Research Article

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POSITIVE EFFECTS OF FLAVONOIDS ON GAMMA-GLUTAMYL TRANSFERASE DYSFUNCTION AND ANTIOXIDANT DEFENCE DEPLETION INDUCED BY ALLOXAN IN RATS

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Abstract

Alloxan (A) is a highly toxic especially the liver and pancreas. The present study was designed to evaluate the antioxidant effect of flavonoids on alloxan-induced hepatic dysfunction, elevation of gamma glutamyl transpeptidase (GGT) and oxidative stress in rats. Adult male Wister rats were administered by alloxan monohydrate (120 mg/kg bw i.p route) This study revealed significant liver enzyme Gamma glutamyl transpeptidase(GGT) elevation, lipid peroxidation and a decline in antioxidant enzyme activities in the liver of alloxan-treated rats compared to control animals. flavonoids significantly increased ($p < 0.05$) antioxidant enzymatic activities (Glutathione, MDA), and reduced elevated blood glucose and GGT levels compared to those given alloxan alone. Thus, the oral administration of flavonoids, significantly ($p < 0.05$) improves alloxan-induced liver dysfunction and stress oxidant in rats. The present results shown that Flavonoids (quercetin, chrysin and hesperidin) has an antihyperglycaemic effect and consequently may alleviate elevation of GGT and oxidative stree in associated with alloxan-induced diabetes mellitus in rats.

Keywords: Alloxan, GGT, MDA, Glutathione.

Introduction

Serum gamma-glutamyl transferase (GGT), an enzyme responsible for extra cellular catabolism of glutathione and a marker of oxidative stress (Lee DH et al., 2004) has been shown to be associated with cardiovascular disease (Lee DH et al., 2006), peripheral arterial disease (Shankar A et al., 2008) and hypertension (Shankar A & Li J. 2007) Several prospective (Nakanishi N et al.,2003, Lee DH et al., 2003, Meisinger C et al., 2005, Lee DH et al.,2004, Perry IJ et al.,1998 and Nilssen O & Forde OH 1994) and cross-sectional studies (Kim DJ et al.,2005, Lee DH et al.,2003, Lim JS et al.,2007) have reported a positive association between serum GGT and diabetes mellitus. In this context, we studied the association between serum GGT and diabetes mellitus. Diabetes is usually accompanied by increased production of free radicals (Baynes JW&Thorpe SR 1999, Baynes JW.1991, Chang KC et al., 1993, Young IS1995) or impaired antioxidant defenses (Halliwell B

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&Gutteridge JM 1990, Saxena AK et al., 1993, McLennan SV et al., 1991). The superoxide anion radicals undergo dismutation to hydrogen peroxide, which if not degraded by catalase or glutathione peroxidase, and in the presence of transition metals, can lead to production of extremely reactive hydroxyl radicals (Jiang ZY et al., 1990, Wolff SP, Dean RT. 1987).Hyperglycemia is also found to promote lipid peroxidation of low density lipoprotein (LDL) by a superoxide-dependent pathway resulting in the generation of free radicals (Tsai EC et al., 1994, Kawamura M et al., 1994). While on the one hand hyperglycemia engenders free radicals, on the other hand it also impairs the endogenous antioxidant defense system in many ways during diabetes (Saxena AK et al., 1993) Antioxidant defense mechanisms involve both enzymatic and nonenzymatic strategies, common antioxidants and the cofactors they work in synergy with each other and against different types of free radicals. Vitamins A and E scavenge free radicals (Young IS et al., 1995, Laight DW et al., 2000, Abdel-Wahab MH& Abd-Allah AR 2000, Chow CK .1991, Asayama K et al., 1989). Diabetes mellitus (DM) is grossly reflected by profound changes in protein metabolism and by a negative nitrogen balance and loss of nitrogen from most organs. (Almdal TP&Vilstrup H. 1987) Increased urea nitrogen production in DM may be accounted for by enhanced catabolism of both liver and plasma proteins. (Jorda A et al., 1982) Management of DM without any side effects is still a challenge to the medical system. There is an increasing demand by patients to use natural products with antidiabetic activity, because insulin and oral hypoglycaemic drugs have undesirable side effects(Kameswara Rao B & Appa Rao CH. 2001) Medicinal plants are a good source of natural antioxidants by scavenging the reactive molecular species to prevent their reaching a target site.(Shanmugasundaram ERB et al ., 1990, Kaleem M et al., 2006, Kaleem M et al., 2005) It has been documented that show their hypoglycaemic effects associated with a significant alteration in the activity of liver and serum enzymes, like alkaline phosphatase (ALT), acid phosphatase and transaminases, aspartate aminotransferase (AST) and alanine amino transferase (ALT). Phytochemicals isolated from plant sources have been are used for the prevention and treatment of cancer, heart disease, DM, and high blood pressure (Waltner-Law ME et al., 2002). Polyphenolics, commonly found in fruits, vegetables and grains, provide chemoprotective effects to combat oxidative stress in the body and to maintain balance between oxidants and antioxidants in order to improve human health (Hsu, 2006). An imbalance caused by oxidant excess leads to oxidative stress, resulting in DNA and protein damages and increases the risk of degenerative diseases such as cancer (Hsu, 2006). The best described property of almost every group of flavonoids is their capacity to act as antioxidants (Nijveldt RJ et al., 2001). The flavones and flavonols (apigenin, luteolin, quercetin, rutin and others) seem to be the most powerful flavonoids for protecting a body against reactive oxygen species.

Materials and methods

Experimental animals

Male wister rats weighing about 150-180 g were used. They were purchased from the mahaveer enterprises hyderabad. They were kept under observation for about 15 days before the onset of the experiment to exclude any intercurrent infection. The chosen animals were housed in plastic well aerated cages at normal atmospheric temperature (25±5 °C) and normal 12- hour light/dark cycle. Moreover, they had free access to water and were supplied daily with standard diet of known composition *ad libitum*. All animal procedures were in accordance with the recommendations of the ethical Committee guidelines for Care and Use of Animals.

Chemical agents

Alloxan monohydrates (A) as well as quercetin (Q),chrysin(Ch). Hesperidin(Hesp) were purchased from Sigma Chemical Company (St.Louis, MO). Pioglitazone gift sample from Natco.pharma. Hyderabad, India.

Induction and treatment of diabetes

Diabetes was induced by a single injection of alloxan (120 mg/kg *i.p*) fasting for at least 16 hours, in freshly prepwered 1% sodium carboxy methyl cellulose, Blood glucose levels were measured 48 hours after alloxan administration, development of diabetes mellitus was proven by sustained hyperglycaemia (diabetic rats had glycaemia > 16 mmol/l).The diabetes developed rats were selected for study and treated with the flavonoids (quercetin(Q) 200mg/kg per oral(*p.o)*.,chrysin(Ch) 100 mg/kg *p.o*.. Hesperidin

(Hesp) 300 mg/kg *p.o*.)and pioglitazone 15 mg/kg *p.o* for 21 consecutive days (after alloxan administration).

Experimental design

The rats were randomly divided into 9 groups (n =6) as follows:

Group I: control animals (sod. carboxymethyl cellulose-1%, orally).

Group II: diabetic animals

Group III: diabetic animals + Quercetin (Q).

Group IV: diabetic animals + Chrysin (Ch)

Group V: diabetic animals + Hesperidin (Hesp)

Group VI: diabetic animals + Pioglitazone(P)

A flavonoids plus pioglitazone was administered in diabetic animals (groups IIIa-Va) which was received Q or Ch or Hesp at the same doses and schedule as groups III – V. The flavonoids were administered orally (by gavage) in sod carboxymethyl cellulose as a vehicle. Doses of flavonoids were assigned on the basis of experience from literature (Mahesh and Menon 2004; De Boer et al.2005).

Biochemical evaluation

Blood samples were collected from the tail vein of rats on 0 day, 7th day, 14th day and 21 days of control, diabetic and flavonoids treated diabetic rats and centrifuged at 1000 *g* for 15 min. In order to determine blood glucose levels (Trinder, P. 1969), Serum Gamma GT (Szasz G 1969) and Glutathione (Ohkawa. H. et al., 1979), & MDA (Beulter. D.V.1963) were estimated after 21 days treatment.

Statistical analysis

The data are presented as mean ± SD Statistical comparisons were made by one-way analysis of variance (ANOVA) and followed by Student-Neuman-Keuls as the *post hoc* test. Data were considered significant when *p* values were lower than 0.05.

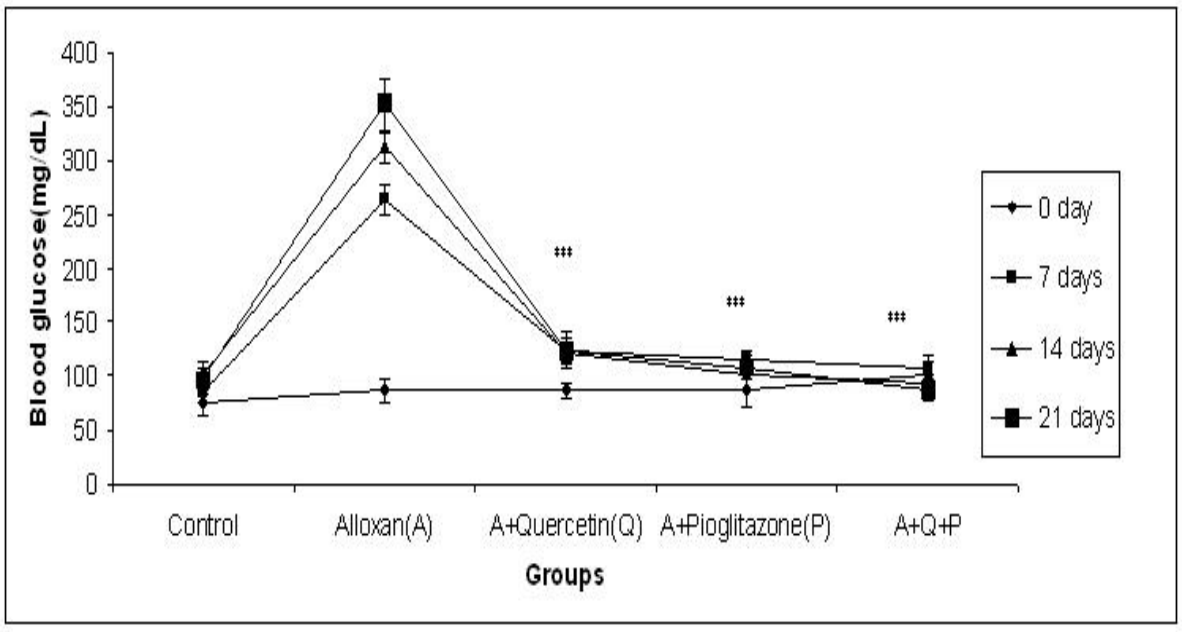

Fig. No. 01: Blood glucose levels of the control, alloxan-induced diabetic, and Alloxan+ Quercetin and treated combination with pioglitazone diabetic rats. Values presented represent Mean±SD of n=6 rats. (Data indicates significant values ***p<0.001vs control ,alloxan diabetic rats and treated with flavonoids)

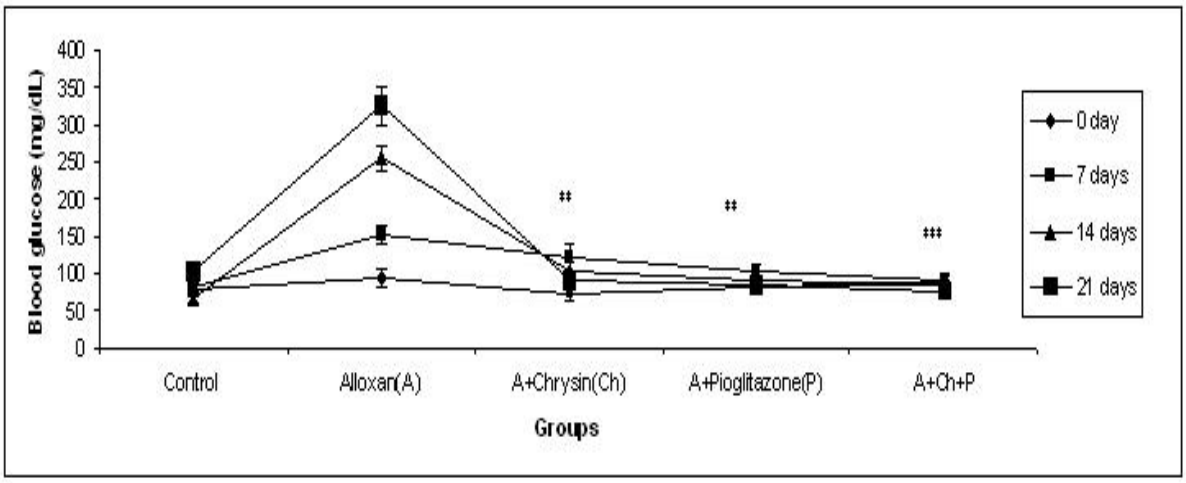

Fig. No. 02: Blood glucose levels of the control, alloxan-induced diabetic, Alloxan+ Chrysin and treated combination with pioglitazone diabetic rats. Values presented represent Mean±SD of n=8 rats. (Data indicates significant values **p<0.05, ***p<0.001vs control ,alloxan diabetic rats and treated with flavonoids)

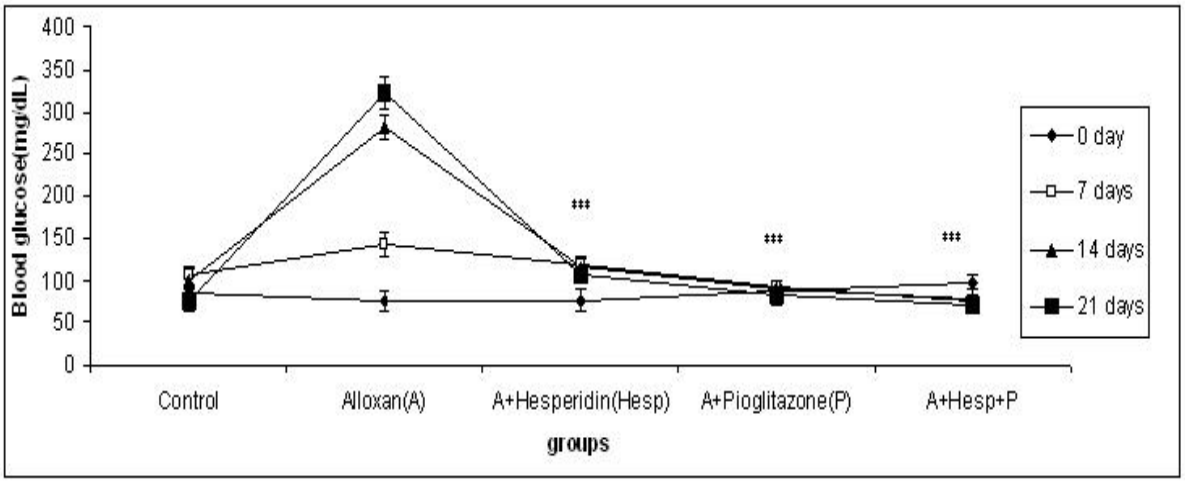

Fig. No. 03: Blood glucose levels of the control, alloxan-induced diabetic, Alloxan+ Hesperidin and treated combination with pioglitazone diabetic rats. Values presented represent Mean±SD of n=6 rats(Data indicates significant values ***p<0.001vs control ,alloxan diabetic rats and treated with flavonoids)

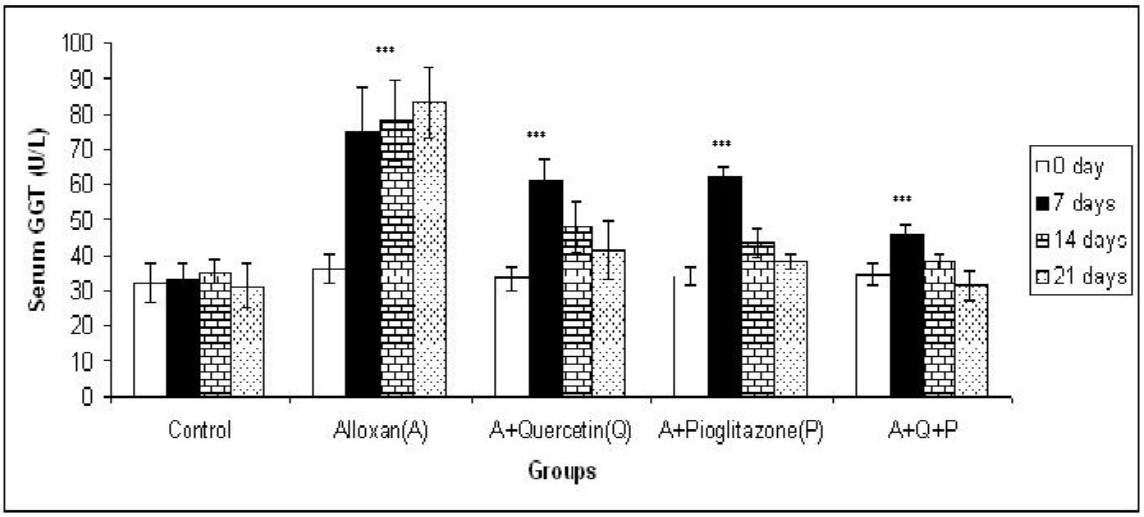

Fig. No. 04: serum GGT activity of the control, alloxan-induced diabetic and Alloxan+ Quercetin and treated combination with pioglitazone diabetic rats. Values presented represent Mean±SD of n=6 rats. (Data indicates significant values ***p<0.001vs control ,alloxan diabetic rats and treated with flavonoids)

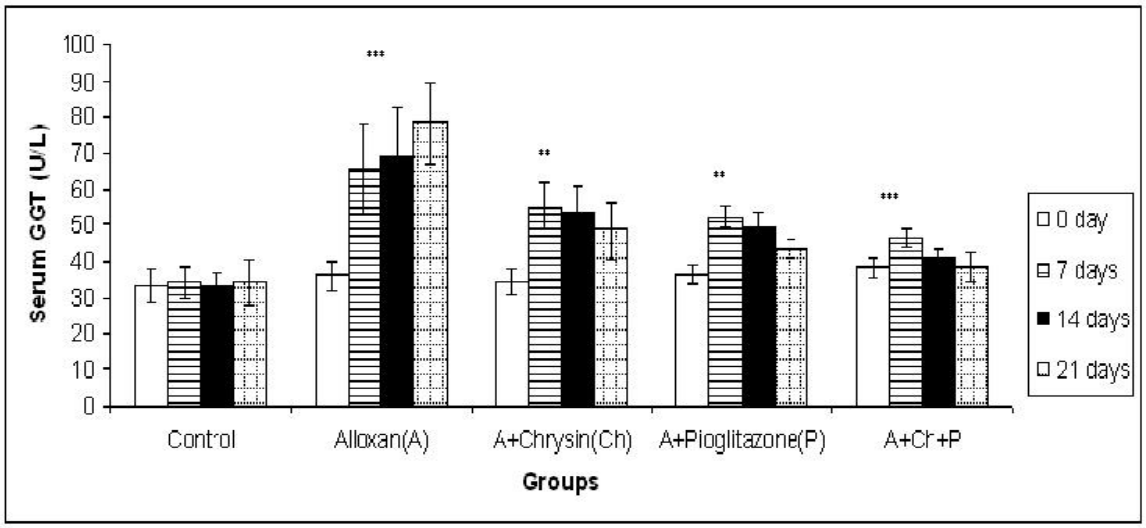

Fig. No. 05: serum GGT activity of the control, alloxan-induced diabetic and Alloxan+ Chrysin and treated combination with pioglitazone diabetic rats. Values presented represent Mean±SD of n=6 rats. (Data indicates significant values **p<0.05, ***p<0.001vs control ,alloxan diabetic rats and treated with flavonoids)

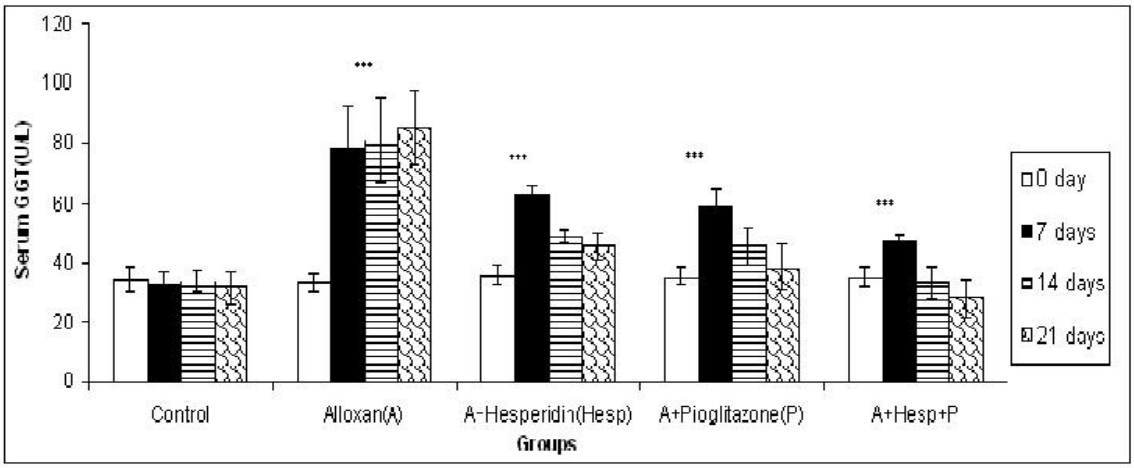

Fig. No. 06: serum GGT activity of the control, alloxan-induced diabetic and Alloxan+ Hesperidin and treated combination with pioglitazone diabetic rats. Values presented represent Mean ±SD of n=6 rats. (Data indicates significant values ***p<0.001vs control ,alloxan diabetic rats and treated with flavonoids)

Table No. 01: Effect of treatment with Quercetin(Q) (200mg/kg) Chrysin(Ch) (100mg/kg) and Hesperidin (Hesp) (300 mg/kg) and pioglitazone combination for 21 days on plama Glutathione and serum MDA levels of control and diabetic rats. (Values are Mean ± SD, n = 6)

Groups/Parametes	MDA(nM)	Glutathione(uM)
Control	6.1±0.92	384.67 ± 15.45
Alloxan(A)	13.8±0.82	103.2±12.71
A+Quercetin (Q)	7.3±2.1**	283.51±31.3**
A+Pioglitazone (P)	7.7±0.25*	296.43±25.2*
A+Q+P	6.7±0.36**	331.23±28.2**
A+Chrysin (Ch)	7.3±1.1**	285.31±22.51*
A+Ch+P	7.0±1.5**	321.46±21.42**
A+Hesperidin(Hesp)	6.7±1.6**	332.31±18.1**
A+Hesp+P	6.4±0.93**	361.26±18.2**

(Data indicates significant values *p<0.05, **p<0.001vs control and alloxan diabetic rats)

Results

The effects of flavonoids (quercetin, chrysin and hesperidin) on blood glucose levels (0 day, 7days, 14 days and 21days) of control, diabetic and flavonoids treated diabetic rats were summarized in Figs. 1, 2 and 3, and the data of MDA, Glutathione, levels were presented in Table 1. Gamma Glutamyl tranferase activity is represented in figs 4, 5 and 6.The quercetin, chrysin , hesperidin and pioglitazone combination had no effect on normoglycaemic animals. On the other hand, the alloxan-induced animals consistently exhibited hyperglycaemia. The simultaneous treatment with quercetin and combination with pioglitazone, significantly reduced the blood glucose levels in diabetics ($p < 0.001$). Completely controlled, elevation of serum glucose by Chrysin, hsperidin alone and combination with pioglitazone ($p < 0.01$). All the flavonoids significantly increased Glutathione levels, reduced MDA levels and the activity of Gamma glutamyl transpeptidase levels as per dose and schedules.

Discussion

The oxidative stress in the pathogenesis of diabetes and diabetic complications has been extensively studied for years both in animal models and in clinical setting. Certain studies have found increased lipid peroxides and/or ROS in different animal models of diabetes (Anjaneyulu et al., 2004; Mehta et al., 2006). However the results in clinical practice are not unambiguous and the usefulness of antioxidant therapy in diabetic patients is far from convincing (Newsholme et al., 2007). Alloxan, a chemical diabetogen, in the presence of glutathione is reduced *via* the alloxan radical into dialuric acid. During this redox cycling process, reactive oxygen species are formed that destroy β-cells in islets of langerhans. Moreover, it is suggested that transitional metals such as iron, zinc and copper may be involved in alloxan toxicity (Szkudelski, 2001). Previous studies that examined the association between serum Gamma glutamyl transpeptidase (GGT) and diabetes mellitus. Plausible mechanisms that support the association of serum GGT with diabetes mellitus include the role of GGT in oxidative stress (Lee DH et al., 2004), insulin resistance (Nilssen.O.& Forde .OH. 1994) and hepatic inflammation which impairs insulin signaling in liver and other organs (Vozarova B et al., 2002). The main study limitation is the cross-sectional nature of NHANES which limits making causal inferences in the association between serum GGT and diabetes. In addition, Gamma-GT catalyses the transfer of the γ-glutamyl group from γ-glutamyl peptides to another peptide or L-amino acids or to water, the estimation of γ-GT is a helpful adjunct in detecting hepatic damage(Lazo M et al., 2008). Previous reports highly significant elevation in the activity of γ-GT was observed in plasma of alloxan-induced diabetic rats, this is in accord with earlier investigations (McLennan SV et al., 1991), wherein a dramatic increase in γ-GT expression was found in the liver of diabetic rats. Elevated activity of γ-GT in plasma takes place as a result of hepatic induction of the enzyme. In addition, hepatocellular damage or cholestasis may also contribute to the elevation in the activity. In our study results specifies increased activity of γ-GT, Melone dialdehyde (MDA) in alloxan-induced diabetic rats was lowered to near normal by Flavonoids (quercetin chrysin and hesperidin) and pioglitazone combination treatment that indicates the possible prevention of necrosis by Flavonoids treatment. The pharmacodynamic profile of quercetin has been well studied (Okamoto, 2005). Its ability to protect against oxidative stress-induced cellular damage as well as its chelatory properties (Mira et al., 2002; Anjaneyulu and Chopra, 2004). As to the chrysin, the data are sparse. (Furusawa et al. (2005) showed that chrysin had only moderate antioxidant. Anti-diabetic potency of flavonoids, particularly hesperidin and quercetin, has been highlighted in many reports increasesd Gltathione levels and attributed in part to their antioxidant and hypoglycaemic effects (Frode and Medeiros, 2008; Lean et al., 1999; Jung et al., 2006). Bioavailability of pioglitazone is higher in diabetic condition but remains unaffected by quercetin treatment. Part of this observation is contrary to a clinical report wherein type II diabetic patients exhibited normal clearance of pioglitazone (Eckland, D.A. & Danhof. M.2000). Phytochemical favonoids like quercetin chrysin and hesperidin longer duration studies of compounds on chronic models are necessary to develop a potent antidiabetic drug.

Conclusions

Our results show that oral administration of quercetin, hesperidin and chrysin has a beneficial effect on the alloxan-induces diabetes by reducing hyperglycaemia, gamma glutamyl transpeptidase and improving the antioxidant status. This study

suggests that the induction of diabetes mellitus by alloxan in rats may be protected by quercetin, hesperidin and chrysin administration. We hypothetized that this effect may be result of antiradical/chelatory properties of flavonoids used. However, inhibition of gamma glutamyl transpeptidase which elevated along with diabetes mellitus effect of these flavonoids.

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