



Antioxidant and Alpha Amylase Inhibitory Activity of Apigenin in Alloxan Induced Diabetic Rats

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Authors' contributions

This work was carried out in collaboration between both authors. Both authors read and approved the final manuscript.

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ABSTRACT

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

Methods: The effect of Apigenin on blood glucose and antioxidant activities were estimated in alloxan induced diabetic and treated rats. The α -amylase inhibitory 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 the 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 increased antioxidant and inhibited the alpha-amylase activity and hypoglycemic effect in alloxan induced diabetes rats.

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1. INTRODUCTION

Diabetes is metabolic disorder, characterised by hyperglycaemia an inadequate insulin secretion from pancreatic Beta-cells. Treatment of Diabetes mellitus by oral hypoglycaemic drugs like Metformin is primarily reduces the hepatic glucose production and stimulates the 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 [3-8].

Apigenin is a flavonoid has posses' antihyperlipidemic, antidepressant, antidiabetic properties [9,10]. and glucose can induces the reactive oxygen species (ROS) from mitochondrial are responsible for hyperglycemias and vascular abnormality [11]. The approaches are to control the hyperglycemia at different mode of actions by increases the glucose uptake and inhibits gluconeogenesis [12] and reduce the hyperglycemias after postprandial [13-17]. The enzyme α -amylase is catalyses and breakdown of carbohydrates in to glucoses [18], So that inhibition of alpha amylase enzyme, leads to control the blood glucose levels [19]. Therefore in this study investigating the Apigenin efficacy in regulating blood glucose levels by inhibition of alpha amylase in alloxan induced type 2 diabetes.

2. MATERIALS AND METHODS

2.1 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, free access to water and standard diet of *ad libitum* and kept for about 7 days before the experiment.

2.2 Chemicals

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

2.3 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 was estimated after 48 hours of alloxan and confirmed the development of diabetes mellitus with increasing of blood glucose > 200 mg/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*) [20] for 21days.

2.4 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 [21].

2.5 Biochemical Evaluation

Blood samples was withdrawn from the eye retro orbital region of all 7 groups of rats, on 0 day, 7th day, 14th day and 21st days after treatment, centrifuged the blood samples at 1000 rpm for 15 minutes and separated the serum, estimated the blood glucose [22], Serum antioxidant [23], Superoxide dismutase (SOD) [24] and α -amylase [25] levels were estimated.

2.6 Statistical Analysis

The data has Mean $\pm$ S.D., Statistical comparisons were made by one-way analysis of variance (ANOVA) and Considered the significancy when the *p*values were lower than 0.05.

3. RESULTS

The effects of Apigenin on blood glucose levels of (0day, 7th day, 14th day and 21 days) Control, Diabetic, Apigenin and Metformin treated rats, results were represented in Figs. 1 and 2. The results of SOD, TAS (after 21days Apigenin treatment) were represented in Table 1. α -amylase inhibitory activity (0 day, 7th days, 14th days and 21days) is represented in Figs. 3 & 4.

Flavonoid Apigenin 40mg/kg, Apigenin80mg/kg alone and Apigenin combination with Metformin treated groups rats were significantly ($p < 0.05$) reduced the levels of blood glucose. The

flavonoid APG40,APG80 doses treated diabetic rats were significantly ($p < 0.001$) increased serum SOD, TAS levels and reduced α -amylase activity.

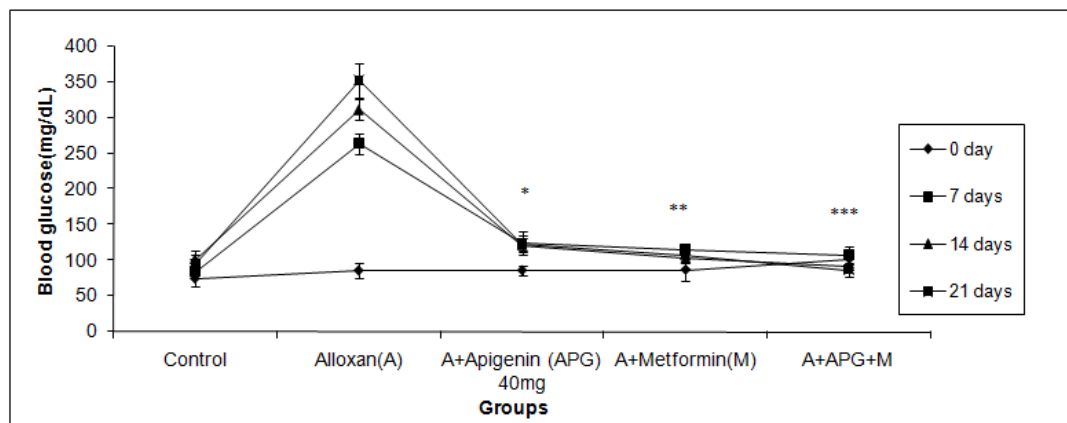


Fig. 1. Blood glucose concentrations (mg/dl) of Control, Diabetic, Apigenin 40 and Apigenin40 (APG40)+Metformin (60mg/kg) treated groups., (Mean $\pm$ SD(n=6)
(The p-values as * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$, Compared control vs. diabetic & treated)

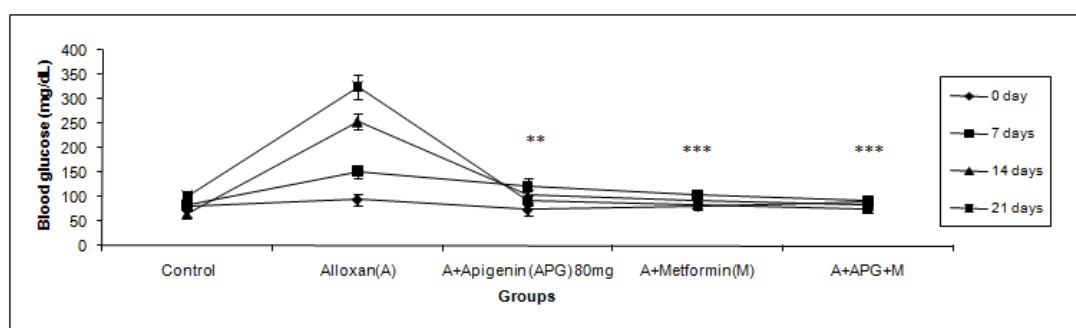


Fig. 2. Blood glucose concentrations (mg/dl) of Control, Diabetic, Apigenin 80 and Apigenin40 (APG80) +Metformin (60mg/kg) treated groups., (Mean $\pm$ SD(n=6)
(The p-values as * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$, Compared control vs. diabetic & treated)

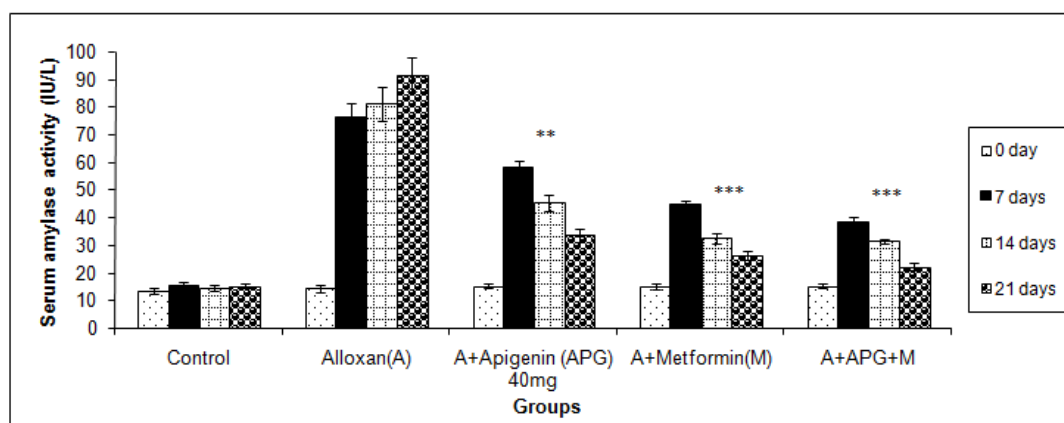


Fig. 3. Serum α -amylase inhibitory activity (IU/L) of Control, Diabetic, Apigenin 40 and Apigenin40 (APG40)+Metformin (60mg/kg) treated groups., Mean $\pm$ SD(n=6)
(The p- values * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$, Compared control vs. diabetic and treated)

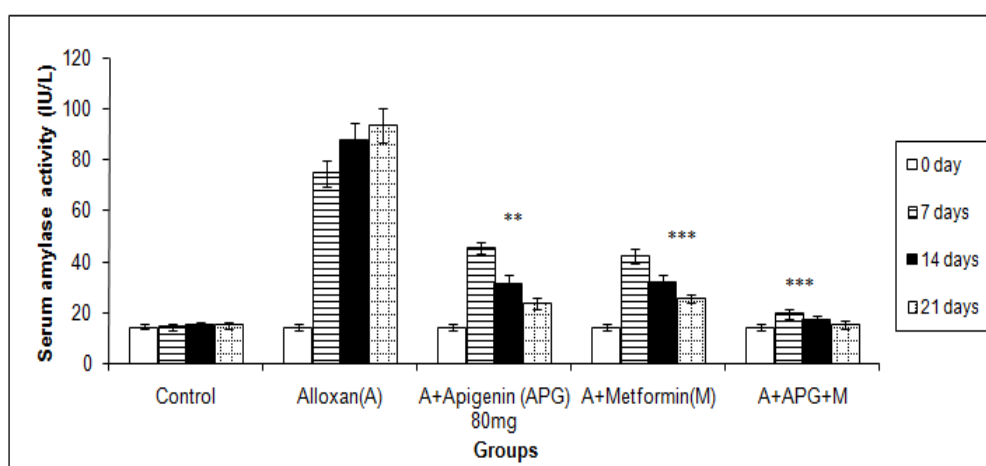


Fig. 4. Serum α-amylase inhibitory activity (IU/L) of Control, Diabetic, Apigenin 80 and Apigenin80 (APG80) +Metformin (60mg/kg) treated groups., Mean±SD(n=6)
(The p-values * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$, Compared control vs. diabetic and treated)

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. (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+Metformin60mg/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***

(p-values * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$, compared control vs diabetic and treated)

4. DISCUSSION

Many flavonoids have been reported to reduce the glucose production from carbohydrates and glucose absorptions. Alpha amylase is an enzyme which is converts the polysaccharides into sugars, they can ready to absorption in intestine. The inhibition of alpha amylase enzyme considered to reduces the hyperglycemia in diabetes.

In our study results has been indicates that inhibition of alpha amylase levels with flavonoids treatment, these findings were support of Tadera et al. [26-28], has reported that inhibition of alpha-glucosidase and alpha amylase with flavonoids therapy. Another study Lo Piparo et al. [29], reported that flavonoids control the carbohydrates degradation by inhibition of alpha-amylase[11] hence, reduces the blood glucose level [30]. Inhibition of α-amylases causes

delaying the hydrolysis of carbohydrates and their absorption can limit the hyperglycemia of post-prandial [31]. The present study results reveals that decreased the blood glucose with flavonoid APG, these study support of Ali et al. [32] reported that antioxidant and hypoglycaemic effects of favonoids [33-34].

“In present study elevated the alpha amylase levels due to cause of reactive oxygen species (ROS) and inflammatory mediators in diabetes mellitus, which was brought to normal by treatment with flavonoids alone and combination of Metformin” [35]. The indirect mechanism of flavonoids and metformin inhibited the alpha amylase levels [36] these findings were support of Mira et al. (2002) [37,38], reported that flavonoids has been antioxidant and anti-inflammatory effects, which are protects the pancreatic β-cells from inflammatory mediators [39-41]. The 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 (6). 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.

5. CONCLUSION

Our study results shows that the treatment of Apigenin alone and combinations has been effectively controlled the alloxan-induced diabetes mellitus by reduction of blood glucose, inhibition of α -amylase activity and improvement of antioxidant status by scavenging the free radical. We suggesting that the diabetes mellitus may be prevented by flavonoids contents like Apigenin

ETHICAL APPROVAL

All procedures followed accordance with the animal ethical Committee approval of viswabharati education society (006/1963/PO/Re/S/17/CPCSEA).

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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