International Journal of Chemical and Biochemical Sciences
(ISSN 2226-9614)

Journal Home page: www.iscientific.org/Journal.html

Metabolic Effects of Ajwa Date Extract against Gentamicin-Induced Hepatotoxicity and Nephrotoxicity in Male Rats

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Abstract

This study elaborates on the chemical composition of Ajwa date extract and investigates its different doses against gentamicin (GM)-induced hepatotoxicity and nephrotoxicity in an animal model. GM-administered rats presented various abnormalities in the kidneys and liver. The major hazards included significantly alleviated antioxidant enzyme activities including glutathione peroxidase (GPx), superoxide dismutase (SOD), and catalase (CAT). However, oral administrations of alcoholic Ajwa date extracts (25, 50, 75, and 100 mg/ kg body weight) to GM-injected rats significantly attenuated malondialdehyde (MDA), total cholesterol, creatinine, triglycerides, low-density lipoprotein cholesterol (LDL-C), and urea in the serum. Contrarily, the results demonstrated a significant rise in high-density lipoprotein cholesterol (HDL-C) serum levels and reduced glutathione (GSH) concentration in the liver. The Ajwa date extract also significantly enhanced antioxidant and liver enzyme activities. The blood glucose levels did not significantly vary in all treatments. The chemical analysis revealed the presence of glucose, polyphenols, copper, flavonoids, potassium, fructose, chromium, phosphorus, and Zinc in Ajwa dates. A significant improvement in studied parameters was noted after Ajwa date extract administrations. The study concludes that significant antioxidant activities of Ajwa date extract facilitate the reversal of GM-related abnormalities in serum oxidative biomarkers, kidneys, and liver. Thus, the positive outcomes could be attributed to the flavonoids and polyphenols-based antioxidant activities.

Keywords: Ajwa date extract; gentamicin; lipid profile; Antioxidant enzymes polyphenols; flavonoids; fruit.

Full length article **Corresponding Author*, e-mail: *hmhmd@qu.edu.sa* https://doi.org/10.62877/37-IJCBS-24-26-20-37

1. Introduction

The hazards of chemical drugs have surged over the years [1]. International medical conferences are increasingly suggesting the exploration of natural plant remedies (extracts and powders) to cure diseases. The suggestions have received positive responses from several countries [2-3]. Natural nutrients might exert therapeutic and preventive impacts on chronic illnesses [3-4]. Ajwa dates (*Phoenix dactylifera* L. var. Ajwa) of Saudi Arabia are rich in fiber, bioactive molecules, and nutrients [5-6]. These dates containing sterols, glycosides, flavonoids, and polyphenols are an important source of vitamins, energy, essential minerals (iron and potassium), carbohydrates, proteins, and dietary fiber [5-7-8]. Multiple studies have reported cardioprotective, antioxidant, gastrointestinal protection, antiviral, hypolipidemic, antifungal, anti-inflammatory, antibacterial, anticancer, and antidiabetic properties of Ajwa dates [2-5-9-11]. Therefore, the exploration of Ajwa dates and extracts' potential for disease treatments has increased in recent decades [2-12-13]. Ajwa dates known to alleviate cisplatin-induced mice nephrotoxicity [14]. Ajwa extract-based rat kidney protection from UV-induced injury has been reported as well [15]. Al-Qarawi et al., [16] have demonstrated corrective effects of date extract on ethanol-associated gastric ulcers in rats. The rat (Rattus Norvegicus) liver protection efficacy of Ajwa dates against Meloxicam effects has also been recognized [17]. Gentamicin (GM) is a highly potential drug against Gram-negative bacterial infections. However, nephrotoxicity is a key drawback of GM administrations. Therefore, this study examined the chemical composition of Ajwa date extract and investigated its amelioration efficacy against GM-induced hepatotoxicity & nephrotoxicity in rats.

2. Materials and Methods

2.1. Dates and Chemicals

Ajwa dates (Alia variety: 10 kg) were obtained from a date palm farm in Madinah, Saudi Arabia during the harvest season of 2023. All chemicals were purchased from Sigma-Aldrich (St. Louis, USA) whereas commercial Kits were obtained from Nanjing Jiancheng Bioengineering Company (Nanjing, China) and Bio-Merieux Laboratory Reagents and Products (France).

2.2. Preparation of Ajwa Extract

Similar fruits (maturity stage, size, and color) weighing about 6-8 g were selected for the experiments. The fruits were carefully washed with water, and the pulp was manually separated from the seeds. The pulp (5 kg) was oven-dried (60 °C) for 2 days followed by homogeneous grounding in an automatic grinder. The obtained powder was stored (25 °C) in brown polyethylene bags until used. The method of Al-Farsi and Lee [10]. Was adopted to prepare date pulp extract. The extract was freeze-dried and stored (-20 °C) in a black bottle until rat administration.

2.3. High-performance Liquid Chromatography (HPLC) of Ajwa extract and Ajwa dates

HPLC apparatus (Agilent 1260 series) fitted with ZORBAX SB-C8 (internal diameter of 4.6 mm × 150 mm, 5 µm) column was used for the separation of metabolites. The mobile phase was comprised of water with TFA (0.01%, pH 2.9), and methanol at 1.5 mL/min flow rate.

2.4. Assessment of Mineral Elements

The standard procedure of AOAC [18] was followed for the mineral elements' assessment.

2.5. Animals and Experimental Design

Thirty-six male Albino Wistar rats (120-140 g) were purchased from the Animal House, College of Pharmacy, King Saud University, Riyadh, Saudi Arabia. The rats were kept in clean regular cages and acclimatized for one week [(23 ± 2 °C room temperature, 40-45% relative humidity, and 12 h light/ dark cycle)] on a basal diet. The guidelines of the College of Agriculture and Food, Qassim University, were followed for the animal experiments. The Ethics Committee of Qassim University approved the experimental protocol (Approval No. 21-04-10).

2.6 Experimental groups

Six random rat groups (6 rats/ group) were used in the experiments. The first negative control (NC) group was fed on the standard basal diet. The second positive control (PC) group received basal diet + GM. The third group was administered with basal diet +GM + 25 mg of Ajwa date extract. The treatment of the fourth group included basal diet + GM + 50 mg of Ajwa date extract. The fifth group was given basal diet + GM + 75 mg of Ajwa date extract. The sixth group was administered with basal diet + GM + 100 mg of Ajwa date extract. Oral administration was followed for the Ajwa date extract whereas intramuscular GM (80 mg/kg/day) administrations were performed for 6 days.

2.7. Sample Collection

The rats were fasted after the experimental period (28 days) for 12 hours, anesthetized (Diethyl ether), and killed via cervical vertebrae dislocation. The collection of blood samples was followed by centrifugation, and serum collection that was stored (-20°C) until biochemical analysis. Then, rats were dissected to retrieve the livers of all experimental rats. Livers were washed (saline solution, 0.9%), and kept in plastic bags, which were tightly wrapped with tin foil and stored at -20°C.

2.8. Biochemical Analysis

Colorimetric assay kits were used to estimate lipid profiles (HDL, TC, and TG). The procedure of Friedewald et al. [19] followed to calculate LDL whereas the methodology of Young and Friedman, [20] adopted to assess creatinine and urea using commercial kits. Randox kits were used to measure ALP, ALT, and AST according to the method of IFCC 1980. Malondialdehyde (MDA) serum level was calculated by following the procedure of Ohkawa et al. [21].

2.9. GSH and Antioxidant Enzyme Activity in Liver

An Ultra-Turrax homogenizer was used to homogenize small pieces of liver tissue (1g) in ice-cold saline buffer (1:9 w/v, 0.85%, pH 7.4). The homogenates were centrifugated (1,000×g, 4°C) for 15 minutes, and supernatants were collected. Supernatants were used in liver antioxidative profile assays. Commercial kits (Nanjing Jiancheng Bioengineering Company, China) were used to measure SOD, GPx, CAT, and GSH levels in the liver homogenate. SOD and CAT assays were performed according to established protocols [47, 48], whereas the technique of Sedlak and Lindsay [22] photometrically demonstrated GSH concentration in the liver homogenates.

2.10. Statistical Analysis

SPSS (Statistical Package for the Social Sciences, 22nd edition) was employed for the data analysis. Statistical analysis involved arithmetic means, standard error, and one-way ANOVA (analysis of variance), whereas means were compared by Tukey's test at a significance level of $p \leq 0.05$.

3. Results and discussion

3.1. Results

3.1.1. Chemical Composition of Ajwa Dates and Extract

Table 1 presents the chemical composition of Ajwa dates and extracts, which contain several flavonoids and phenols such as Syringic acid, Gallic aid, Coumaric acid, Chlorogenic acid, Caffeic acid, and Cinnamic acid. Gallic acid had the highest concentration and abundance in Ajwa dates (218.68 µg/g) and extract (256.76). The concentration of most compounds was found to be higher in the Ajwa extract than in dates.

3.1.2. Sugar content of Ajwa dates and date extract

Fructose and glucose are common sugars of date fruits (Table 2). Glucose concentration in Ajwa dates was noted as 31.03 g/100 g, which was lower than the glucose level (34.47 g/100 g) of Ajwa extract. Similarly, Ajwa extract had a slightly higher fructose concentration (32.19 g/100 g) than Ajwa dates (30.07 g/100 g). Contrarily, sucrose was not detected in Ajwa dates and extract.

3.1.3. Mineral Content of Ajwa Ddates and Extract

Table 2 depicts the concentrations of mineral elements in Ajwa dates (mg/kg) and the extract (mg/l). Ajwa dates pulp

and extract contained large quantities of phosphorus (700 and 360), calcium (1290 and 380), potassium (4600 and 5900), sodium (100 and 930), and aluminum (10, 100) whereas nickel, zinc, cadmium, lead, and chromium noted in smaller quantities.

3.1.4. Serum Glucose Level

The serum glucose levels did not significantly vary in the different rat groups (Table 3). However, an insignificant rise was noted in the positive control group. The groups administered with different concentrations of Ajwa extract exhibited a gradual insignificant alleviation in glucose levels.

3.1.5. Serum Lipid profile

Table 3 demonstrates serum HDL, LDL, cholesterol, and triglycerides levels. GM administration alone significantly enhanced LDL, total cholesterol (TC), and triglycerides (TG) levels while simultaneously mitigating HDL levels. Contrarily, Ajwa extract-treated groups presented significant attenuation in LDL, TC, and TG levels with a rise in HDL levels.

3.1.6. GSH and Antioxidant Enzymes in Liver Homogenate and Serum MDA Levels

GM administration resulted in a significant decrease in reduced glutathione (GSH) levels in the liver homogenate (Table 4). However, different concentrations of Ajwa extract significantly improved liver GSH levels. Table 4 presents the superoxide dismutase (SOD), glutathione peroxidase (GPx), and catalase (CAT) enzymes activities in the liver. GM administration significantly reduced CAT, GPx, and SOD activities as compared to the normal control. Ajwa date extract treatment in different groups significantly enhanced these enzymes' activities in comparison to the positive control. MDA level remained significantly higher in the positive control (GM only) than in the negative control (Table 4). All concentrations of Ajwa date extract significantly decreased the MDA levels in all groups.

3.1.7. Treatment Impacts on Kidney Function

Table 5 depicts impact of different treatments on kidney function based on serum creatinine and urea concentrations. Creatinine & urea concentrations considerably increased in positive control group. However, Ajwa date extract treatment significantly improved parameters by reducing creatine & urea serum concentrations.

3.1.8. Treatment Impacts on Liver Function

Table 6 presents the liver function-related enzyme activities. ALP, ALT, and AST significantly increased post-GM administration. However, oral administration of Ajwa extract concentrations considerably attenuated these enzymes' levels. Thus, Ajwa extract's dose-dependent positive impact was noted in liver function-related enzymes.

3.2. Discussion

Gentamicin (GM) is commonly administered to investigate kidney damage in rats [23-25]. GM disintegrates into superoxide anions, hydroxyl radicals, water, and hydrogen peroxide [26-27]. These oxygenated metabolites have been linked to GM nephrotoxicity in several studies. The dates are well-known to contain biologically active compounds including beneficial antioxidants. Therefore, this study elucidated the health promotion and disease-curing (liver diseases and nephrotoxicity) efficacy of Ajwa dates and extract. Ajwa extract was found to be rich in flavonoids and polyphenols (Table 1). Polyphenols are considered the most effective antioxidants [28]. Ajwa extract intake did not yield significant differences among serum glucose levels of all rat groups. The group that received the highest Ajwa date extract dose (100 mg) demonstrated the lowest glucose level. Thus, a higher Ajwa extract dose could help in lowering serum glucose levels (Table 3), which is in line with the previous findings [29]. These positive impacts could be attributed to the flavonoids in Ajwa date extract, which affected the islet-β cells, and alleviated gastric emptying [30-31]. Moreover, phenols can inhibit α-glucosidase and α-amylase, and enhance glucose entry into the skeletal muscles [32].

The results revealed the presence of large amounts of flavonoids, phenols, glucose, minerals, and fructose in date pulp and extract (Table 1 and Table 2). Minerals participate in various functions in the body such as metabolism, maintenance of internal balance, and protection against common disorders and diseases [33]. The antioxidant potential of polyphenols facilitates their wide usage as therapeutic and prophylactic agents against various diseases [34-36]. GM-induced detrimental alterations in the liver tissues could lead to damage of liver cells and seriously disturbed fatty acid phosphorylation mechanism. The liver regulates total cholesterol level in blood by converting it into bile salts. Therefore, GM toxicity to the liver tissues can lead to hyperlipidemia condition [37]. The Ajwa date extract successfully ameliorated GM-associated hepatotoxicity by improving GSH and antioxidant enzyme levels in the liver. It explains the lipid profile improvement after Ajwa date extract administration. The results revealed a dose-dependent improvement in lipid profile in Ajwa date extract treated groups. Liver and kidney are highly susceptible to ROS (reactive oxygen species)-associated injury due to presence of long-chain polyunsaturated fatty acids in their lipid composition [38].

Rise in creatinine and urea levels indicates the kidneys' pathological changes. During this study, a significant dose-dependent reduction was noted in creatinine urea concentrations after Ajwa extract administration. Al-Qarawi et al., [16] have also reported protective efficacy of dates against renal toxicity. The significant attenuation of liver function enzymes' activities in the serum could be due to the hepatic cell membrane's reduced lipid peroxidation after Ajwa date extract treatment. The repair/ regeneration of injured hepatic cells could also be the reason behind this phenomenon [39].Antioxidant enzyme (SOD, GP_X, and CAT) activity was significantly improved post-Ajwa extract administration. All doses of Ajwa date extract yielded antioxidant enzyme enhancement in the liver whereas the maximum improvement was noted at the highest dose of 100 mg. Osman [40] and Alqarni [10] have also reported significantly increased antioxidant enzymes after Ajwa date extract administration. GM treatments often damage the liver's antioxidant defense, which lower CAT, GPx, and SOD activities characterize. However, treatment with Ajwa extract considerably ameliorated CAT, GPx, and SOD levels in rats. Ajwa extract administration also significantly increased the liver's GSH (reduced glutathione) levels. The highest GSH level was noted in the group treated with 75 mg of the Ajwa date extract.

Table 1. HPLC-based detection of phenolic and flavonoid compounds (µg/g) in Ajwa dates and Ajwa date extract.

No	Item	Type	Dates (µg/g)	Extract (µg/g)
1	Gallic aid	Phenolic acid	218.68	256.76
2	Chlorogenic acid	phenolic acid	20.74	23.38
3	Catechin	Polyphenol	ND	ND
4	Methyl gallate	Polyphenol	2.84	2.94
5	Caffeic acid	Phenolic compound	11.32	16.18
6	Syringic acid	Phenolic compound	2.26	5.20
7	Pyro catechol	Phenolic compound	6.19	7.90
8	Rutin	Phenolic compound	ND	ND
9	Ellagic acid	Phenolic compound	3.09	3.14
10	Coumaric acid	Phenolic compound	8.13	7.18
11	Vanillin	phenolic aldehyde	0.67	2.98
12	Ferulic acid	phenolic aldehyde	22.02	39.80
13	Naringenin	Polyphenol	ND	2.58
14	Daidzein	Isoflavone	1.97	2.42
15	Quercetin	Flavonoids	ND	ND
16	Cinnamic acid	Phenolic compound	3.97	2.41
17	Apigenin	Flavone	1.03	5.54
18	Kaempferol	Flavonoid	ND	ND
19	Hesperetin	Flavone	4.46	12.66

* ND= not detected

Table 2. Mineral and Sugar contents in Ajwa dates and Ajwa date extract.

	Date(mg/kg)	Extract mg/ L
Element		
Calcium	1290	380
Sodium	100	930
Potassium	4600	5900
Phosphorus	700	360
Copper	6	2
Aluminum	100	10
Zinc	4.5	3.6
Chromium	4	0.4
Lead	<0.05	0.1
Cadmium	<0.05	<0.001
Nickel	0.2	---
Sugar content		
Glucose	31.03	34.47
Fructose	30.07	32.19
Sucrose	ND	ND

* ND= not detected.

Table 3. Impact of Ajwa date extract on serum glucose and lipid profile.

Groups	Glucose (mg/dl)	Cholesterol (mg/dl)	Triglycerides (mg/dl)	HDL-CH (mg/dl)	LDL-CH (mg/dl)
NC	90.50 ± 3.42 ^{a}	100.8 ± 4.11 ^{b}	77.75 ± 4.11 ^{b}	40.50 ± 1.29 ^{a}	44.50 ± 1.29 ^{a}
PC	95.0 ± 4.40 ^{a}	128.5 ± 5.45 ^{a}	90.25 ± 4.27 ^{a}	36.75 ± 0.96 ^{c}	49.21±1.12 ^{b}
PC + 25 mg extract	92.0 ± 5.61 ^{a}	104.6 ± 7.40 ^{b}	80.80 ± 7.12 ^{b}	37.60 ± 1.95 ^{bc}	45.0 ± 1.58 ^{a}
PC + 50 mg extract	90.20 ± 6.98 ^{a}	100.6 ± 7.57 ^{b}	80.20 ± 2.68 ^{b}	39.40 ± 2.07 ^{ab}	43.60 ± 2.30 ^{a}
PC + 75 mg extract	86.75 ± 5.12 ^{a}	95.25 ± 5.56 ^{b}	75.25 ± 4.57 ^{b}	39.0 ± 0.82 ^{ab}	45.50 ± 1.29 ^{a}
PC + 100 mg extract	85.40 ± 5.94 ^{a}	99.40 ± 12.28 ^{b}	81.80 ± 6.76 ^{b}	39.60 ± 1.14 ^{ab}	44.20 ± 2.39 ^{a}

* Data represent means ± standard deviation. Means with different letters in the same column are significantly different at P≤0.05. NC= negative control that received only a standard diet. PC= positive control that received a standard diet and gentamicin.

Table 4. Impact of different treatments on antioxidant enzymes (CAT, SOD, GPx) and GSH in liver homogenate and serum MDA level.

Groups	Catalase (U/l)	SOD (U/l)	GPx (U/l)	MDA (nmol/l)	GSH (mg/l)
NC	345.5 ± 4.43 [a]	4.98 ± 0.17 [a]	37.75 ± 1.78 [a]	7.68 ± 0.68 [c]	29.0 ± 2.58 [a]
PC	274.8 ± 6.02 [d]	2.73 ± 0.22 [d]	17.63 ± 2.27 [c]	17.53 ± 1.12 [a]	16.90 ± 2.82 [b]
PC+ 25 mg extract	326.0 ± 5.48 [c]	3.72 ± 0.65 [c]	31.60 ± 3.78 [c]	12.14 ± 1.77 [b]	26.74 ± 2.38 [a]
PC + 50 mg extract	324.8 ± 4.76 [c]	4.44 ± 0.80 [abc]	31.68 ± 2.16 [b]	11.70 ± 2.88 [b]	26.92 ± 2.64 [a]
PC+ 75 mg extract	322.0 ± 5.89 [c]	3.85 ± 0.13 [bc]	30.68 ± 3.68 [b]	10.93 ± 1.72 [b]	28.75 ± 2.22 [a]
PC + 100 mg extract	336.2 ± 5.67 [b]	4.56 ± 0.43 [ab]	32.56 ± 3.01[b]	10.14 ± 3.0 [bc]	28.54 ± 1.20 [a]

* Data represent means ± standard deviation. Means with different letters in the same column are significantly different at P≤0.05. NC= negative control that received only a standard diet. PC= positive control that received a standard diet and gentamicin.

Table 5. Serum urea and creatinine levels in different treatments.

Groups	Urea (mg/dl)	Creatinine (mg/dl)
NC	27.90 ± 1.56 [d]	0.40 ± 0.04 [c]
PC	49.15 ± 2.52 [a]	0.86 ± 0.07 [a]
PC + 25 mg extract	36.28 ± 1.86 [b]	0.55 ± 0.09 [b]
PC + 50 mg extract	38.64 ± 2.11 [b]	0.54 ± 0.08 [b]
PC + 75 mg extract	36.70 ± 0.93 [b]	0.52 ± 0.07 [b]
PC + 100 mg extract	32.52 ± 2.11 [c]	0.50 ± 0.05 [b]

* Data represent means ± standard deviation. Means with different letters in the same column are significantly different at P≤0.05. NC= negative control that received only a standard diet. PC= positive control that received a standard diet and gentamicin.

Table 6. Impact of the different treatments on liver enzymes.

Groups	ALT (U/l)	AST(U/l)	ALP (U/l)
NC	37.50 ± 4.80 [b]	29.75 ± 2.50 [d]	105.0 ± 3.92 [c]
PC	49.0 ± 4.16 [a]	41.0 ± 2.58 [a]	135.0 ± 4.40 [a]
PC + 25 mg extract	38.60 ± 3.21 [b]	34.80 ± 4.66 [bc]	118.8 ± 11.37 [b]
PC + 50 mg extract	38.60 ± 3.29 [b]	34.0 ± 1.58 [b]	122.4 ± 4.72 [b]
PC + 75 mg extract	39.0 ± 5.77 [b]	31.0 ± 3.37 [cd]	113.0 ± 6.27 [bc]
PC + 100 mg extract	39.0 ± 5.61 [b]	28.0 ± 2.74 [d]	117.0 ± 11.31 [bc]

Data represent means ± standard deviation. Means with different letters in the same column are significantly different at P≤0.05. NC= negative control that received only a standard diet. PC= positive control that received a standard diet plus gentamicin.

The reduced GSH is the first physiological defense against free radicals. Phenolic compounds (vanillic, gallic, syringic, chlorogenic, ferulic, catechin, and coumaric acids) of Ajwa dates might have contributed to improved liver GSH levels [41]. Multiple studies have reported similar changes in serum metabolites and antioxidant enzymes [42-43]. It suggests the potential role of oxidative stress in GM toxicity. However, exact role of oxidative damage to liver antioxidant enzyme alterations remains unclear [44]. This study's results align with previous investigations also reported alleviation of GSH and antioxidant enzymes in liver against ROS.

4. **Conclusion**

The promising findings of this study encourage the investigations and applications of medicinal plants with higher antioxidant potential to counter drug toxicity. Ajwa dates are an important source of micro and macro elements. Therefore, the study also provides a scientific rationale for Ajwa date extract intake as a dietary protocol in the case of continuous chronic disease-curing drug administrations.

Author Contributions: Conceptualization, (W.E.A. and H.M.M.) ; Data curation, (W.E.A. and M.S.A.); Formal analysis, (O.A.A., S.S.A. and H. M.); Investigation, (O.A.A., S.M.A., and S.S.A.); Methodology, (O.A.A.); Resources, (M.S.A.); Software, (N.A.A.); Supervision, (H.M.M.) ; Validation, (S.A.A.); Writing – original draft, (S.A.A.); Writing – review & editing, (S.A.A. and N.A.A.). All authors have read and agreed to publish version of the manuscript.

Funding: The Researchers would like to thank the Deanship of Graduate Studies and Scientific Research at Qassim University for financial support (QU-APC-2024-9/1).

Institutional Review Board Statement: The Ethics Committee of Qassim University, Saudi Arabia, approved the experimental protocol (Approval No. 21-04-10).

Informed Consent Statement: Not applicable.

Data Availability Statement: Data are contained within the article.

Acknowledgments: The Researchers would like to thank the Deanship of Graduate Studies and Scientific Research at Qassim University for financial support (QU-APC-2024-9/1).

Conflicts of Interest: The authors declare no conflicts of interest.

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