaditis). The *p*-value < 0.05 was considered to be significant.

Results

Mean liver weights of experimental groups (C, D, and E) were lower than both groups A and B. The difference of group D only was significant from group A (*p*-value = 0.03) and B (*p*-value = 0.02). Liver to body weight ratio was significantly high in group B, in comparison to group A (*p*-value = 0.03), group D (*p*-value = 0.02) and group E (*p*-value = 0.07). Differences between experimental groups C, D, and E were not statistically significant for hepatic weight, alone, or hepatic weight to body weight ratio. Table 1 shows the mean and standard deviation of hepatic weight and the hepatic weight to body weight ratio.

Administration of anti-TB drugs for 14 days to group B resulted in degeneration, necrosis, steatosis, triaditis, and fibrosis as evident on histological examination of rabbit liver. Concurrent administration of Ajwa date whole to group C, flesh to group D and seed powder to group E prevented the histological damage to liver and caused a significant reduction in all histopathological parameters (Table 2).

Hepatocytes were arranged in cords in relation to central vein and sinusoids as seen in histological sections of liver from the control group (Figure 1a). General architecture of hepatic tissue was lost in liver sections from rabbits treated with anti-TB drugs (group B) and showed steatosis with vacuolation (Figure 1b). Changes in cytoplasmic content and pyknotic nuclei confirming degenerative changes were observed in group B (Figure 1c and d). Furthermore, liver sections of anti-TB drugs treated group showed marked bridging fibrosis (Figure 1e).

The groups treated with Ajwa date whole, flesh and seed powder (group C, D, and E, respectively), showed marked ameliorations in hepatic lesions, with less obvious

Table 1. Effect of Ajwa date on hepatic weight and hepatic weight to body weight ratio (n = 6).

Groups	Hepatic weight (g)	Hepatic to body weight ratio
Group A (normal control)	38.5 ± 1.2	0.029 ± 0.002
Group B (hepatotoxic control)	38.6 ± 1.1	0.033 ± 0.002 [#]
Group C (Ajwa date whole)	37.1 ± 0.7	0.031 ± 0.002
Group D (Ajwa date flesh)	36.6 ± 1.1	0.029 ± 0.001*
Group E (Ajwa date seed)	37.3 ± 1.0	0.029 ± 0.001*
p-value	0.009	0.001

[#] p-value < 0.05 versus group A, * p-value < 0.05 versus group B.

Table 2. Histological changes after administration of Ajwa date following hepatotoxicity induced by antituberculosis drugs in Groups B-E (n = 6).

Histological parameters	Group A n (%)	Group B n (%)	Group C n (%)	Group D n (%)	Group E n (%)	p-value
Degeneration	0 (0.0)	6 (100.0)	1 (16.7)	2 (33.3)	2 (33.3)	0.004
Necrosis	0 (0.0)	5 (83.3)	1 (16.7)	1 (16.7)	2 (33.3)	0.023
Steatosis	0 (0.0)	6 (100.0)	1 (16.7)	2 (33.3)	2 (33.3)	0.004
Fibrosis	0 (0.0)	6 (100.0)	1 (16.7)	2 (33.3)	2 (33.3)	0.004
Triaditis	0 (0.0)	5 (83.3.0)	0 (0.0)	2 (33.3)	2 (33.3)	0.010
Regeneration	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	-

inflammatory and degenerative changes. Steatosis and fibrosis were markedly reduced along with almost normal hepatic strands with lesser dilatation of central veins and sinusoids. Although some vacuolated and degenerative cells were also seen but they were few in number (Figure 2).

Discussion

The therapeutic use of isoniazid and rifampicin, for the treatment of TB, is a well-known cause of liver injury.¹⁷ This study was designed to evaluate the protective role of Ajwa date in anti-TB drugs induced hepatotoxicity in the animal model. The current study reports hepatocytes degeneration, necrosis, steatosis, triaditis, and fibrosis of the rabbit's liver after administration of isoniazid and rifampicin. However, the concurrent administration of Ajwa date whole, its flesh, and seed powder prevented the histological damage to the liver and caused a significant reduction in all histopathological changes.

According to the previous studies, the measurement of single organ weight is more reliable, as compared to whole-body weight.¹⁸ Since body weight decreases in hepatotoxic studies, therefore, liver-to-body weight ratio was also estimated.

In this study, hepatic weight and hepatic to whole body weight ratio were decreased in Ajwa treated groups in comparison to the diseased group, but the difference between the treated groups was statistically insignificant. These results are similar to a Chinese study in which the effect of a herb was observed on DILI in rats and no significant difference

was observed in hepatic weight and hepatic to body weight ratio.¹⁹ However, hepatic to body weight ratio was observed to decrease significantly, with the administration of date seed powder, against CCl₄-induced hepatotoxicity.¹²

It was observed that administration of anti-TB drugs resulted in degeneration, necrosis, steatosis, triaditis, and fibrosis of the rabbit's liver. However, the concurrent administration of Ajwa date whole, its flesh, and seed powder preserved the histological structure of the liver by significantly reducing these changes. A similar study was done by Kosasih and colleagues.²⁰ to evaluate the hepatoprotective and antioxidant potential of Ajwa fruit powder. The results were by the current study. The authors deduced that the presence of flavonoids and vitamin C, in Ajwa date fruit powder, was the major reason for the prevention of hepatocellular injury. Similarly, histopathological changes were seen in another study, including portal inflammation, necrosis, fatty changes, and ballooning degeneration of hepatocytes pretreated with isoniazid and rifampicin. These changes were reduced markedly when co-treated with pyrrolidine dithiocarbamate.²¹ Similarly, work done by Abdelaziz and Ali¹² demonstrated the hepatoprotective effect of Hayani date seed powder against CCl₄-induced hepatotoxicity by decreasing degeneration, vacuolization, and fibrosis. Antioxidant activity seems to be the most probable mechanism of hepatoprotective effect of Ajwa dates. Lipid peroxidation is mainly responsible for inflammatory changes and damage to liver tissue. Ajwa date causes a decrease in lipid peroxidation of hepatocellular membrane and increases the process of repair possibly due

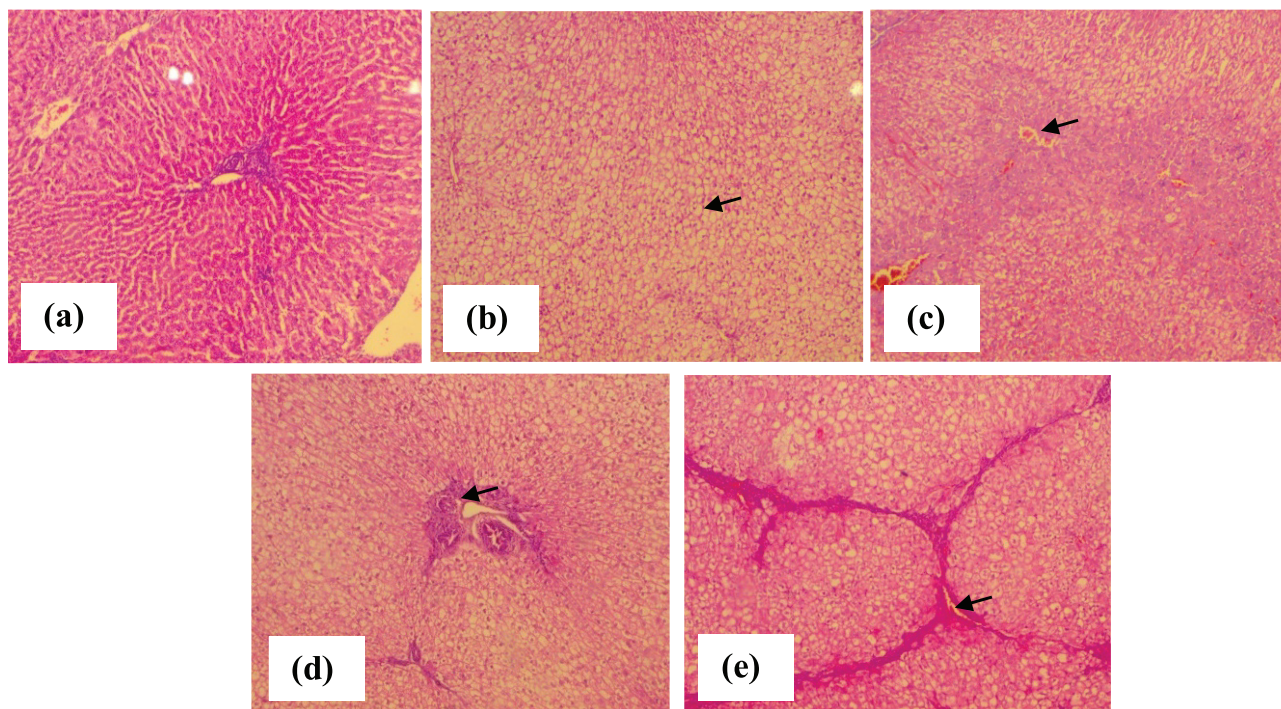


Figure 1. Photomicrographs of liver tissue showing (a) normal architecture arranged as sheets of hepatocytes (b) steatotic changes (c) triaditis along with degenerative and necrotic changes (d) feathery degeneration (e) bridging fibrosis (H&E, ×40).

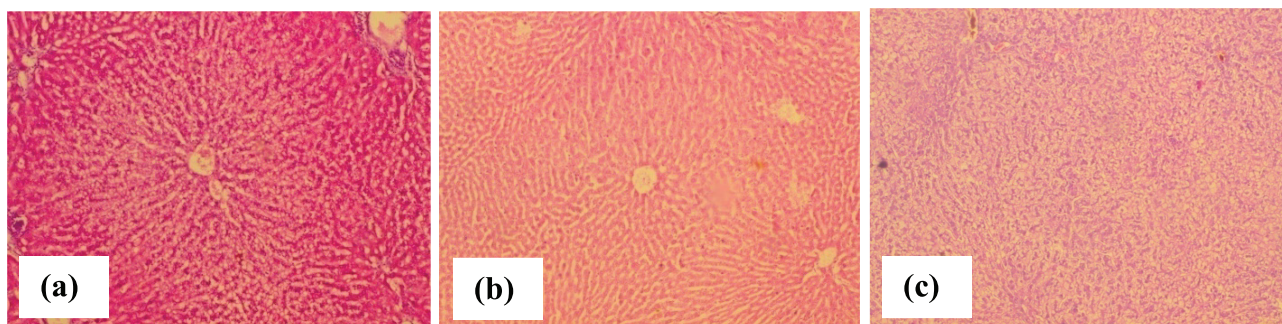


Figure 2. Photomicrographs of liver tissue showing (a) cords of hepatocytes and almost normal architecture in anti-TB drugs and whole date treated group (b) cords of hepatocytes around central vein in anti-tuberculous drugs and Ajwa date flesh group (c) less hydropic and necrotic changes in anti-TB drugs and Ajwa date seed powder group (H&E, ×40).

to the presence of antioxidants and vitamins, such as vitamin E.¹¹ Multiple studies have documented an increase in antioxidant activity as a result of administration of date fruit and seed powders. Increased levels of superoxide dismutase and glutathione-S-transferase, reported in these studies, have been linked to the enhanced free radical scavenging capacity that counters oxidative stress and protects the cellular injury.¹²⁻¹⁴

The hepatoprotective role of Ajwa date may also be related to its inhibitory effect on inflammation. Inflammatory changes have a profound impact on causing drug-induced hepatitis, as evident by the abundance of arachidonic acid derivatives, commonly seen during the inflammatory process. Ajwa date has been shown to possess anti-inflammatory

activity.²² Therefore, hepatoprotection can be reliably linked to anti-inflammatory properties as well.

Conclusion

It is concluded that Ajwa date whole, flesh, and seed powder can protect the liver against isoniazid and rifampicin-induced damage. Whole dates are more beneficial in preventing hepatic cellular injury as compared to flesh or seed powder alone.

Limitations of the study

This study has few limitations. First, only one variety of dates was used. Second, biochemical parameters could have been added to augment the histological parameters of hepatotoxicity.

Acknowledgment

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List of Abbreviations

CCl ₄	Carbon tetrachloride
DILI	Drug-induced liver injury
H&E	Hematoxylin and eosin
NADH	Nicotinamide acetyl dehydrogenase
TB	Tuberculosis

Conflict of interest

None to declare.

Grant support and financial disclosure

None to disclose.

Ethical approval

The Institutional Ethical Review Board of Postgraduate Medical Institute (PGMI), Lahore, Pakistan approved the study with ethical approval number 00-08-5-2016 dated 15.04.2016.

Authors' contribution

SM: Conception and design of the study, data collection and drafting of the manuscript.

UA: Acquisition, analysis and interpretation of data.

SZ: Analysis of data, important intellectual input and drafting of the manuscript.

SC: Conception and design of the study, Important intellectual input.

ALL AUTHORS: Approval of the final version of the manuscript to be published.

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