Comparative Cardioprotection Analysis Of Phoenix Dactilyfera. Ajwa Dates (an Ace Inhibitor) with Captopril , In Clozapine Generated Cardiotoxic Rat Model

Faiza Khan,[1] Muhammad Faheem Anwer,[2] Qura-tul-Ain,[3] Wardah Anwar,[4] Irum Imran,[5] Saadia Shahzad Alam[6]

Abstract

Objective: To compare the cardioprotective effects of Ajwa dates fruit and pit extracts with Captopril in Clozapine induced cardiotoxic rats.

Material and Methods: Place and duration of study at Postgraduate Medical Institute Lahore for 21 days. In an experimental study, forty-eight rats were randomly divided into six groups of eight rats per group. Group 1 was healthy Control group. Myocarditis was induced in all other groups by Clozapine injection. Group 2 was diseased control group while groups 3 – 6 were given Captopril and Ajwa fruit and pit extracts respectively. After 21 days, blood samples were collected from heart and serum was used for further analysis. Tissue samples were collected by homogenizing hearts in phosphate buffer and supernatant used for biochemical testing. Oxidative stress parameters, CK-MB, GSH, GSH-Px, LDH and MDA were studied in all groups.

Results: LDH and CK-MB values of group 6, having captopril 5mg and ajwa fruit and pit both extract, was closest to the control group. Similarly GSH, GSH-Px and MDA values in group 6 were closest to the control group. P-Value came out to be significant for both comparisons hence ensuring the significance of the results.

Conclusion: Captopril and Ajwa dates have strong cardioprotective and antioxidant effects. Patients who are experiencing adverse effects, may benefit from the combination of Ajwa dates and low-dose captopril.

Keywords: LDH (Lactate dehydrogenase), CK-MB (Creatinine kinase), GSH,GSH-Px (Glutathione peroxidase), MDA (Malondialdehyde)

How to cite: Khan F, Anwer MF, Ain QU, Anwar W, Imran I, Alam SS. Comparative Cardioprotection Analysis of Phoenix Dactilyfera. Ajwa Dates (An Ace Inhibitor) with Captopril , in Clozapine Generated Cardiotoxic Rat Model. Esculapio - JSIMS 2024;20(03): *335-340*

DOI: https://doi.org/10.51273/esc24.25132039

Introduction

Clozapine has been extensively employed as a pharmacotherapeutic agent for managing psychosis over several decades. Its noteworthy efficacy, particularly in addressing refractory cases of schizophrenia, has been a subject of considerable recognition. A study has revealed its superior effectiveness compared to alternative antipsychotic medications in the context of treating resistant forms of schizophrenia.[1] While being a globally utilized pharmaceutical, it is imperative to acknowledge that clozapine is not without significant adverse effects. Agranulocytosis stands out as a prevalent adverse reaction associated with the drug, and additionally, myocarditis has been identified as a serious and noteworthy adverse effect in certain patient populations. These adverse events necessitate careful consideration and monitoring in clinical practice.[2]

In a recent research study, findings indicated a notable occurrence of cardiomyopathy, with an incidence rate ranging from 3-4%. These observations were specifi-

1,3. Pak Red Crescent Medical College, Dina Nath, Lahore
2. CMH Lahore Medical College, Lahore
4. Al Aleem Medical College Lahore
5. Amna Inayat Medical College Lahore
6. Shaikh Zayed Medical College Lahore

Correspondence:
Dr Faiza Khan: *email: drfaizakhan@hotmail.com Associate Professor Pharmacology. Pak Red Crescent Medical College, Dina Nath, Lahore.*

Submission Date: 25-06-2024
1st Revision Date: 15-07-2024
Acceptance Date: 05-09-2024

cally documented in a middle-aged female patient who was hospitalized for the management of myocarditis, an adverse event that manifested during the course of her clozapine treatment.[3]

Ajwa dates are renowned for their nutritional prowess. Revered for their myriad health benefits, these dates offer a multitude of advantages for human well-being on a significant scale.[4] Ajwa dates exert positive influences on hormone levels, red blood cells (RBCs), platelets, and hemoglobin (Hb). Functioning as antioxidants, they play a role in antihypertensive, anti-inflammatory, and anticancer activities. Moreover, they contribute to the prevention of allergies, diabetes, diarrheal diseases, and exhibit antibacterial properties, serving as effective agents against cancer.[5] Also at cellular and chemical level, Ajwa dates has been considered very beneficial in many medical conditions.[6] In accordance with research findings, Ajwa dates have demonstrated efficacy in lowering cholesterol levels, managing diabetes, exhibiting anticancer properties as previously discussed, and contributing to the control of cardiovascular diseases.[7] Extensive examination and practical observation have revealed that date seeds possess numerous beneficial properties. Specifically, on a cellular level, these seeds augment antioxidant activity by enhancing the functionality of relevant enzymes.[8] These attributes serve as a safeguard against the oxidative stress associated with myocardial injury. Additionally, a study involving pediatric cancer patients has substantiated that the consumption of Ajwa dates enhances their treatment efficacy and overall outcomes.[9] Furthermore, it is well-established that captopril is an extensively utilized medication for hypertension management, effectively alleviating the strain on cardiac tissue. This pharmaceutical agent belongs to the angiotensin-converting enzyme (ACE) inhibitors, a prominent class of antihypertensive drugs.[10]

Moreover, findings from a study conducted on diabetic rats demonstrated the drug's efficacy in efficiently reducing the apoptosis of myocardial cells induced by diabetes. The medication enhances the function of the renin-angiotensin system, thereby effectively improving ventricular function. The consequential impact of this drug holds a noteworthy position in the therapeutic approach to managing myocardial workload.[11] Despite the extensive positive effects of captopril in addressing cardiac issues, it is essential to acknowledge the presence of a range of adverse effects that impact various aspects of human health. Certain studies have indicated that it may contribute to fibrosis in the heart and lung tissues following a myocardial infarction.[12]

Several well-known adverse effects associated with captopril and related drugs include: paroxysmal cough occurring in approximately 10% of patients, proteinuria in almost 1% of patients, inadequate renal function in nearly 0.2% of patients, neutropenia/agranulocytosis, pruritic rash in about 0.7% of patients, altered taste or loss of taste in approximately 2-4% of patients, and, importantly, anaphylactic and allergic reactions observed in a majority of patients.[(13)] The objective of our study is to to explore the protective effects of various extracts of Phoenix dactylifera (AJWA DATES) on Clozapine's cardiotoxicities in rats.

Material and Method s

An experimental case control study. Place of study was Postgraduate Medical Institute Lahore. After taking the IRB approval No. F-39/NHU/Admin/IRB dated 06-11-2014. Sample size was 48 rats. Study duration was 21 days and Study subjects Forty eight rats were randomly divided into six groups with eight rats in each group. Sample size was Forty eight and Data Collection procedure. Forty eight rats were randomly divided into six groups with eight rats in each group. Group 1 was healthy Control group. Myocarditis was induced in all other groups by Clozapine injection intraperitoneally. Group 2 was diseased control group while groups 3 – 6 were given Captopril and Ajwa fruit and pits extracts respectively. After 21 days, blood samples were collected from heart and serum was used for further analysis. Tissue samples were collected by homogenizing hearts in phosphate buffer and supernatant used for biochemical testing. Myocardial damage was determined by Oxidative stress parameters LDH, CK-MB, MDA, GSH, GSH-Px were studied in all groups.

Table 1: *A tabular description of various animal groups and the diet/ treatment they were given.*

Groups	Diet/ treatment given
Group 1(healthy control)	Standard Laboratory diet
Group2(diseased control)	Clozapine Only
Group3	CLZ & CAP 5mg
Group4	CLZ & CAP 10mg
Group5	CLZ & Aq. ADFE + Aq. ADPE
Group6	CLZ & CAP 5mg & Aq. ADFE + Aq. ADPE

Mean	Control	CLZ	CLZ & CAP 5mg	CLZ & CAP 10mg	CLZ & aq. ADFE + aq. ADPE	CLZ & CAP 5mg & aq. ADFE + aq. ADPE	p-value
LDH(IU/L)	205.63±11.08	312.63±10.54	215.38±17.31	214.13±9.95	211.00±9.62	212.00±10.32	<0.001
	196.36-214.89	303.81-321.44	200.90-229.85	205.81-222.44	202.96-219.04	203.37-220.63	
CK-MB(IU/L)	240.25±10.24	332.13±3.76	252.75±8.07	206.50±7.46	242.38±16.77	252.25±7.96	<0.001
	231.69-248.81	328.98-335.27	246.01-259.49	200.26-212.74	228.36 -256.39	245.60-258.90	
MDA(µmol/g protein)	321.13±5.92	433.88±14.96	324.88±7.14	321.13±5.92	324.88±7.14	322.88±6.85	<0.001
	316.18-326.07	421.37-446.38	318.91-330.84	316.18-326.07	318.91-330.84	317.14-328.61	
GSH value (nmol/g protein)	34.75±1.67	17.63±1.19	31.13±1.13	34.25±1.83	31.50±1.51	33.75±1.49	<0.001
	33.35-36.15	16.63-18.62	30.18-32.07	32.72-35.78	30.24-32.76	32.51-34.99	
GSH-Px(IU/g protein)	27.50±1.20	16.13±1.73	24.88±2.90	26.00±3.02	26.00±2.62	26.25±2.61	<0.001
	26.50-28.50	14.68-17.57	22.45-27.30	23.47-28.53	23.81-28.19	24.07-28.43	

Data was analysed by SPSS Version 20.0. Comparison was performed between various groups using Tukey's test. P-value of 0.05 was kept as reference and the results were compared to check for the significance of the outcome.

Results

The study was conducted on 48 rats divided into 6 groups (8 rats/group). Group 1 was the healthy control group. Group 2 was diseased control group, it was given CLZ only. Group 3 and 4 were given CLZ and Captopril 5 and 10 mg respectively. Group 5 was given CLZ and Aq. ADFE & Aq. ADPE. While Group 6 was given CLZ and Captopril 5mg plus Aq. ADFE and Aq. ADPE.

The overall difference among groups was found significant with $p<0.001$. In group-wise comparison, it was noted that group 2 had significantly higher LDH levels as compared to all other groups with $p<0.05$. All groups other than group 2 had significantly lower levels as compared to other groups. The group 3, 4, 5 and 6 had no significant difference from each other. For CK-MB, the overall difference was found significant with p-value <0.001. In group-wise comparison, it was observed that the group 1 had significantly lower CK-MB levels. The group 3 had insignificant difference with group 4, while 2, 5 and 6 had significantly higher levels of CK-MB as compared to group 4. Group 3 had no significant difference from group 5 and 6 but had significantly higher levels as compared to group 1. The group 5 and 6 had no difference from each other.

The mean MDA levels were noted and found highest were also noted and found to be and highest for CLZ injected group 2.433.88umol/g protein (p-value <0.001). The values of groups 3 and 5 were comparable to each other and closer to control group (324.88 umol/g). The value of group 4 was the closest to control group (321.13 umol/g). Value of group 6 was found closer to group 1 (322.88 umol/g). The GSH value was in reverse order in comparison to previous variables and mean value was highest for group 1 and lowest for group 2, and were recorded to be 17.6±1.2 and 34.8±1.7 respectively. Again group 4 had relatively closer mean values to group 1. Group 3 had significantly lower levels than group 4. After overall significance the group wise comparison revealed that group 4 had no significant difference from group 1 while all other groups had significantly lower values as compared to group 1.

The GSH-Px levels had almost similar results as for GSH. The table and figure below show the mean levels of GSH-Px levels for all groups. The overall difference was significant and in group-wise comparison. The group 1 had significantly higher values than all. Group 2 had significantly low levels, while the group 3-6 had no significant difference from group 1. The mean values of biochemical parameters of aforementioned groups (Table-1) are illustrated by bar charts. The overall difference among groups was found significant with $p < 0.001$. After 21 days of injections of Clozapine, group 2

(I) Group	(J) Group	LDH (IU/L)	CK-MB (IU/L)	MDA (µmol/g protein)	GSH value (nmol/g protein)	GSH value (nmol/g protein)
Control	CLZ	0.000	0.000	0.000	0.000	0.000
	CLZ & CAP 5mg	0.567	0.138	0.951	0.000	0.280
	CLZ & CAP 10mg	0.701	0.000	1.000	0.984	0.819
	CLZ & aq. ADFE + aq. ADPE	0.941	0.998	0.951	0.001	0.819
	CLZ & CAP 5mg & aq. ADFE + aq. ADPE	0.885	0.169	0.998	0.760	0.906
CLZ	CLZ & CAP 5mg	0.000	0.000	0.000	0.000	0.000
	CLZ & CAP 10mg	0.000	0.000	0.000	0.000	0.000
	CLZ & aq. ADFE + aq. ADPE	0.000	0.000	0.000	0.000	0.000
	CLZ & CAP 5mg & aq. ADFE + aq. ADPE	0.000	0.000	0.000	0.000	0.000
CLZ & CAP 5mg	CLZ & CAP 10mg	1.000	0.000	0.951	0.002	0.938
	CLZ & aq. ADFE + aq. ADPE	0.975	0.306	1.000	0.996	0.938
	CLZ & CAP 5mg & aq. ADFE + aq. ADPE	0.992	1.000	0.997	0.012	0.867
CLZ & CAP 10mg	CLZ & aq. ADFE + aq. ADPE	0.995	0.000	0.951	0.008	1.000
	CLZ & CAP 5mg & aq. ADFE + aq. ADPE	0.999	0.000	0.998	0.984	1.000
CLZ & aq. ADFE + aq. ADPE	CLZ & CAP 5mg & aq. ADFE + aq. ADPE	1.000	0.359	0.997	0.046	1.000

(Diseased control) had highest levels of LDH and CK-MB isoenzymes, 312.6±10.5 and (332.13IU/L) respectively as compared to Groups 1,3,4, 5, 6 having (P-value < 0.001). *Tukey's test*

Discussion

Comparative cardio-protection analysis of Phoenix dactylifera (Ajwa dates), often hailed for its potential cardiovascular benefits, against captopril, a well-known ACE inhibitor, in a clozapine-generated cardiotoxic rat model presents an intriguing avenue for exploration.[14]

In a clozapine-induced cardiotoxic rat model, the table displays mean values and confidence intervals for a range of biochemical markers suggestive of heart injury and oxidative stress across treatment groups. Treatment options include combination therapies, clozapine (CLZ), clozapine with aqueous extract of Ajwa dates (ADFE) and Ajwa date pits (ADPE), clozapine with captopril (CLZ & CAP), and control. P-values show the statistical significance of differences between groups. Particularly in cardiac tissue, LDH is a sign of cellular injury. The increased LDH levels in the CLZ group as opposed to the control group point to myocardial damage brought on by clozapine. LDH levels were considerably lower in the captopril-treated group than in the CLZ group after both 5 mg and 10 mg of captopril, as well as ADFE and ADPE, suggesting possible cardioprotective effects. LDH levels were significantly reduced by the combined therapy as well, indicating possible synergistic effects.

Phoenix dactylifera, particularly Ajwa dates, is rich in bioactive compounds like polyphenols, flavonoids, and antioxidants, which have demonstrated potential in promoting cardiovascular health. These compounds exhibit anti-inflammatory, antioxidant, and vasodilatory properties, which could mitigate the adverse effects of cardiotoxicity induced by clozapine.[15]

An enzyme called CK-MB, which is frequently employed as a diagnostic for myocardial infarction, is released from injured myocardial cells. As with LDH, increased CK-MB levels in the CLZ group indicate clozapine-induced myocardial injury. When compared to CLZ alone, treatment with captopril—especially at a dose of 10 mg—as well as ADFE and ADPE dramatically decreased CK-MB levels, suggesting cardioprotective benefits. The combo therapy significantly decreased the levels of CK-MB as well.

On the other hand, captopril, a conventional ACE inhibitor, is widely prescribed for managing hypertension and heart failure. Its mechanism of action involves inhibiting the angiotensin-converting enzyme, thereby reducing the production of angiotensin II and promoting vasodilation, ultimately alleviating cardiac workload and improving cardiac function.[16] Lipid peroxidation

and oxidative stress are indicated by MDA. The clozapine toxicity-induced increase in oxidative stress in cardiac tissue is indicated by the considerably higher MDA levels in the CLZ group as compared to the control. MDA levels were significantly reduced after treatment with captopril, ADFE, and ADPE, indicating potential antioxidant effects. MDA levels were significantly reduced by the combined therapy as well, suggesting possible synergistic or additive antioxidant effects.

While GSH-Px is an enzyme that catalyzes the reduction of hydrogen peroxide and organic hydroperoxides by GSH, GSH itself is a significant antioxidant. The CLZ group's significantly lower GSH and GSH-Px levels in comparison to the control group suggest compromised antioxidant defense systems in the context of clozapine-induced cardiotoxicity. GSH and GSH-Px levels were significantly elevated after treatment with captopril, ADFE, and ADPE, indicating a possible restoration of antioxidant ability. GSH and GSH-Px levels were also significantly elevated by the combo therapy. In a clozapine-generated cardiotoxic rat model, where clozapine-induced myocardial damage is mimicked, evaluating the efficacy of Ajwa dates versus captopril offers valuable insights into their comparative cardioprotective effects. Parameters such as cardiac biomarkers (troponins, creatine kinase), histopathological changes in cardiac tissue, oxidative stress markers, and cardiac function assessments (echocardiography, ECG) can be monitored to gauge the extent of cardio-protection provided by each intervention.[17]

Ajwa dates, with their rich array of bioactive compounds, may exert cardioprotective effects by scavenging free radicals, reducing oxidative stress, modulating inflammatory pathways, and enhancing endothelial function. These mechanisms could potentially mitigate clozapine-induced cardiotoxicity by attenuating oxidative damage, inflammation, and apoptotic pathways in cardiac tissue.[18] Overall, the comparative cardio-protection analysis of Ajwa dates and captopril in a clozapine-generated cardiotoxic rat model holds promise for uncovering novel therapeutic interventions for managing drug-induced cardiovascular complications, while also shedding light on the mechanisms underlying the cardioprotective effects of natural dietary constituents versus conventional pharmacotherapy.[19]

Conclusion

The results of this comparative study indicate that in a rat model of cardiotoxicity induced by clozapine, captopril and Ajwa dates, especially in the form of aqueous extracts, have strong cardioprotective and antioxidant effects. Additionally, combination therapy including Ajwa dates and captopril shows synergistic benefits that may improve antioxidant activity and cardio-protection. These findings encourage future research into Ajwa dates as a natural adjunct therapy for the treatment of drug-induced cardiotoxicity, possibly providing a more secure and comprehensive method of managing cardiovascular health. Patients whose tolerance to larger doses of captopril is being limited by adverse effects may benefit from the combination of Ajwa dates and low-dose captopril.

Conflict of Interest: *None*

Funding Source: *None*

References

1. Siskind D, McCartney L, Goldschlager R, Kisely S. Clozapine v. first-and second-generation antipsychotics in treatment-refractory schizophrenia: systematic review and meta-analysis. The British Journal of Psychiatry. 2016;209(5):385-92.
2. De Berardis D, Serroni N, Campanella D, Olivieri L, Ferri F, Carano A, et al. Update on the adverse effects of clozapine: focus on myocarditis. Current drug safety. 2012;7(1):55-62.
3. Ronaldson K, Fitzgerald P, McNeil J. Clozapine-induced myocarditis, a widely overlooked adverse reaction. Acta Psychiatrica Scandinavica. 2015;132(4):231-40.
4. Parvez R, Gautam A, David J. Study on antioxidant activity and health benefits of Ajwa dates. Energy. 2021; 277:14.
5. Al-Alawi RA, Al-Mashiqri JH, Al-Nadabi JS, Al-Shihi BI, Baqi Y. Date palm tree (Phoenix dactylifera L.): natural products and therapeutic options. Frontiers in plant science. 2017;8:845.
6. Zhao C-N, Meng X, Li Y, Li S, Liu Q, Tang G-Y, et al. Fruits for prevention and treatment of cardiovascular diseases. Nutrients. 2017;9(6):598.
7. Saryono S, Rahmawati E, Proverawati A, Hisni D. Effect of antioxidant status and oxidative stress products in pre-menopausal women after treatment with date seed powder (Phoenix dactylifera L.): a study on women in Indonesia. Pak J Nutr. 2017;16(6):477-81.

8. Hamad I, AbdElgawad H, Al Jaouni S, Zinta G, Asard H, Hassan S, et al. Metabolic analysis of various date palm fruit (Phoenix dactylifera L.) cultivars from Saudi Arabia to assess their nutritional quality. Molecules. 2015;20(8):13620-41.
9. Tastemur Y, Gumus E, Ergul M, Ulu M, Akkaya R, Ozturk A, et al. Positive effects of angiotensin-converting enzyme (ACE) inhibitor, captopril, on pentylenetetrazole-induced epileptic seizures in mice. Tropical Journal of Pharmaceutical Research. 2020; 19(3): 637-43.
10. Dong LY, Yao LP, Zhao J, Jin KK, Qiu XX. Captopril inhibits calpain-mediated apoptosis of myocardial cells in diabetic rats and improves cardiac function. Molecular Medicine Reports. 2018;18(2):2300-6.
11. Sahin B, Ergul M. Captopril exhibits protective effects through anti-inflammatory and anti-apoptotic pathways against hydrogen peroxide-induced oxidative stress in C6 glioma cells. Metabolic Brain Disease. 2022:1-10.
12. Rajaram CU. Review of Captopril Drug Formulation, Mechanism of action, Dosage, Use and Adverse drug reactions. Research Journal of Pharmaceutical Dosage Forms and Technology. 2021;13(2):157-60.
13. Sabbah MF, Alshubali F, Baothman OA, Zamzami MA, Shash L, Hassan IA. Cardioprotective Effect of Ajwa Date Aqueous Extract on Doxorubicin-Induced Toxicity in Rats. Biomedical and pharmacology Journal. 2018;11(3):1521-36.
14. Khalid S, Arshad M, Saad B, Imran M, Ul HB, Ain TT, et al. Cardioprotective effects of dates. International Journal of Biosciences. 2020;6655:110-23.
15. Aboubakr EM, Rushdi MI, Babekir EY. Improving Captopril cardioprotective activities against Doxorubicin induced cardiotoxicity by co-administration with allicin. SVU-International Journal of Medical Sciences. 2024;7(1):49-61.
16. Kanniah G, Kumar S. Clozapine associated cardiotoxicity: issues, challenges and way forward. Asian Journal of Psychiatry. 2020;50:101950.
17. Jubayer F, Kayshar S, Rahaman M. Effects of Ajwa date seed powder on serum lipids in humans: A randomized, double-blind, placebo-controlled clinical trial. Journal of Herbal Medicine. 2020;24:100409.
18. Prabhakaran S. In Vitro and In Vivo studies on Anti-Cataract Activity of Phoenix Dactylifera L., Seed against Naphthalene Induced Cataractogenesis in Rats: Nandha College of Pharmacy, Erode; 2021.

Authors Contribution

FK: Conceptualization of Project
MFA: Data Collection
QTA: Literature Search
WA: Statistical Analysis
II: Drafting, Revision
SSA: Writing of Manuscript