

## BIOCHEMICAL AND HISTOPATHOLOGICAL INVESTIGATIONS OF HEPATOPROTECTIVE POTENTIAL OF *Phoenix dactylifera* AGAINST ISONIAZID INDUCED TOXICITY IN ANIMAL MODEL

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*Phoenix dactylifera* (Ajwa khajur) is pharmacologically important plant because of its hepatoprotective, nephroprotective, gastroprotective, antihyperlipidemic and anticancerous properties. In present study, hepatoprotective effect of *Phoenix dactylifera* was observed in adult albino rabbits. Isoniazid (INH) is a first line antitubercular agent but it has hepatotoxicity causing potential due to the generation of reactive oxygen species on its prolong use. Thirty adult albino rabbits of either sex were used in this study. Hepatoprotective potential of date palm was screened by using its two doses i.e. 150mg/kg/day and 300mg/kg/day up to 14 days. Results have shown that experimental animals treatment with date palm significantly prevented elevation of liver function enzymes (AST, ALT and ALP). Alteration in oxidative status parameters (TAC, TOS, MDA and catalase) significantly prevented as compared to isoniazid induced hepatotoxic animals. Also, complete blood count evaluation showed increase in blood cells number. All above findings were also confirmed by histopathological studies. Liver tissue samples showed normal hepatocytes architecture in date palm treated groups. Therefore, from the results we can conclude that *Phoenix dactylifera* fruit possesses hepatoprotective activity and have a mild hematopoietic effect.

**Keywords:** Tuberculosis, drugs, antioxidants, hepatotoxicity, hematopoietic, hepatoprotective, isoniazid, silymarin.

### INTRODUCTION

Tuberculosis which is one of the major diseases responsible for high mortality rate throughout the world (Murray, 2004). It spread through airborne droplets of infected individual. According to the standard guidelines streptomycin, isoniazid (INH), rifampicin (RIF), ethambutol and pyrazinamide are used as first line therapeutic agents for recommended duration (Chaulk and Grady, 2000). Treatment requires prolonged use of these drugs which are associated with some serious health issues like hypersensitivity reactions, jaundice, hepatitis, blood disorders (anemia, agranulocytosis and thrombocytopenia), hepatotoxicity, neuropathy and nephropathy (Bruton *et al.*, 2006).

The impairment of normal hepatic function can be termed as hepatic injury or hepatotoxicity. Deviation in normal range of alanine aminotransferase (ALT) enzyme in serum is indication of liver injury (Navarro and Senior, 2006). Hepatic injury is a consequence of impaired mitochondrial function which is main target of many drugs. Intracellular oxidant stress promotion due to generation of reactive oxidant species (ROS) by CYP2E1 enzyme system plays major role in hepatotoxicity (Jaeschke *et al.*, 2002). Hepatotoxicity induced by hepatotoxic substances can be treated by substances termed as hepatoprotective agents having potential to counter toxic effects.

Treatment of hepatic problems with synthetically prepared drugs is not much effective. Traditional medicines are more effective to treat liver diseases as compared to synthetic medicines (Saleem and Naseer, 2014). Herbal medicines are considered as less toxic and associated with less incidents of side effect. In most of Asian countries, liver diseases are being treated by herbal products manufactured by leading pharmaceutical companies (Radha and Yogesh, 2005; Bhawna and Kumar, 2009). In modern medicine, medicinal plants are still a significant source of pharmacological active substances but traditional medicines are become less important to deal with health issues worldwide (Gupta and Briyal, 2004; Krentz and Bailey, 2005).

*Phoenix dactylifera* L. also known as date palm belongs to Arecaceae family, is being cultivated in Asian countries from centuries, is used in diet and traditional medicine due to its nutritive and pharmacological importance (Baliga *et al.*, 2011). Date palm fruit is rich in vitamins (ascorbic acid, carotenoids, folate, niacin, pyridoxal, riboflavin, retinol and thiamin), minerals (P, Se, Na, Zn, K, Fe, Mn, Mg, Cu and Ca), carbohydrates (sucrose, glucose and fructose), amino acids (arginine, alanine, aspartic acid, cysteine, glycine, glutamic acid, histidine, leucine, isoleucine, proline, serine, tyrosine and threonine) fiber and fats. The phenolic contents (protocatechuic acid, p-hydroxybenzoic acid, p-coumarin, ferulic acid, sinapic acid, gallic acid, vanillic acid, synergic

acid and derivatives of cinnamic acid) and flavonoids (quercetin, luteolin and apigenin) have antioxidant activity. The main uses of *Phoenix dactylifera* studied in animal models are hepatoprotective, nephroprotective, anticancer, anti-inflammatory, gonadotropic and antihyperlipidemic activities (Copley *et al.*, 2001; Ateeq *et al.*, 2013). In this study, our aims were to investigate the antioxidant and hepatoprotective activities of date palm in isoniazid induced hepatotoxic adult albino rabbits.

## MATERIALS AND METHODS

**Plant material:** *Phoenix dactylifera* (ajwa khajur) purchased from the local market of Faisalabad and authenticated by Department of Horticultural Science, University of Agriculture, Faisalabad. Fruit flesh was manually separated from the pits, minced and was subjected to freeze drying until moisture free material was obtained. Fine powder was obtained by the use of mechanical grinder and stored in airtight container at 4°C.

**Experimental animals:** A total number of thirty healthy adult albino rabbits of either sex (weight ranges from 1000g to 1500g) were purchased from local animal market of Faisalabad. The animals were housed at Institute of Microbiology, University of Agriculture, Faisalabad at 25±5°C in a well-ventilated animal room under twelve-hour light and dark cycle. Seasonal fodder and water ad-libitum provided to all animals and they were acclimatized for one week. The experiment was conducted according to the guidelines of Directorate of Research and Advance Studies and with the consent of the Society of Ethics of Animals, University of Agriculture, Faisalabad, Pakistan.

**Chemicals and drugs:** Tablet isoniazid 100mg was obtained from Unexo Labs (Pvt) Ltd. Lahore, Pakistan. Tablet Silliver® (Silymarin) 200mg was obtained from Abbott Laboratories (Pvt) Ltd, Karachi, Pakistan. All the reagents were used of analytical grades.

**Experimental design:** Rabbits were divided in five equal groups. Each group was carried 6 animals. Different treatments administered intragastrically in rabbits for 14 days are given in Table 1.

**Blood sampling and biochemical assay:** Blood samples were taken in heparinized tubes before drug administration and then after 14<sup>th</sup> day of drug administration. The samples were allowed to clot for 20 minutes at refrigeration temperature and then were centrifuged at 4000xg for 5 minutes. Serum thus was obtained and stored at -4°C. Serum alanine aminotransferase (ALT), alkaline phosphate (ALP) and aspartate aminotransferase (AST) activities were estimated by using Randox reagent kits (Catalogue # AL1200, AP8002, AS2800) and oxidative status (total oxidant status, total antioxidant capacity, catalase activity and malondialdehyde) were assessed for hepatotoxicity by methods developed by Erel (2004) and Erel (2005) using spectrophotometer.

**Table 1. Feeding and drug administration schedule in adult albino rabbits during the experimental period of 0-14 days.**

Groups	Treatments
Group I: Normal control	Normal saline.
Group II: Untreated control	Isoniazid (100mg/kg/day, orally)
Group III: Silymarin	Isoniazid (100mg/kg/day, orally) + silymarin (100mg/kg/day, orally)
Group IV: Treated I	Isoniazid (100mg/kg/day, orally) + low dose of <i>Phoenix dactylifera</i> fruit powder (150mg/kg/day, orally)
Group V: Treated II	Routine feed + isoniazid (100mg/kg/day, orally) + High dose of <i>Phoenix dactylifera</i> fruit powder (300mg/kg/day, orally)

Whole blood samples were collected for complete blood count (CBC) by using automated hematology system. The following parameters Red blood cells (RBC), Total white blood cells (TWBC), Platelets (PLT), Hemoglobin (Hgb), Hematocrit (Hct), Mean corpuscular volume (MCV), Mean corpuscular hemoglobin (MCH) and Mean corpuscular hemoglobin concentration (MCHC) have been performed.

**Histopathological analysis:** Histopathology was performed on collected tissue sample according to the method as described by (Bancroft and Gamble 2002). Liver samples were collected at the end of experiment and stored in formalin. After treatment with graded ethanolic dilutions, tissue fragments were prepared by blocking and mounted on glass slides. Staining was performed with hematoxylin and eosin (H & E stain). Finally, slides were examined under Olympus PM-10ADS automatic light microscope (Olympus optical Co., Tokyo, Japan) with 40X objective.

**Statistical analysis:** SPSS software version 3.16 was used for the statistical analysis presented in the experiment. The values were expressed as mean ± SEM. Statistical analysis was performed by one way of analysis of variance (ANOVA) and statistical differences among different treatment groups was determined by Duncan Multiple Range test at 5% level of significance.

## RESULTS

**Hematology profile:** Results showed that date palm treatment significantly ( $P \leq 0.01$ ) increased RBC, hemoglobin, hematocrit, platelet and TWBC count as compared to reduced levels in groups treated with isoniazid alone. Non-significant ( $P \geq 0.01$ ) change was observed in mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH) and mean corpuscular hemoglobin concentration (MCHC) in all treated groups (Table 2).

**Liver function biomarkers:** Isoniazid treatment significantly ( $P \leq 0.01$ ) increased liver function biomarkers (ALT, AST and ALP). While treatment with date palm showed significant

**Table 2. Effect of Date palm on blood (complete blood count) in isoniazid induced hepatotoxicity in rabbits.**

Parameters	Control	Untreated control	Silymarin	Treated I	Treated II
RBC ( $\times 10^{12}/L$ )	6.188 $\pm$ 0.029a	6.017 $\pm$ 0.017c	6.327 $\pm$ 0.025a	6.177 $\pm$ 0.030ab	6.302 $\pm$ 0.060bc
Hgb ( $\times 10g/dL$ )	12.017 $\pm$ 0.130b	10.283 $\pm$ 0.125c	12.983 $\pm$ 0.130a	11.700 $\pm$ 0.052ab	12.267 $\pm$ 0.126b
Hct (%)	39.433 $\pm$ 0.535a	34.317 $\pm$ 0.436b	40.700 $\pm$ 0.245a	35.883 $\pm$ 0.358a	39.967 $\pm$ 0.216b
PLT ( $\times 10^9/L$ )	244.333 $\pm$ 2.692a	211.833 $\pm$ 2.330b	250.833 $\pm$ 1.167a	237.000 $\pm$ 1.506a	247.667 $\pm$ 2.290a
WBC ( $\times 10^9/L$ )	8.383 $\pm$ 0.114a	6.850 $\pm$ 0.112b	7.983 $\pm$ 0.079a	6.900 $\pm$ 0.077b	7.767 $\pm$ 0.196b
MCV (fL)	62.517 $\pm$ 0.322a	61.117 $\pm$ 0.682a	62.700 $\pm$ 0.819a	61.717 $\pm$ 0.574a	62.000 $\pm$ 0.573a
MCH (pg)	20.883 $\pm$ 0.290a	20.233 $\pm$ 0.514a	21.100 $\pm$ 0.428a	19.650 $\pm$ 0.232a	20.050 $\pm$ 0.673a
MCHC ( $\times 10g/L$ )	34.467 $\pm$ 0.279a	33.650 $\pm$ 0.278a	34.283 $\pm$ 0.396a	33.900 $\pm$ 0.271a	34.183 $\pm$ 0.277a

Means sharing similar letters in a row are statistically non-significant ( $P>0.05$ )

**Table 3. Effect of Date palm on liver function biomarkers (ALT, AST and ALP) in isoniazid induced hepatotoxicity in rabbits.**

Parameters	Control	Untreated control	Silymarin	Treated I	Treated II
ALT (U/L)	53.333 $\pm$ 0.615c	102.500 $\pm$ 1.057a	61.667 $\pm$ 1.282c	83.500 $\pm$ 2.742bc	67.833 $\pm$ 0.703b
AST (U/L)	50.333 $\pm$ 0.615c	109.667 $\pm$ 1.145a	60.167 $\pm$ 1.537c	86.167 $\pm$ 2.414bc	72.333 $\pm$ 1.498ab
ALP (U/L)	56.000 $\pm$ 0.856c	115.000 $\pm$ 1.291a	64.000 $\pm$ 1.155c	90.000 $\pm$ 2.828bc	72.167 $\pm$ 2.227ab

Means sharing similar letters in a row are statistically non-significant ( $P>0.05$ )

**Table 4. Effect of Date palm on liver function biomarkers (ALT, AST and ALP) in isoniazid induced hepatotoxicity in rabbits.**

Parameters	Control	Untreated control	Silymarin	Treated I	Treated II
TOS ( $\mu\text{mol}/L$ )	2.128 $\pm$ 0.027c	3.835 $\pm$ 0.127a	2.567 $\pm$ 0.025bc	3.022 $\pm$ 0.058bc	2.697 $\pm$ 0.057b
TAC ( $\mu\text{mol}/L$ )	0.640 $\pm$ 0.029a	0.253 $\pm$ 0.013b	0.553 $\pm$ 0.033a	0.328 $\pm$ 0.012ab	0.452 $\pm$ 0.030b
CAT (KU/L)	51.462 $\pm$ 0.861a	15.955 $\pm$ 0.844c	41.240 $\pm$ 1.125b	22.382 $\pm$ 1.559c	34.393 $\pm$ 2.528c
MDA (nmol/L)	7.247 $\pm$ 0.413c	36.335 $\pm$ 1.024c	16.838 $\pm$ 1.191bc	28.977 $\pm$ 0.904ab	20.457 $\pm$ 0.969bc

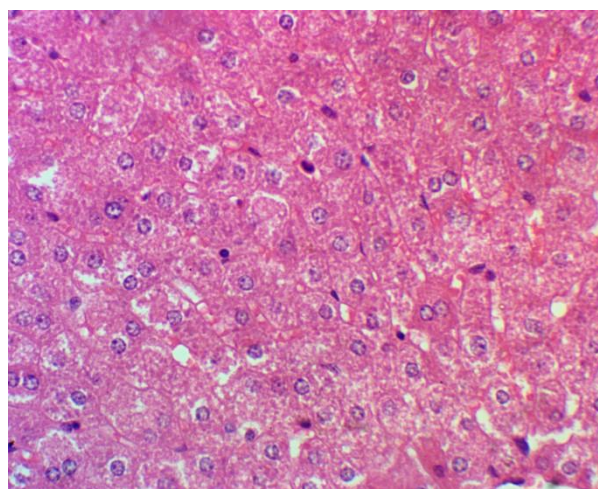
Means sharing similar letters in a row are statistically non-significant ( $P>0.05$ )

( $P\leq 0.01$ ) maintenance of the normal serum ALT, AST and ALP levels (Table 3).

**Oxidative status:** Serum TOS and MDA level was significantly increased ( $P\leq 0.01$ ) in isoniazid treated group as compared to control. Date palm treatment showed significant decrease ( $P\leq 0.01$ ) in TOS and MDA. Treatment with date palm showed significant ( $P\leq 0.01$ ) increase in serum TAC and catalase level as compared to isoniazid alone treated group (Table 4).

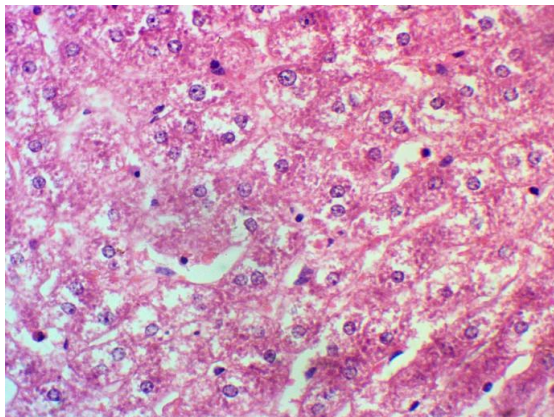
**Histopathological analysis:** Liver tissue samples of control group exhibiting normal parenchyma in appearance. Hepatocytes appeared normal containing normal nuclei with chromatin material (Fig. 1a). In isoniazid treated group II hepatic parenchyma exhibiting mild to moderate degree of congestion. Nuclei of hepatocytes are condensed and pyknotic. Sinusoidal spaces are not prominent indicating mild degree of cell swelling. Individual cell necrosis at few places and mild degree of biliary cellular hyperplasia are the prominent features (Fig. 1b). Silymarin and *P. dactylifera* treated group exhibiting hepatocytes are normal in appearance. Mild degree of congestion is present in parenchyma. Nuclei of hepatocytes are normal however cytoplasm is hazy in appearance which is indicating mild

degree of cell swelling. Sinusoidal spaces are normal (Fig. 1c & d).

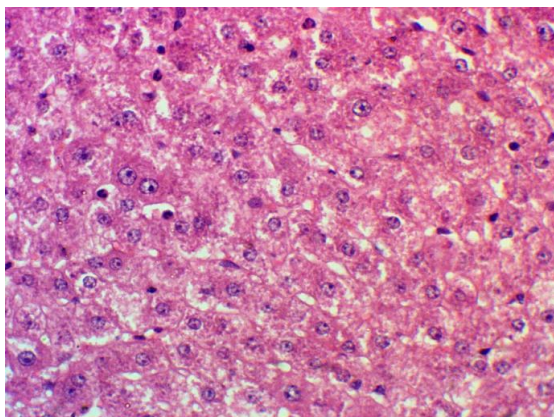


**Figure 1a. Hepatocytes of Control group indicating a normal lobular architecture of liver of rabbit (H & E, 40X).**

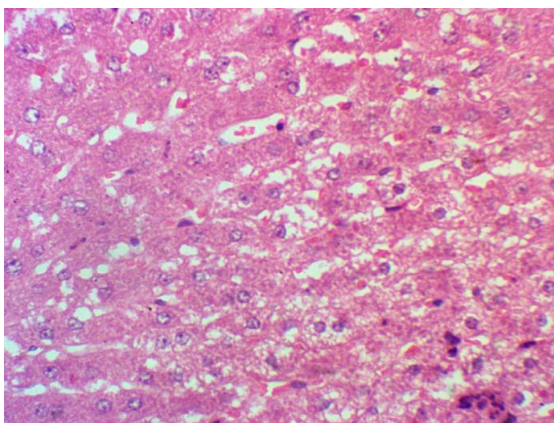




**Figure 1b.** Liver of rabbit treated with INH 100mg/kg body weight with daily oral administration for 14 days (H & E, 40X).



**Figure 1c.** Liver of rabbit treated with INH 100mg/kg body weight and silymarin 100mg/kg body weight with daily oral administration for 14 days (H & E, 40X).



**Figure 1d.** Liver of rabbit treated with INH 100mg/kg body weight and *P. dactylifera* 300mg/kg body weight with daily oral administration for 14 days (H & E, 40X).

## DISCUSSION

The number of patients diagnosed with tuberculosis are increasing globally. Prolonged treatment with antitubercular agents exposed the patients to hepatotoxic effects (Steel *et al.*, 1991). In tuberculosis treatment isoniazid is used as first line agent but it is the main drug which causes hepatotoxicity (Huang *et al.*, 2002). Isoniazid is metabolized to acetylisoniazid in body by hepatic enzyme *N*-acetyltransferase-2. Then, it is hydrolyzed into acetylhydrazine and isonicotinic acid by cytochrome P450 enzymes (Huang *et al.*, 2003). *Phoenix dactylifera* L. (ajwa khajur) belongs to family Arecaceae is a good source of minerals, fiber, carbohydrates, proteins, antioxidants and vitamins (Al-Orfi *et al.*, 2012). Herbal formulations have gained importance due to their efficacy and fewer side effects compared to the synthetic drugs. These effects are due to the presence of different phytochemicals like flavonoids, saponins, terpenoids which are reported to have antidiabetic and antioxidant potential (Mallhi *et al.*, 2014).

Marked alterations in blood cells count demonstrated hematotoxicity in this experiment. Total white blood cells (TWBC), platelets, red blood cells (RBCs), hemoglobin (Hgb) and hematocrit (Hct) were significantly ( $P \leq 0.01$ ) decreased with isoniazid treatment as it causes bone marrow depression which consequently decreases blood cells count. Treatment with *P. dactylifera* increases blood cells count due to its hematopoietic activity and its positive effects on peripheral blood parameters. Whereas, non-significant ( $P \geq 0.01$ ) difference in mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH) and mean corpuscular hemoglobin concentration (MCHC) was observed.

Hepatoprotective activity of *Phoenix dactylifera* is evident as serum level of hepatic function markers i.e. ALT, AST and ALP were significantly ( $P \leq 0.01$ ) reduced as compared to isoniazid alone treated groups which suggest that hepatic damage caused by free radicals generated by toxic dose of isoniazid. Treatment with *P. dactylifera* prevented liver damage as it contains wide variety of flavonoids and vitamins which exert antioxidant effect and scavenge free radicals generated by isoniazid as observed.

For normal cell functioning there must be a balance between oxidative stress and antioxidant status. Increased oxidative stress under certain pathophysiological conditions exposes the cell to deleterious damaging effect (Harma *et al.*, 2005). Total oxidative status (TOS) and catalase level significantly ( $P \leq 0.01$ ) increased total antioxidant capacity (TAC), catalase (CAT) and malondialdehyde (MDA). The values of total oxidative status and malondialdehyde increased due to increased generation of reactive oxygen species and resulted hepatocellular damage while total antioxidant capacity and catalase activity were decreased in isoniazid treated groups as compared to normal control. The treatment with silymarin and

*P. dactylifera* fruit aqueous extract prevented these parameters to deviate from normal level due to its antioxidant activity.

Isoniazid metabolites cause increase in oxidative stress which has cell damaging potential (Sarich *et al.*, 1995). Necrosis of tubular epithelial cells was observed in isoniazid treated group while performing histopathological studies. Treatment with standard hepatoprotective drug i.e. silymarin and treatment with *P. dactylifera* prevented the hepatotoxicity. The flavonoids and vitamin C present in *P. dactylifera* are major hepatoprotective agents which prevented the hepatic injury and release of liver specific enzymes. So, gross analysis showed that hepatic injury caused by isoniazid to liver of rabbits was markedly prevented with the use of *P. dactylifera*.

**Conclusion:** Biochemical analysis and histopathological studies showed that aqueous extract of *Phoenix dactylifera* fruit have hepatoprotective effect against isoniazid induced toxicity. Hematological parameters showed that *Phoenix dactylifera* fruit has hematopoietic activity. It is also concluded that the tested dose of plant has no side effects. Therefore, present study suggests that *Phoenix dactylifera* has promising hepatoprotective activity.

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