Antioxidant Effect of Aqueous Extract of *Triticum aestivum* Grass on Insulin Resistance models in Wistar Albino Rats

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ABSTRACT

Context: Prolonged use of hypoglycaemic drugs is problematic due to their toxic side effects and diminution of response. Oral antihyperglycaemic agents from plant sources are therefore being explored for use in traditional medicine. **Aims:** To study antihyperglycaemic, hypolipidemic and antioxidant effect of aqueous extract of *Triticum aestivum* grass (TAGE) in two different models of T2DM in wistar albino rats such as High Fat Diet with Low dose STZ and High Fructose Diet models. **Settings and Design:** Two models of insulin resistance were used (single i.p injection of STZ (45 mg/kg) with high fat diet and fructose 10% w/v, p.o, *ad libitum*). **Methods and Material:** STZ with high fat diet and fructose 10% w/v, p.o, in rats was administered for a period of 21 days. Three doses of TAGE (40, 60 and 80 mg/kg, p.o) were used. Pioglitazone (PG) 20 mg/kg was used as the reference standard. At the end of the experimental period, serum biochemical parameters like fasting blood sugar, plasma insulin, lipid profile and MDA and SOD activity were studied in both the models. **Statistical analysis used:** one way ANOVA followed by Turkey's multiple comparison test. $p<0.05$ was considered as significant. **Results:** In both the models, TAGE significantly lowered plasma glucose, lipid, MDA level and increased SOD activity. It also improved insulin resistance which is comparable to that of normal and standard group. **Conclusion:** TAGE may be a potent drug, for treatment and prevention of complications of T2DM used alone or in combination with other oral hypoglycaemic agents.

Key words: Diabetes mellitus, Fructose, Insulin resistance, Streptozotocin, *Triticum aestivum*.

Key Messages: *T.aestivum* grass aqueous extract may be a useful and safer adjunctive to other oral hypoglycemic agents for treatment and prevention of insulin resistance Type-2 Diabetes Mellitus.

INTRODUCTION

Type 2 diabetes mellitus (T2DM) is one of the world's most common chronic diseases and projected to increase to 438 million in 2030.[1] Many risk factorscontribute to insulin resistance and its negative metabolic consequences including pancreatic β-cell exhaustion.[2,3] High fat diet, high fructose feeding give rise to insulin resistance andimpaired glucose tolerance.[4-6] Experimental animal models of T2DM exhibit high degree of oxidative stress due to persistent and chronic hyperglycaemia.[7,8] Hence, compounds with antioxidant properties appear to be promising candidates. Prolonged use of antidiabetic agents is problematic due to their toxic side effects and diminution of response. Oral antihyperglycaemic agents from plant sources are therefore being explored for use in therapeutics.[9-11] Fresh juice of *Triticum aestivum* grass contains a number of aminoacids, vitamins, phytochemicals which is used as health improving adjuvants in several diseases. There are few reports of showing that *T. aestivum* has hypolipidemic properties in normal rat due to presence of such phytochemicalshaving antioxidant properties.[12-14]

Therefore, the present study was conducted to evaluate the antihyperglycaemic, hypolipidemicinsulin sensitizing

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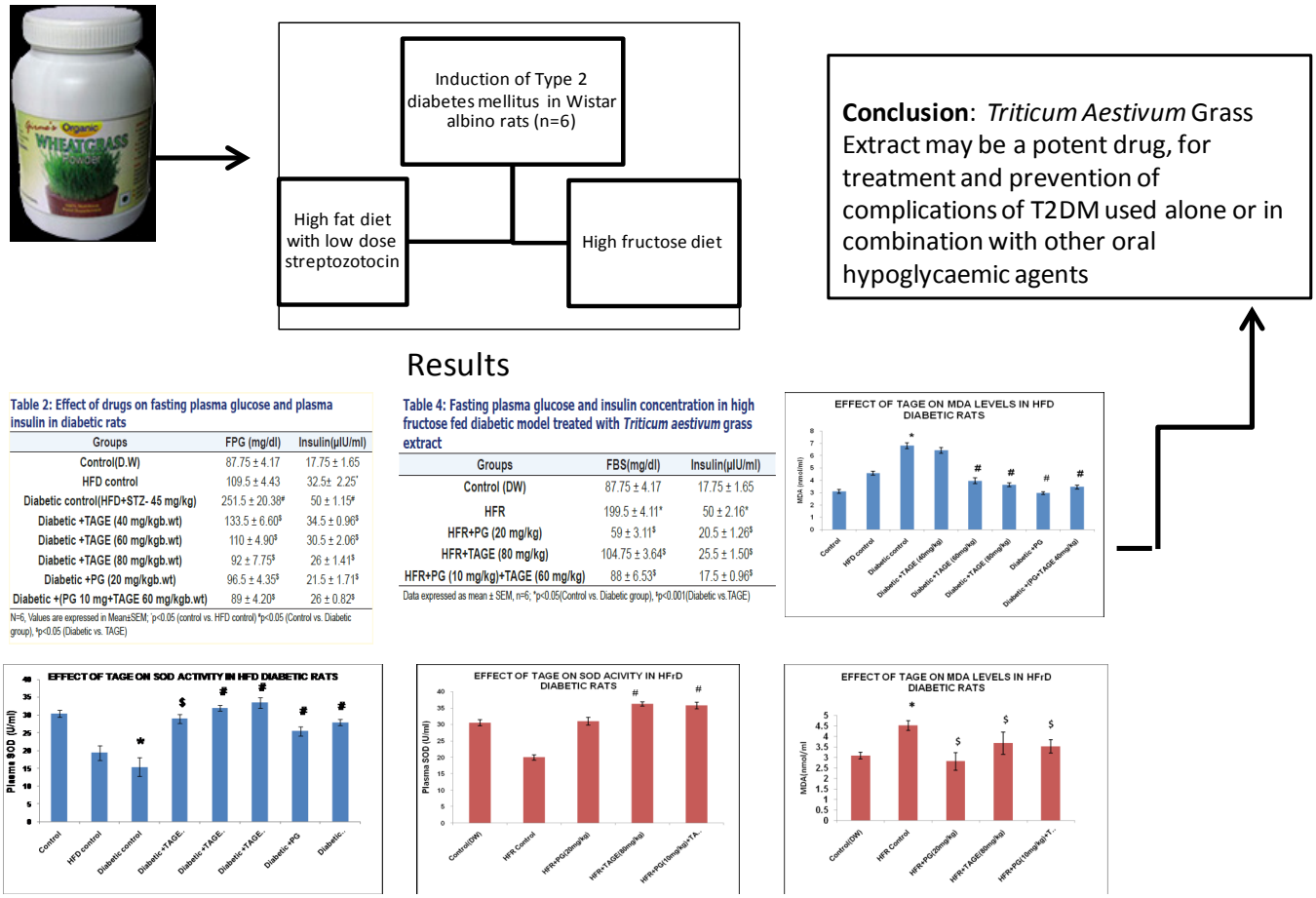

Graphical Abstract

and antioxidant activity of aqueous extract of *T. aestivum* grass (TAGE) in two different models of insulin resistance.

SUBJECTS AND METHODS

Chemicals

For induction of diabetes, Streptozotocin was obtained from Hi media Lab. Ltd. Different biochemical parameters like, Fasting plasma glucose (GOD/POD method), plasma cholesterol (CHOD/PAP method), triglyceride(GPO/PAP method) and HDL (Peg Precipitation Method) were estimated by using commercially available kits(Crest Bio system). The reagents used were procured from Hi media laboratory and were of analytical grade.

Preparation of grass extract

The *T. aestivum* grass powder was obtained from Girme's Wheat grass, Anand Nagar, Solapur, Maharastra. Aqueous extract from the dry powder was prepared.[15] Dry powder of *T. aestivum* (50 gms) was suspended in 1 litre of distilled water and kept for 24h with occasional shaking. Thereafter it was filtered, lyophilized and freeze dried [Yield = 12.6%]. Then the extract was and used as oral preparation in different doses. Preliminary phytochemical screening of the extract was done.

Animals

Male Wistar albino rats weighing between 150-200 g were used in the study. They were housed in clean polypropylene cages(six rats/cage) and maintained under controlled room temperature(25 ± 1°) with relative humidity of 45-55% under 12:12 hr light and dark cycle for one week with free access to food and water *ad libitum*. All procedures using animals obtained the approval of the Institutional Animal Ethical Committee, and the experiment was carried out in compliance with the Guidelines for CPCSEA [472/CPCSEA].

Acute toxicity studies

Acute oral toxicity was determined using male Wistar albino rats weighing 150-200 g. OECD guideline no. 423 was followed for toxicity studies.[16] Animals were administered orally with a single dose of aqueous extract of *T. aestivum* and were observed for their mortality during 14 days.

Induction of Type 2 diabetes mellitus

High fat diet with low dose streptozotocin induced Type-2 Diabetes Mellitus: The model was developed by feeding high fat diet [18%v/w hydrogenated coconut oil along with normal laboratory diet] for 2weeks followed by Intra peritoneal injection of single dose of streptozotocin (45 mg/kg b.w).[17] To avoid an early fatal hypoglycaemia 5% glucose solution was given on 1st day to all animals. The animals having fasting glucose level above 226 mg/dl after 7th day of STZ administration were selected for the study.

High Fructose fed Type-2 Diabetes Mellitus: Insulin resistance was induced by feeding fructose 10% w/v, p.o., *ad libitum* along with standard laboratory diet for 21 days.[18]

Experimental design

High fat diet with low dose streptozotocin induced Type-2 Diabetes Mellitus

A total of 60 rats were divided into ten groups (n=6). They were fed with high fat diet and oral treatments of aqueous extract of *T. aestivum* grass (TAGE) and pioglitazone (reference standard) as a suspension in 1% Tween 80 solution for three weeks (21 days) daily between 08.00 and 09.00 h.

Group I: Normal control -normal lab diet (NLD) with Distilled Water (DW)

Group II: Tween 80 (1ml)

Group III: HFD control - (NLD with 20%v/w hydrogenated coconut oil)

Group IV: Diabetic control - (HFD + STZ)

Group V: Diabetic rats - TAGE 40 mg/kg

Group VI: Diabetic rats - TAGE 60 mg/kg

Group VII: Diabetic rats - TAGE 80 mg/kg

Group VIII: Diabetic rats - Pioglitazone 20 mg/kg

Group IX: Diabetic rats - Pioglitazone10 mg/kg + TAGE 40 mg/kg

Group X: NLD -TAGE 60 mg/kg

High Fructose fed Type-2 Diabetes Mellitus

Eighteen high fructose diet (10% fructose solution) induced diabetic rats were selected and randomly divided into four groups containing six animals each (n=6).

Group I: Aqueous extract of *T. aestivum* grass (80 mg/kg)

Group II: Pioglitazone (20 mg/kg)

Group III: Pioglitazone (10 mg/kg), with aqueous extract of *T. aestivum* grass (40 mg/kg)

All the drugs and vehicles were administered by oral route with the help of oral feeding canula for rats whereas STZ was given by IP route.

Biochemical assays

Blood samples were collected from the overnight fasted animals through retro orbital puncture under mild ether anaesthesia in the morning for estimation of biochemical parameters on day 0, 8, 15 and 22. Plasma was collected by centrifuging the sample at 3000 rpm for 10 minutes and FPG, insulin, total cholesterol (TC), triglyceride (TG)

Table 1: Qualitative phytochemical screening of aqueous extract of *Triticum aestivum*

Phytoconstituents	Methods	Aqueous extract
Alkaloid	Wagner's Reagent Test, Mayer's Reagent Test, Dragendorff's test, Hager's test	+
Carbohydrate	Benedict's Test, Molisch's test, Fehling's Test	+
Glycoside	General test	+
Cardiac Glycoside	Keller-Killiani Test, Legal's Test, Baljet's test	-
Tanins and phenolics	*Test with heavy metals*, Ferric Chloride test, Nitric acid test, Gelatin test	+
Proteins & amino acids	Biuret's Test, Ninhydrin's Test, Xanthoproteic's test, Million's test	+
Gum & mucilage	Test with 95% Alcohol, Molisch's Test	+
Flavonoids	Test with NaOH, Ferric chloride test, Shinoda test, Lead acetate test	+
Saponin glycosides	Foam Test	+
Steroids	Salkowski's Test, Libermann Burchard Reagent Test	-
Fats and oils	Spot test	-
Triterpinoids	Test with Tin and Thionyl Chloride	+

Table 2: Effect of drugs on fasting plasma glucose and plasma insulin in diabetic rats

Groups	FPG (mg/dl)	Insulin(µlU/ml)
Control(D.W)	87.75 ± 4.17	17.75 ± 1.65
HFD control	109.5 ± 4.43	32.5± 2.25˙
Diabetic control(HFD+STZ- 45 mg/kg)	251.5 ± 20.38#	50 ± 1.15#
Diabetic +TAGE (40 mg/kgb.wt)	133.5 ± 6.60$	34.5 ± 0.96$
Diabetic +TAGE (60 mg/kgb.wt)	110 ± 4.90$	30.5 ± 2.06$
Diabetic +TAGE (80 mg/kgb.wt)	92 ± 7.75$	26 ± 1.41$
Diabetic +PG (20 mg/kgb.wt)	96.5 ± 4.35$	21.5 ± 1.71$
Diabetic +(PG 10 mg+TAGE 60 mg/kgb.wt)	89 ± 4.20$	26 ± 0.82$

N=6, Values are expressed in Mean±SEM; ˙p<0.05 (control vs. HFD control) #p<0.05 (Control vs. Diabetic group), $p<0.05 (Diabetic vs. TAGE)

Table 3: Effect of drugs on plasma lipid profile in diabetic rats

Groups	Total cholesterol (mg/dl)	TG (mg/dl)	LDL (mg/dl)	HDL (mg/dl)
Control(D.W)	63.75 ± 2.39	82.75 ± 0.48	20.35 ± 1.60	25.75 ± 2.06
HFD control	81.5 ± 2.63*	120.5 ± 11.61*	29.65 ± 2.85	12.5 ± 1.89*
Diabetic control	123 ± 3.79*	215.5 ± 3.59*	69.65 ± 3.62*	9.75 ± 0.75*
Diabetic +TAGE (40 mg/kg bw)	86.5 ± 4.57$	146.75 ± 10.06$	25.9 ± 3.26$	31.25 ± 2.43$
Diabetic +TAGE (60 mg/kg bw)	69.75 ± 2.95$	114.75 ± 2.25$	14.15 ± 2.17$	32 ± 1.83$
Diabetic+TAGE (80 mg/kgb.w)	66.75 ± 0.48$	103 ± 5.05$	8.85 ± 0.34$	36 ± 1.63$
Diabetic+PG (20 mg/kgb.w)	98 ± 2.94$	80.75 ± 1.11$	54.35 ± 3.36$	27.5 ± 0.96$
Diabetic +(PG 10 mg+TAGE 60 mg /kgb.w)	67.25 ± 0.75$	73.5 ± 1.11$	9.4125 ± 0.72$	33.5 ± 0.87$

n=6, Values expressed in Mean±SEM, *p<0.05 (Control vs. Diabetic), $p<0.05, (Diabetic vs. TAGE)

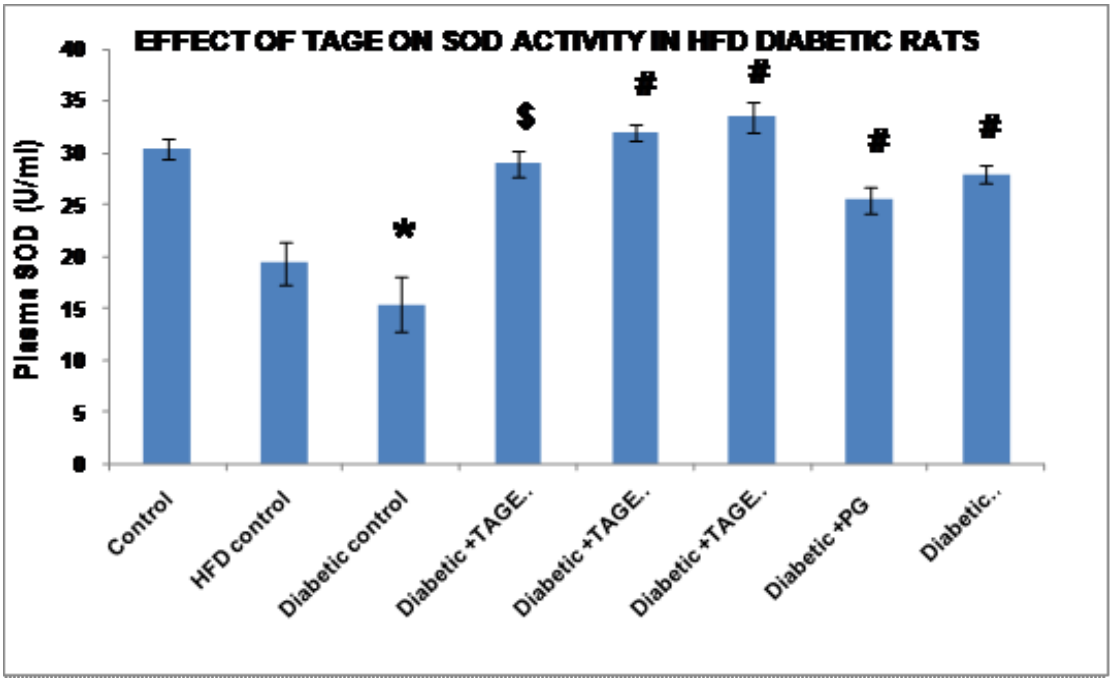

Figure 1: Effect of Tage on SOD Activity in HFD Diabetic Rats

Data expressed as Mean ± SEM *p<0.05 (Control Vs Diabetic group) # p<0.05(HFD Vs DC), $p<0.05 (Diabetic group Vs treatment group).

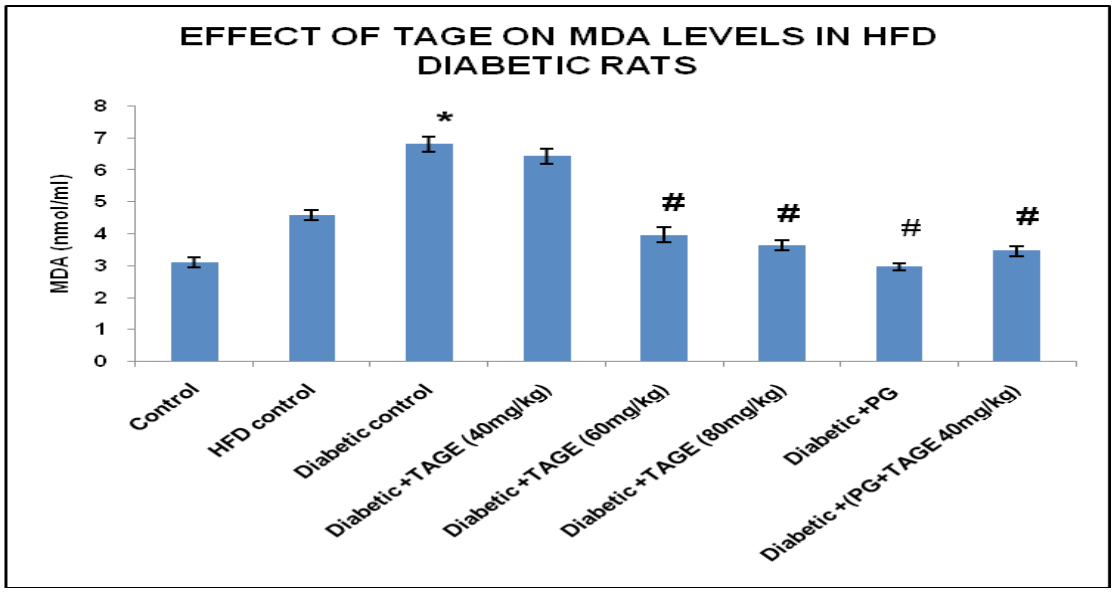

Figure 2: Effect of Tage on MDA Levels in HFD Diabetic Rats

Data expressed as Mean ± SEM *p<0.05 (Control Vs Diabetic group) # p<0.05(HFD Vs DC), $p<0.05 (Diabetic group Vs treatment group).

Table 4: Fasting plasma glucose and insulin concentration in high fructose fed diabetic model treated with *Triticum aestivum* grass extract

Groups	FBS(mg/dl)	Insulin(µlU/ml)
Control (DW)	87.75 ± 4.17	17.75 ± 1.65
HFR	199.5 ± 4.11*	50 ± 2.16*
HFR+PG (20 mg/kg)	59 ± 3.11$	20.5 ± 1.26$
HFR+TAGE (80 mg/kg)	104.75 ± 3.64$	25.5 ± 1.50$
HFR+PG (10 mg/kg)+TAGE (60 mg/kg)	88 ± 6.53$	17.5 ± 0.96$

Data expressed as mean ± SEM, n=6; *p<0.05(Control vs. Diabetic group), $p<0.001(Diabetic vs.TAGE)

Table 5: Plasma lipid in high fructose fed diabetic model treated with *Triticum aestivum* grass extract

GROUPS	TC(mg/dl)	TG(mg/dl)	LDL(mg/dl)	HDL(mg/dl)
Control (DW)	63.75 ± 2.39	82.75 ± 0.48	20.35 ± 1.60	25.75 ± 2.06
HFR	104.75 ± 6.60*	112.75 ± 2.53*	69.2 ± 6.09*	12 ± 1.73*
HFR+PG (20 mg/kg)	81 ± 1.73$	68.5 ± 4.27$	47.3 ± 1.84$	20 ± 0.82$
HFR+TAGE (80 mg/kg)	56.75 ± 1.11$	63.5 ± 2.22$	14.8 ± 2.54$	29.25 ± 2.14$
HFR+PG (10 mg/kg)+TAGE (60 mg/kg)	61 ± 1.73$	59 ± 0.58$	12.2 ± 1.19$	29.25 ± 0.75$

Data are expressed as Mean±SEM, n=6, *p<0.05(Control vs. Diabetic), $p<0.05, (Diabetic vs.TAGE)

and high density lipoprotein(HDL) were estimated by using diagnostic kits and low density lipoprotein (LDL) cholesterol was calculated as per Friedewald's[19] equation i.e. LDL-C=TC – (HDL-C + TG/5).

Estimation of plasma superoxide dismutase (SOD)

Plasma SOD was estimated as per the method of P. Kakkar *et al*, 1984.[20] *Estimation of plasma MDA*

Plasma MDA estimation was done as per the method of K. Satoh *et al*, 1978.[21]

Statistical analysis

Results are expressed as Mean ± SEM. Statistical analysis was performed using one way ANOVA followed by Turkey's multiple comparison test. p<0.05 was considered as significant.

RESULTS

Preliminary phytochemical screening of aqueous extract of *T. aestivum* showed presence of alkaloids, carbohydrates, glycosides, tannins, phenolics, gums, mucilage, saponins, flavins, flavonoids and triterpenoids. (Table 1)

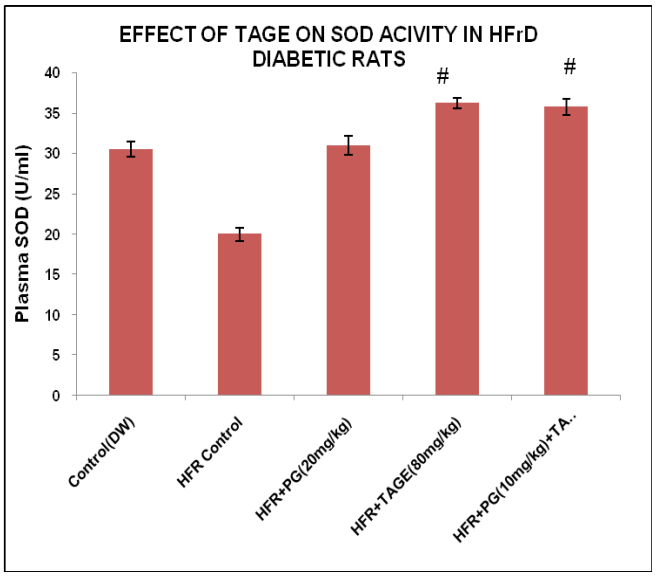

Figure 3: Effect of Tage on SOD Activity in HFrD Diabetic Rats

Data expressed as Mean ± SEM, *p<0.05 (Control vs. Diabetic group), # p<0.05 (Diabetic group vs.TAGE).

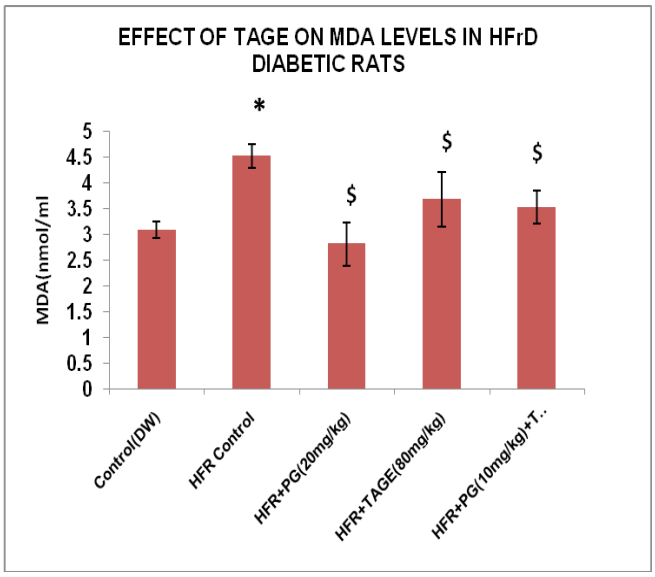

Figure 4: Effect of Tage on MDA Levels in HFrD Diabetic Rats

Data expressed as Mean ± SEM, *p<0.05 (Control vs. Diabetic group), $p<0.05 (Diabetic group vs.TAGE).

Acute oral toxicity

The oral administration of TAGE in rats up to the dose 2000 mg/kg did not show any sign of toxicity and no mortality for 14 days. It was shown that TAGE was safe up to oral dose of 2000 mg/kg of body weight.

High fat diet with low dose streptozotocin induced Type-2 Diabetes Mellitus

Effects on fasting Plasma Glucose and plasma insulin level

Fasting plasma glucose and insulin levels in the diabetic control group (HFD and STZ (45 mg /kg) significantly increased compared to that of normal control. TAGE in 40, 60, 80 mg /kg doses produced significant decrease in FPG than that of diabetic control group whereas it shows highly significant effect at 80 mg dose (92 ± 7.75 mg/dl).The standard drug PG (20 mg/ kg) showed significant decrease in FPG. TAGE at low dose (40 mg/kg) in combination with half dose of PG (10 mg/kg) also had significant effect on FPG. Plasma insulin levels in HFD (32.5 ± 2.25 µIU/ml) as well as in diabetic group (50 ± 1.15 µIU/ml) were significantly increased than that of control group (17.75 ± 1.65 µIU/ml) showing insulin resistance. TAGE alone as well as in combination decreased the plasma insulin level significantly in the above disease models. There was no significant difference in plasma insulin between drug treatment groups and normal control (Table 2).

Table 6: Effect of TAGE (60 mg/kg) on fasting plasma glucose, plasma insulin, and lipid profile and in normal rats

Biochemical Parameters	Normal Control	TAGE (60 mg/kg)
Fasting plasma glucose (mg/dl)	87.75 ± 4.17	89.5 ± 5.91
Plasma insulin (µlU/ml)	17.75 ± 1.65	19.5 ± 0.96
Total cholesterol (mg/dl)	63.75 ± 2.39	66.25 ± 1.55
TG (mg/dl)	82.75 ± 0.48	82.75 ± 2.63
LDL (mg/dl)	20.35 ± 1.60	13.55 ± 1.56
HDL (mg/dl)	25.75 ± 2.06	36 ± 1.56

n=6, Values are expressed in Mean±SEM, p>0.05; there was no significant difference in any of the above biochemical parameters of TAGE (60 mg/kg) vs. normal control.

Effects on plasma lipid profile

Diabetic control group showed significant increase in TC, TG and LDL in comparison to that of normal control whereas HDL decreased significantly. TAGE in different doses (40, 60 & 80 mg /kg) produced decrease in plasma TC, TG, and LDL level and increase HDL level significantly. Pioglitazone (20 mg/kg) produced significant improvement in lipid profile in comparison to diabetic control group. Combination of TAGE low dose with half dose of PG (10 mg/kg) showed significant decrease in lipid profile (Table 3).

Effect of drugs on oxidative parameters

Diabetic control group showed significant decrease in plasma SOD activity in comparison to that of control. TAGE in all three doses produced highly significant increase in SOD activity. PG (20 mg/kg) showed significant increase whereas TAGE 40 mg/kg in combination with

half dose of PG showed highly significant increase in plasma SOD activity when compare to that of diabetic control (Figure 1). Similarly diabetic group elevated the plasma MDA levels which was significantly reduced by the TAGE in different doses (Figure 2).

High Fructose fed Type-2 Diabetes Mellitus

TAGE (80 mg/kg) produced significant decrease in plasma glucose and insulin levels compared to the disease control. Also combination of TAGE+PG significantly reduced the plasma glucose and insulin. A similar effect on lipid profile was observed with TAGE (80 mg/kg) and its combination with pioglitazone. (Table 4&5). TAGE in 80 mg/kg produced highly significant increase in SOD activity when compared to that of diabetic control (Figure 3). Similarly diabetic group elevated the plasma MDA levels which was significantly reduced by the TAGE (Figure 4).

Effect of TAGE (40 mg/kg) on fasting plasma glucose, plasma insulin, and lipid profile and in normal rats

The minimum effective dose of TAGE (40 mg/kg) did not produce any significant changes in the above parameters compared to the normal control which shows that TAGE itself has no hypoglycemic or hypolipidemic effect (Table 6).

DISCUSSION

Hyperglycaemia the primary clinical manifestation of T2 DM, associated with its micro and macro vascular complications is life threatening.[22] Lifestyle changes such as weight reduction, exercise and dietary modifications are often recommended, but have been difficult to maintain over a long term. Therapeutic strategy to improve insulin sensitivity with newer medications ispractised in recent years, because early treatment and prevention play a pivotal role in reducing the population burden of diabetes, but at the cost of unwanted side effects. Thus, there has been a growing interest in herbal remedies with least side effects and the maximal preventive outcome. *Triticum aestivum*, a common herb has been used for treating various ailments in traditional medicine and is reported to possess hypoglycemic, hypolipidemic as well as antioxidant activities.[23] With this above background the present study was undertaken to evaluate the antidiabetic effect of aqueous extract of *TAGE* in wistar albino rats. HFD and Low dose STZ with high fructose diet induced diabetes are two experimental models of hyperinsulinemia in rodents which represent the metabolic abnormalities seen in patients with insulin resistance and its consequent complications. High-fat diet following low-dose STZ closely mimics the natural history of the disease events (from insulin resistance to β cell dysfunction) as well as metabolic characteristics of human type 2 diabetes.[24-26] Addition of fructose (10% w/v) in drinking water for a period of 1 week or longer has been found to be most suitable for the production of insulin resistance in rats.[18] In the present study, administration of fructose for 21 days significantly increased the glucose, insulin and triglyceride levels. TAGE in different doses were evaluated for its effect on FPG, lipid profile and antioxidant activity and compared with standard drug pioglitazone. The results of the study show that, TAGE has got beneficial effect against diabetes mellitus associated with hyperlipidaemia and oxidative stress. In all the given doses it produced significant decrease in fasting plasma glucose and insulin level compared to disease control. TAGE also produced significant decrease in TC, TG and LDL level with increase in HDL level in a dose dependant manner. This result corroborates with observation made by Saroj Kothari *et al* in 2008.[27] Euglycemicrats treated with TAGE did not show any significant change in FPS and insulin level and no significant effects on lipid profile which reflects that the aqueous extract of wheat grass is antihyperglycemic without hypoglycaemic action. Our observations corroborate with Shaikh *et al* (2011) and Shirude (2011) who have reported antidiabetic effect of *Triticum aestivum* grass in alloxan induced diabetic rats. Yogesha Mohan *et al* also have proved the antidiabetic effect of ethanolic extract of *T. aestivum*.[23]

CONCLUSION

To conclude, the present study showed potency of TAGE to ameliorate the hyperglycemia and insulin resistance in diabetic rats. Furthermore it has also beneficial effect against dyslipidemia and oxidative stress commonly associated with this disease.Aqueous extract of *T. aestivum* grass contains tannins, saponins, flavinoids which may be responsible for its observed hypoglycaemic and anti-oxidant effects. Therefore it can be concluded that TAGE may be a potent drug, for treatment and prevention of complications of T2DM used alone or in combination with other oral hypoglycaemic agents. However further chemical and pharmacological interventions are required and the active constituents responsible for these effects need to be isolated to establish its use in future.

Highlights of Paper

- *Triticum aestivum*, has been used for treating various ailments in traditional medicine and is reported to possess hypoglycemic, hypolipidemic as well as antioxidant activities.
- The present study was undertaken to evaluate the antidiabetic effect of aqueous extract of *Triticum aestivum* in wistar albino rats.
- HFD and Low dose STZ with high fructose diet induced diabetes are two experimental models of hyperinsulinemia in rodents which were used in this study.
- The results of the study show that, *Triticum aestivum* has got beneficial effect against diabetes mellitus associated with hyperlipidaemia and oxidative stress.

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