Journal of Chemical Health Risks

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JCHR (2024) 14(2), 2048-2053 | ISSN:2251-6727

# Apigenin Inhibits A-Amylase Activity in Alloxan Induced Type-2 Diabetic Rats

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*(Received: 07 January 2024 Revised: 12 February 2024 Accepted: 06 March 2024)*

**KEYWORDS**

Apigenin, α-amylase, flavonoids, blood glucose, antioxidant activity.

**ABSTRACT**

**Purpos**e: to study the inhibitory activity of alpha amylase, antidiabetic, antioxidant effects of flavonoid Apigenin in diabetic rats.

**Methods**: The effect of Apigenin on blood glucose and antioxidant activities were estmated in alloxan induced diabetic and treated rats. The **α**-amylase activity in the serum was assessed after oral administration of 7th day, 14th day, and 21st days treatment of Apigenin.

**Results**: The Apigenin was reduced serum glucose, **α**-amylase levels and increased antioxidant levels significantly (P<0.05) in Apigenin alone and combination with Metformin treated groups.

**Conclusion**: The present investigation concludes that flavonoid Apigenin, single drug and combination with Metformin was inhibited alpha-amylase activity, antioxidant and hypoglycemic effect in alloxan induced diabetes rats

## Introduction

Diabetes is a metabolic disorder, characterised by hyperglycaemia an insufficient insulin secretion from pancreatic Beta-cells. Treatment of Diabetes mellitus by oral hypoglycaemic drugs like Metformin is a biguanide that primarily reduces the hepatic glucose production and stimulates peripheral insulin sensitivity [1]. Diabetes mellitus was regulated by several natural source of plant and their extracts [2] and also hyperglycaemia has been regulates the phytochemicals and flavonoids[3].

Apigenin is a flavonoid has posses' antihyperlipidemic, antidepressant, antidiabetic properties[4].and the glucose-induced mitochondrial reactive oxygen species (ROS) are responsible for hyperglycemia and vascular complications[5] The approaches are to control the hyperglycemia at different mode of actions by increases the glucose uptake and inhibits gluconeogenesis [5] and reduce the hyperglycemias after postprrandial [6]. The inhibition of carbohydrates hydrolyzing enzyme α-amylase is a one of that catalyses the breakdown of starch to glucose[7]. So that inhibition of alpha amylase enzymes leads to a control the blood glucose [8]. Therefore in this study investigating the Apigenin efficacy in regulating blood glucose levels and inhibition of alpha amylase in alloxan induced type 2 diabetes.

## Materials and Methods

### Experimental animals

Male Wister rats weight 150-180gm were procured and housed in well aerated cages at normal atmospheric temperature (25±5 °C) and 12- hours light/dark cycle and kept for about 7 days observation before the experimentation and free access to water and standard diet of *ad libitum.* All animal procedures were followed accordance with the ethical Committee guidelines (006/1963/PO/Re/S/17/CPCSEA).

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**Chemicals**

Alloxan (A), Apigenin (APG) was purchased from Sigma (USA) Metformin as gift sample from Natco.pharma. Hyderabad, India.

**Induction and treatment of diabetes**

Diabetes mellitus was induced by using a single dose of alloxan (120 mg/kg *i.p*) injection for atleast 16 hours after fasting rats, Blood glucose levels were estimated after 48 hours of alloxan and confirmed the development of diabetes mellitus by increasing of blood glucose > 200mg/dl. All the diabetic rats were selected for this study, divided them as groups and treated with Apigenin 40mg/kg (*p.o)* (APG40), Apigenin 80mg/kg (*p.o)* (APG80) and Metformin 60mg/kg (*p.o)* [9] for 21days.

**Experimental design**

The rats were divided into 7 groups (n =6) and treated as follows:

Group I: normal control (Sod. carboxymethyl cellulose-1% (CMC), orally).

Group II: diabetic control (Alloxan)

Group III: diabetics + Apigenin (APG40)

Group IV: diabetics + Apigenin(APG80)

Group V: diabetics + Metformin (60mg/kg)

Apigenin plus Metformin 60mg/kg combination treated groups are IIIa & IVa. Which were received APG40 and APG 80.[10].

**Biochemical evaluation**

Blood samples was collected from eye retro orbital region of all 7 groups of rats on 0day, 7$^{th}$ day, 14$^{th}$ day and 21$^{st}$ days after treatment, centrifuged the blood samples at 1000 rpm for 15 minutes and separated the serum, estimated the blood glucose[11] and Serum antioxidant [12], Superoxide dismutase (SOD) [13] and α-amylase [14] levels were estimated.

**Statistical analysis**

The data has Mean ± S.D., Statistical comparisons were made by one-way analysis of variance (ANOVA) and data considered significant when the *p* values were lower than 0.05.

**Results**

The effects of Apigenin on blood glucose levels of (0day, 7$^{th}$ day, 14$^{th}$ day and 21 days) Control, Diabetic, Apigenin and Metformin treated rats results were represented in Figs. 1 and 2. The results of SOD, TAS levels (21days after Apigenin treatment) were represented in Table 1. α-amylase inhibitory activity (0 day, 7$^{th}$ days, 14$^{th}$ days and 21days) is represented in figs 3& 4 . Flavonoid Apigenin 40mg/kg, Apigenin80mg/kg alone and Apigenin combination with Metformin treated groups were significantly ($p$ < 0.001) reduced the blood glucose levels. The flavonoid APG40, APG80 doses were significantly ($p$ < 0.001) increased the SOD, TAS levels and reduced α-amylase activity.

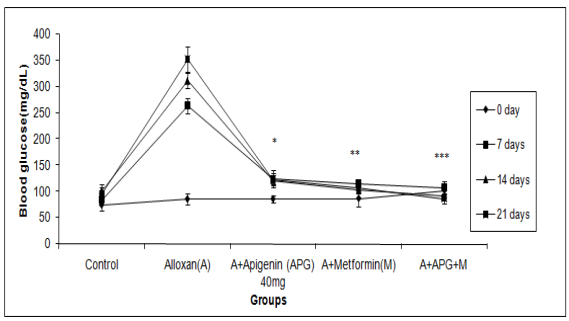


**Figure 1:** Blood glucose concentrations (mg/dl) of Control, Diabetic, Apigenin 40 and Apigenin40 (APG40) +Metformin (60mg/kg) groups., values presented Mean±SD( n=6) (The significant values as *****p<0.05, ****** p<0.01, ******* p<0.001,Compared control vs. diabetic and treated groups)

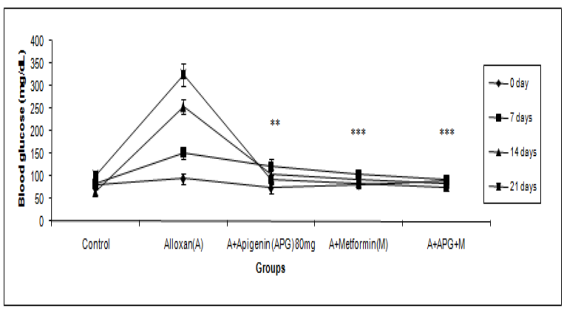


**Figure 2:** Blood glucose concentrations (mg/dl) of Control, Diabetic, Apigenin 40 and Apigenin80 (APG80) +Metformin (60mg/kg) groups., values

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presented Mean±SD( n=6) (The significant values as *p<0.05, ** p<0.01, *** p<0.001,Compared control vs. diabetic and treated groups).

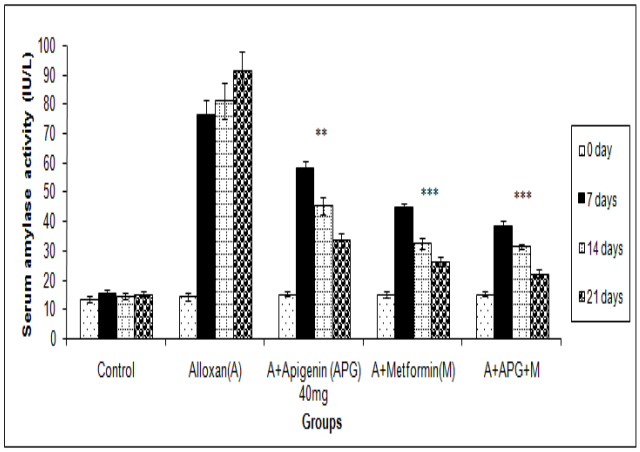


**Figure 3:** serum α-amylase inhibitory activity (IU/L) of Control, Diabetic, Apigenin 40 and Apigenin40 (APG40)+Metformin (60mg/kg) groups., values presented Mean±SD( n=6) (The significant values as *p<0.05, ** p<0.01, *** p<0.001,Compared control vs. diabetic and treated groups)

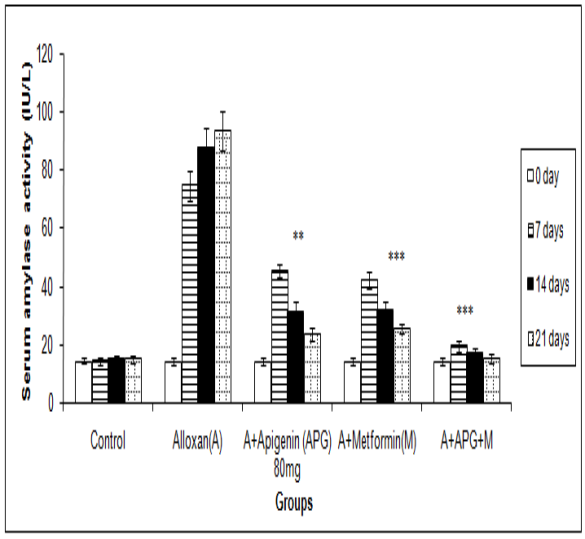


**Figure 4:** serum α-amylase inhibitory activity (IU/L) of Control, Diabetic, Apigenin 80 and Apigenin80 (APG80) +Metformin (60mg/kg) groups., values presented Mean±SD( n=6) (The significant values as *p<0.05, ** p<0.01, *** p<0.001,Compared control vs. diabetic and treated groups).

**Discussion**

Many flavonoids have been reported to reduce the glucose production from carbohydrates and glucose absorptions. Alpha amylase is an enzyme which is catalyses the hydrolysis of 1, 4-glucosidic linkages of starch, glycogen and oligosaccharides into sugars which can readily absorption in intestine. So inhibition of alpha amylase enzyme activity in digestive tract is being considered to be effective in controlling diabetes .

**Table 1:** Effect of Apigenin 40mg , Apigenin 80mg and Metformin (60mg/kg) alone and combinations for 21 days treatment on serum SOD and Total antioxidant status (TAS) of control, diabetic and treated groups. (Data values are Mean ± SD, n = 6)

| **Groups/ Parametes** | **SOD(IU)** | **Total antioxidant status(TAS) ( nM of ascorbic acid))** |
|---|---|---|
| **Control** | 38.61±10.26 | 25.89 ± 9.41 |
| **Alloxan(A)** | 11.3±2.82 | 5.2±1.11 |
| **A+** Apigenin 40 (APG) | 17.3±11.1* | 8.3±1.68* |
| **A+Metformin 60mg/kg(M)** | 22.6±8.25* | 9.7±3.25* |
| **A+APG40+M** | 26.23±0.36* | 12.26±3.56* |
| **A+** Apigenin 80 | 30.31±9.1** | 19.42±1.41** |
| **A+APG80+M** | 36.57±12.5*** | 22.11±3.5*** |

(Data values significant as follows *p<0.05, ** p<0.01, *** p<0.001, compared with control vs diabetic and treated groups)

In our study results has been indicates that inhibition of alpha amylase levels with flavonoids treatment, these findings were support of Tadera et al. (2006)[15], has reported that inhibition of alpha-glucosidase and alpha amylase with flavonoids therapy. Another study[16], reported that flavonoids control the carbohydrates degradation by inhibition of alpha-amylase[17] hence, reduces the glucose levels in diabetic patients [18].

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JCHR (2024) 14(2), 2048-2053 | ISSN:2251-6727

Inhibition of α-amylases can limit the hyperglycemia of post-prandial by delaying the hydrolysis of carbohydrates and their absorption [19].

The present study results reveals that decreased the blood glucose with flavonoid APG, these study support of Ali et al. (2006), reported that antioxidant and hypoglycaemic effects of favonoids [20-21].

In present study elevated the alpha amylase levels due to cause of reactive oxygen species (ROS) and inflammatory mediators in diabetes mellitus which were controlled to normal by treatment with flavonoids and combination with Metformin. The indirect mechanism of flavonoids and metformin inhibited the alpha amylase levels [22] these findings were support of [23], reported that flavonoids has been antioxidant and anti-inflammatory effects, which are protects the pancreatic β-cells from inflammatory mediators[24-27]. The present study indicates that flavonoids posse's antidiabetic as well as inhibiting the alpha amylase activity in type 2 diabetic rats. Free radical scavenging activity and reduced hepatic lipidperoxidations by Apigenin[28-31]. The Apigenin may have potential agent to regulate the hyperglycemias in diabetes mellitus by inhibition of alpha amylase and hepatic protection.Therefore further investigations needs for its possible use in human.

## Conclusion

Our results shows that oral administration of Apigenin has been a beneficial effect on the alloxan-induced diabetes mellitus by reducing the hyperglycaemia, inhibition of α-amylase activity and improving the antioxidant status. This study suggests that the diabetes mellitus may be prevented by flavonoids like Apigenin administration,

## Acknowledgements

The authors are thankful to Chaitanya Deemed to be University, Warangal, Telangana, India., for providing the facility for research work.

## Conflict of Interest

Authors have no conflict of interest.

## References

1. Ali H, Houghton PJ and Soumyanath A. Alpha-Amylase inhibitory activity of some Malaysian plants used to treat diabetes; with particular reference to *Phyllanthus amarus. J. Ethnopharmacol.* **107**: 449 (2006);.DOI: 10.1016/j.jep.2006.04.004
2. Ami Naik , Sherif Babatunde Adeyemi, Bhavin Vyas, Ramar Krishnamurthy. Effect of co-administration of metformin and extracts of Costus pictus D.Don leaves on alloxan-induced diabetes in rats. Journal of Traditional and Complementary Medicine., **12** :269 (2022). DOI: 10.1016/j.jtcme.2021.08.007
3. Bailey CJ. Biguanides and NIDDM. Diabetes Care.;**15**(6):755(1992)..DOI: 10.2337/diacare.15.6.755
4. Blios, M.S. Antioxidant determination by the use of stable free radical. *Nature.* **26**:1199 (1958). http://dx.doi.org/10.1038/1811199a0.
5. Andrade-Cetto A, Becerra-Jiménez J, Cárdenas-Vázquez R. Alfa- glucosidase-inhibiting activity of some Mexican plants used in the treatment of Type 2 diabetes. J Ethnopharmacol;**116**(1):27 (2008). DOI: 10.1016/j.jep.2007.10.031
6. David SH, Bell MB. Type 2 diabetes mellitus: What is the optimal treatment regimen? *Am J Med*, **116**: 23 (2004). DOI: 10.1016/j.amjmed.2003.10.017
7. De boer VCJ, Dihal AA,Van der woude H, Arts ICW,Wolffram S, Alink GM,Rietjens IMCM, keijer J, Hollman PCH: tissue distribution of quercetin in rats and pigs. *J nutr*, **135**: 1718 (2005). DOI: 10.1093/jn/135.7.1718
8. Della-Logia, A., Ragazzi, E. and Tubaro. Anti-inflammatory activity of benzopyrones that are inhibitors of cyclo-and lipo-oxygenases. *Pharmacol. Res. Commun.*, **20**: 91 (1988). DOI: 10.1016/s0031-6989(88)80849
9. Funke I and Melzig MF. Traditionally used plants in diabetes therapy-phytotherapeutics as inhibitors of α-amylase activity. *Braz. J. Pharmacogn.* **16**: 1 (2006). https://doi.org/10.1590/S0102-695X2006000100002

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10. Jung UJ, Lee M-K, Park YB, Kang MA ET Choi MS. Effect of citrus flavonoids on lipid metabolism and glucose-regulating enzyme mrna levels in type-2 diabetic mice. *Int. J. Biochem. Cell. Biol.* **38**: 1134 (2006). doi: 10.1016/j.biocel.2005.12.002.
11. Kaiser N, Sasson S, Feener EP, Boukobza N, Higashi S, and Moller DE. Differential regulation of glucose transport and transporters by glucose in vascular endothelial and smooth muscle cells. *Diabetes*, **42**:80 (1993). DOI: 10.2337/diab.42.1.80
12. Kim YM, Jeong YK, Wang MH, Lee WY and Rhee HI. Inhibitory effect of pine extract on alpha-glucosidase activity and postprandial hyperglycemia. *Nutrition*, **21**: 756 (2005). DOI: 10.1016/j.nut.2004.10.014
13. Kim, H. P., Mani, I. and Ziboh, V. A. Effects of naturally-occuring flavonoids and bio-flavonoids on epidermal cyclooxygenase from guinea-pigs. Prostaglandins Leukot. *Essent. Fatty Acids*, **58**: 17 (1998). DOI: 10.1016/s0952-3278(98)90125-9
14. Kotowaroo MI, Mahomoodally MF, Gurib-Fakim A and Subratty AH. Screening of traditional antidiabetic medicinal plants of Mauritius for possible alpha-amylase inhibitory effects in vitro. *Phytother. Res.* **20**: 228 (2006). DOI: 10.1002/ptr.1839
15. Kwon YI, Jang HD and Shetty K. Evaluation of *Rhodiola crenulata* and *Rhodiola rosea* for management of type II diabetes and hypertension. *Asia Pac. J. Clin. Nutr.* 2006; **15**: 425 (2006).
16. Lean MEJ, Noroozi M, Kelly I, Burns J, Talwar D, Sattar N, Crozier A. Dietary flavonols protect diabetic human lymphocytes against oxidative damage to dna. *Diabetes*, ; **48**:176 (1999). DOI (2006).doi: 10.2337/diabetes.48.1.176
17. Lee, J. S. (2006) Effects of soya protein and genistein on blood glucose, antioxidant enzyme activities and lipid profile in streptozotocin induced diabetic rats. *Life Sci*., **13**: 1578 (2006) DOI: 10.1016/j.lfs.2006.06.030
18. Li, W., Dai, R. J., Yu, Y. H., Li, L., Wu, C. M., Luan, W. W., Meng, W. W., Zhang, X. S., Deng, Y. L. (2007) Antihyperglycemic effect of *Cephalotaxus sinensis* leaves and GLUT-4 translocation facilitating activity of its flavonoid constituents. *Biol. Pharm. Bull*.**30**: 1123(2007). DOI: 10.1248/bpb.30.1123
19. Lo Piparo, E.; Scheib, H.; Frei, N.; Williamson, G.; Grigorov, M.; Chou, C.J. Flavonoids for controlling starchdigestion: Structural requirements for inhibiting human alpha-amylase. *J. Med.Chem.* **51**, 3555 (2008). DOI: 10.1021/jm800115x
20. Mahesh T, Menon VP. Quercetin alleviates oxidative stress in streptozotocin-induced diabetic rats. *Phytother res*, **18**: 123 (2004).. DOI: 10.1002/ptr.1374
21. Mai TT, Thu NN, Tien PG and Van Chuyen N. Alpha-glucosidase inhibitory and antioxidant activities of Vietnamese edible plants and their relationships with polyphenol contents. *J. Nutr. Sci. Vitaminol.***53**: 267 (2007). DOI: 10.3177/jnsv.53.267
22. Mira L, Fernandez M.T, and Santos M, Rocha R, Florencio M.H, Jennings K.R. Interactions of flavonoids with iron and copper ions: a mechanism for their antioxidant activity. *Free Radical Res*., **36**, 1199 (2002). DOI: 10.1080/1071576021000016463
23. Misra, H. P., and Fridowich, I. Superoxide dismutase, a photochemical augmentation assay. *Archives of Biochemistry and Biophysics*, **181**, 308 (1977).. DOI: 10.1016/0003-9861(77)90509-4
24. Sharma, K. K., Gupta, R. K., Gupta, S., Samuel, K. C. Antihyperglycaemic of onion: effect on fasting blood sugar and induced hyperglycemia in man. *Ind. J. Med. Res*. 1977; **65**: 422–429.
25. Shunkla, R., Anand, K., Prabhu, K. M., Murthy, P. S. Hypolipidemic effect of water extract of Ficus bengalensis in alloxan induced diabetes mellitus rabbits. *Ind. J.Clin. iochem*. **3**: 321 (2000).. DOI: 10.1016/s0378-8741(02)00366-5
26. Singh, J. P., Selvendirank, K., Banu, S. M., Padmavathi, R., Sakthisekeran, D. (2004) Protective role of apigenin on the status of lipid peroxidation and antioxidant defense against hepatocarcinogenesis in Wistar albino

rats. *Phytomedicine.* **11**: 309 (2004). doi: 10.1078/0944711041495254

27. Street HV and Close JR. Assay method of amylase activity. *Clin. chim. Acta*, **1**: 256 (1956). DOI: 10.1016/s0008-6215(00)84496-1
28. Tadera, K.; Minami, Y.; Takamatsu, K.; Matsuoka, T. Inhibition of alpha-glucosidase and alphaamylase by flavonoids. *J. Nutr. Sci. Vitaminol. (Tokyo),* ***52***, 149 (2006). DOI: 10.3177/jnsv.52.149
29. Trinder, P. Determination of blood glucose using an oxidaseperoxidase system with a non-carcinogenic chemogen. *J Clin Pathol;* **22**:158 (1969). doi: 10.1136/jcp.22.2.158.
30. Valcheva-Kuzmanova, S., Kuzmanov, K., Tancheva, S., Belcheva, A. Hypoglycemic and hypolipididemic effects of *Aronia melanocarpa* fruit juice in streptozotocin induced diabetic rats. *Methods Find. Exp. Clin. Pharmacol*., **29**: 101 (2007). DOI: 10.1358/mf.2007.29.2.1075349
31. Youn JY, Park HY and Cho KH. Anti-hyperglycemic activity of *Commelina communis* L.: inhibition of alpha-glucosidase. *Diabetes Res. Clin. Pract.***66**: 149(2004). DOI: 10.1016/j.diabres.2003.08.015