

IN VIVO ANTIDIABETIC POTENTIAL AND SAFETY OF AQUEOUS EXTRACT OF *TRITICUM AESTIVUM* (WHEATGRASS)

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ABSTRACT

Primary goal in management of diabetes mellitus is to realize normoglycaemia. Like many other plants, Triticum aestivum has been used widely in complementary and alternative medicine but minimal data is available on its effectiveness and toxicity effects. This study was done to evaluate in vivo glycemic and toxicity effects of Triticum aestivum. 10% alloxan monohydrate was administered intraperitoneally in Swiss White Albino rats to induce diabetes in determination of efficacy. Rats with blood glucose levels above 200 mg/L were orally administered with aqueous extracts of Triticum aestivum plant at 50, 90.9, 165.1 and 300mg/kg body weight. Glibenclamide was used as the positive control. Toxicity studies were done post oral administration of either 300, 448.14, 669.4 or 1000mg per kilogram body weight of plant extracts for 28 days. The weights of the rats organs, haematological and biochemical parameters were used for toxicity studies. $p \leq 0.05$ was considered statistically significant. The results obtained in this study indicated that the young plant of Triticum aestivum has therapeutic benefits in treatment of diabetes mellitus. No chronic toxicity effects were established. Use of Triticum aestivum as mono therapy for diabetes should be recommended on carrying out clinical studies in humans.

KEYWORDS : Normoglycemia, *Triticum aestivum*, Glibenclamide, In vivo, Alloxan, Efficacy & Toxicity

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INTRODUCTION

Despite great development of therapeutic and other measures for mitigating diabetes, there has been a steady global rise in its prevalence. In 1980, 108 million people were living with diabetes but the prevalence quadrupled to 422 million in 2014. The increase in diabetic cases has been more in the low and middle income countries over the last decade (WHO, 2016a). In Kenya, a low middle income economy, diabetes has been on a steady rise and the prevalence is currently at 4% in a population of 46 million people (WHO, 2016b). Diabetes and its related complications cause huge economic losses in individuals, families and national economies which is attributed to loss of work and wages, disability, high health budget and high costs of treatment. An individual is said to be diabetic if his or her glycaemic levels are above the normal reference level of fasting plasma glucose of above or equal to 7.0 mmol/l (126 mg/dl) or a 2 hour post prandial glucose level equal to or above 11.1 mmol/L (200 mg/dl) or a deranged oral glucose tolerance test (OGTT) (WHO, 2016a; Aronson and Rayfield, 2002; Piero et al., 2014).

Upon diagnosis, the primary goal in the management of diabetes mellitus is to realize optimal glycaemic control and maintain normoglycaemia as much as possible. When diet therapy fails in normalizing glycemia, oral

insulin secretagogues and alpha glucosidase inhibitors as well as parenteral insulin are used as antidiabetics. However, the drugs have serious side effects and a search of more efficient, safer and affordable drugs continues (Nolte and Karam, 2001).

Triticum aestivum (common wheat), of the Kingdom plantae, family *Poaceae* (*Gramineae*) and genus *Triticum L.* has been a major nutritional source since the history of man. The consumption of the young plant (wheatgrass) of *Triticum aestivum* has grown tremendously in both developed and developing countries in the twentieth century (Ronald, 1990) driven by claims on numerous therapeutic benefits in mitigating human diseases (Swati et al., 2010; Singh et al., 2010). Recent studies has demonstrated wheatgrass potential in treatment of cancer (Khan et al., 2015; Patel, 2016), diabetes (Yogesha et al., 2013), thalassemia (Marwaha, 2004) and ulcerative colitis (Ben-Arye et al., 2002) as well as antimicrobial activity (Sundaresan et al., 2015). Phytochemical and nutritional analysis on wheatgrass has indicated its rich contents (Chauhan, 2014; Polshetiwar and Khorate 2016). However, the data on its effectiveness is limited and safety data on the *Triticum aestivum* plant is not available. This study was done to evaluate *in vivo* antihyperglycaemic activity and provide safety data on the aqueous extracts of locally indoor grown *Triticum aestivum* plant (wheatgrass).

MATERIALS AND METHODS

Preparation of Aqueous Extracts of *Triticum Aestivum* Plant

Triticum aestivum (wheatgrass) was grown indoors in a customized building in Ondiri Village, Kikuyu Division, Kiambu County of Kenya using the standard procedure for growing and processing of wheatgrass (SOP/WGP/SHC1). The aqueous extract preparation and freeze drying was done at the Department of Pharmacy laboratories of Kenyatta University.

In Vivo Evaluation of Glycemia Effects

The study used 8 weeks old Swiss Albino rats of a mean weight of 120 grams (Frodes and Medeiros, 2007). The rats were bred and housed at temperature of 25⁰C with 12 hours/12 hours darkness/photoperiod and fed on rodent pellets and water *ad libitum* in the Animal house at the Department of Biochemistry and Biotechnology of Kenyatta University. Diabetes was induced into the Swiss Albino rats by a single intraperitoneal administration of 186.9 mg/kg body weight (Karau et al., 2012) of freshly prepared 10% alloxan monohydrate (2,4,5,6 tetraoxypyrimidine; 5-6-dioxyuracil) obtained from Sigma (Steinhein, Switzerland). 72 hours after alloxan administration, the animals were left to fast overnight and fasting blood glucose levels were determined using Accu-Chek® perfoma model Accu-Chek® perfoma strips. Rats with blood glucose levels above 200 mg/L (11.1mmol/l) were considered diabetic and used in this study.

The experimental rats were arranged into seven groups of five animals each. Group I (normal rats) for normal control and group II diabetic rats for negative control were orally administered with 0.5 ml physiological saline. Group III (positive control) were administered with 3mg per kilogram body weight glibenclamide. Diabetic rats in group IV to VII were orally administered with logarithmic therapeutic doses (Thompson, 1985) of *Triticum aestivum* aqueous extracts at 50, 90.9, 165.1 and 300 mg per kilogram body weight respectively. The animals were denied feeds but allowed free access to water until the end of the experiment. The experiment was carried out for 24 hrs. Blood glucose determination was done at the start of the experiment (0 hr) and repeated at 1, 2, 3, 4, 6, 12 and 24 hours.

In Vivo Logarithmic Dose Toxicity Evaluation

Normal 8 weeks old rats were randomly placed into five groups of five rats each. Group I was orally administered 0.5 ml physiological saline. Group II to V rats were orally administered daily with non-therapeutic doses of aqueous extracts of *Triticum aestivum* 300, 448.14, 669.4 and 1000mg/kg body weight for 28 days. During this period, the rats were allowed free access to mice pellet and water. The body weight of each rat was established at the start of the experiment and after every seven days during the dosing period up to and including the 28th day. At the end of 28 days, the rats were euthanized. Blood samples and body organs were collected for toxicity studies.

Determination of Biochemical Parameters

Serum was analyzed for the levels of alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (ALP), γ -Glutamyltransferase (GGT), total protein, albumin, blood urea nitrogen (BUN), creatinine (CREAT), sodium (Na⁺), potassium (K⁺), amylase (AMY), creatinine kinase (CK) and lactate dehydrogenase (LDH) using an Automated Clinical Chemistry Analyzer.

Determination of Hematological Parameters

Whole blood was analyzed for red blood cells counts (RBC), hemoglobin, mean cell hemoglobin, mean cell hemoglobin concentration, mean cell volume, white blood cells counts (WBC), WBC differentials counts for neutrophils, lymphocytes, eosinophils, basophils, monocytes and platelets using the Coulter Counter System (Beckman Coulter®, ThermoFisher, UK). Air-dried thin blood films stained with giemsa stain were examined microscopically using magnification 400 and 1000 for cell morphologies.

Qualitative Phytochemical Analysis

Phytochemicals screening was done to establish presence or absence of tannins, flavonoids, terpenoids, saponins, steroids, phlobatannins, anthraquinones, cardiac glycosides and alkaloids using standard methods (Pradeep et al., 2014). The results were reported as faintly present, moderately present, highly present or absent.

Data Management and Statistical Analysis

Data for glycaemic activity and toxicity evaluation are expressed as mean \pm SD of five animals. Variation between experimental groups was statistically determined by one way analysis of variance (ANOVA) primary test followed by Tukeys post Anova test using Minitab version 17. Means with $p \leq 0.05$ were considered significantly different.

RESULTS AND DISCUSSIONS

Effect on Glycemia

Table 1 presents the blood sugar levels obtained for normal and treated or non-treated alloxan induced diabetic rats at hourly determination for 24 hrs. Blood sugar levels for the nondiabetic animals and diabetic animals were significantly different. Treatment with glibenclamide and therapeutic doses of aqueous extracts of *Triticum aestivum* lowered the levels of glycaemia in the diabetic animals up to the 24th hour while the non-treated diabetic rats exhibited significant hyperglycemia. Blood sugar lowering rates of *Triticum aestivum* at doses of 165.1 and 300mg per kilogram body weight were comparable to those of glibenclamide. Glycemia lowering effects of *Triticum aestivum* have equally been recorded on the ethanolic extract of wheatgrass (Yogesha et al., 2013).

In Vivo Toxicity Effects

Effect on the General Body and Organ Weights

The effects of a daily administration of aqueous extracts of *Triticum aestivum* for 28 days on the general weight of Swiss albino rats and percentage organ weight is shown in Table 2. No significant difference in general body weight between the untreated and treated animals in respective periods of weight determination. The percentage organ to body weight of the liver for the 1000mg per kilogram body *Triticum aestivum* treated group was significantly lower than the control group and *Triticum aestivum* treated animals at 300, 448.14 and 669.4 milligram per kilogram body weight.

Effect on Biochemistry Parameters

Table 3 shows the results for evaluation of liver function. The gammaglutamyl transferase (GGT) levels for the normal control group (3.5±2.1IU/L) did not differ significantly with those of the 300 (4.6±2.3IU/L), 669.4 (8.3±3.7 u/l) and 1000 milligram per kilogram body weight (7.6±4.0 u/l) *Triticum aestivum* treated groups but the 448.14mg/kilogram body weight *Triticum aestivum* treated group had the lowest Gamma glutamyltransferase levels at 1.4±1.2 IU/L that was significantly different from the rest of the treatment groups. Although the levels for GGT for the 669.4 and 1000mg/kilogram body weight *Triticum aestivum* treated groups were significantly high than the normal control, the levels obtained did not indicate a dose related effect as the 669.4 mg/kilogram body weight had lower levels than the 1000mg/kilogram body weight treated group, the 448.14 mg/kilogram body weight treated group had lower levels than the 300mg/kilogram body weight and the non-treated groups.

Table 4 presents the results for Lactate dehydrogenase (LDH), Creatine phosphokinase (CK), Blood urea nitrogen (BUN), Creatinine (CREAT), Sodium (Na⁺) and Potassium (K⁺). There was no significant difference in the values obtained between the normal group and the *Triticum aestivum* treated groups. The Potassium (K⁺) levels that were affected by delayed time of serum/cells separation was significantly high at 22±8mmol/l for the normal control than the *Triticum aestivum* treated groups whose potassium levels ranged between 9 and 11 mmol/l.

Effect on Haematological Indices

As shown in Table 5, haemoglobin levels for the normal control were significantly lower than the 448.1mg/kg body weight *Triticum aestivum* treated animals but did not vary significantly with other *Triticum aestivum* treated groups. No significant variation was established in other red blood cell parameters, platelets and white blood cells.

Table 1: Effects of Oral Administration of Aqueous Extracts of *Triticum aestivum* at Therapeutic Doses on Blood Glucose Levels in Alloxan Induced Diabetic Rats

Treatment Group	Blood Glucose Levels at Varying Times (mmol/l)							
	0hr	1hr	2hr	3hr	4hr	6hr	12hr	24hr
Normal control	5.8±0.9 ^{aB}	6.0±0.6 ^{aB} (104)	5.9±0.2 ^{aB} (102)	5.8±0.3 ^{aB} (100)	5.6±0.5 ^{abC} (97)	5.3±0.5 ^{abCB} (91)	4.6±0.3 ^{bcBC} (79)	4.5±0.7 ^{cB} (78)
Diabetic untreated	18.04±2.5 ^{aA}	18.6±2.9 ^{aA} (103)	19.1±2.6 ^{aA} (106)	19.9±2.6 ^{aA} (110)	20.2±2.0 ^{aA} (112)	19.4±1.6 ^{aA} (108)	15.3±1.3 ^{aA} (85)	9.6±3.6 ^{bA} (53)
Diabetic plus <u>glibenclamide</u>	18.8±2.8 ^{aA}	17.9±1.9 ^{abA} (95)	15.2±1.7 ^{abcA} (81)	14.4±2.0 ^{bcA} (77)	12.6±2.2 ^{cABC} (67)	7.4±1.5 ^{dB} (39)	4.3±1.4 ^{dB} (23)	4.6±0.9 ^{dB} (25)
Therapeutic Aqueous Extract Doses (mg/kg body weight) of <i>Triticum Aestivum</i>								
50	20.1±6.2 ^{aA}	18.6±4.5 ^{aA} (93)	16.9±4.6 ^{aA} (84)	16.3±5.0 ^{aA} (81)	13.9±4.8 ^{abAB} (69)	7.4±3.1 ^{bcB} (37)	3.2±0.9 ^{cC} (16)	3.3±0.7 ^{cB} (16)
90.9	18.8±5.8 ^{aA}	17.1±6.2 ^{aA} (91)	15.4±5.2 ^{aA} (82)	12.7±3.7 ^{abAB} (68)	11.1±2.9 ^{abcBC} (59)	6.6±2.5 ^{bcB} (35)	3.8±1.5 ^{cC} (20)	3.5±1.1 ^{cB} (19)
165.1	21.3±6.0 ^{aA}	18.0±6.5 ^{abA} (85)	16.9±6.6 ^{abcA} (79)	15.2±6.8 ^{abcA} (71)	13.3±7.2 ^{abcABC} (62)	8.2±4.7 ^{bcB} (39)	6.6±1.3 ^{bcB} (31)	5.9±2.4 ^{cAB} (28)
300	19.7±3.2 ^{aA}	15.4±3.6 ^{abA} (78)	13.6±3.4 ^{abcAB} (69)	12.4±4.1 ^{bcAB} (63)	10.2±4.0 ^{bcdBC} (52)	7.3±2.0 ^{cdB} (37)	4.0±1.3 ^{dC} (20)	4.8±1.7 ^{dB} (24)

Results are expressed as Mean \pm Standard Deviation (SD) of five rats per group. Means in each group that do not share a superscripted small letter in a row or capital letter in a column are significantly different at $p < 0.05$ by ANOVA and post ANOVA (Tukeys) test. Numbers in brackets indicate percentage glycemia.

Table 2: Effects of Oral Administration of High Non-Therapeutic Aqueous Extract

Doses of *Triticum aestivum* in Rats Daily for 28 Days on the Percent Organ to Body Weight

Treatment Group	Average Weekly Body Weight Change (g)	Percent Organ to Body Weight (g/100g)					
		Heart	Kidney	Lungs	Spleen	Liver	Brain
Normal Control	11.8 \pm 11.2 ^a	0.35 \pm 0.04 ^a	0.88 \pm 0.09 ^a	0.94 \pm 0.18 ^a	0.71 \pm 0.19 ^a	5.48 \pm 0.46 ^a	0.75 \pm 0.19 ^a
High Non-Therapeutic Aqueous Extract Doses (mg/kg Body Weight) of <i>Triticum Aestivum</i>							
300	7.8 \pm 6.9 ^a	0.35 \pm 0.04 ^a	0.80 \pm 0.14 ^a	1.04 \pm 0.59 ^a	0.59 \pm 0.24 ^a	4.19 \pm 0.36 ^{ab}	0.83 \pm 0.15 ^a
448.14	7.6 \pm 8.2 ^a	0.38 \pm 0.12 ^a	0.88 \pm 0.26 ^a	0.90 \pm 0.16 ^a	0.67 \pm 0.24 ^a	4.48 \pm 1.36 ^{ab}	0.90 \pm 0.22 ^a
669.4	9.8 \pm 7.7 ^a	0.41 \pm 0.04 ^a	0.82 \pm 0.08 ^a	0.84 \pm 0.03 ^a	0.66 \pm 0.17 ^a	4.00 \pm 0.49 ^{ab}	0.85 \pm 0.14 ^a
1000	4.9 \pm 11.1 ^a	0.43 \pm 0.03 ^a	0.86 \pm 0.07 ^a	0.90 \pm 0.19 ^a	0.48 \pm 0.10 ^a	3.49 \pm 0.45 ^c	1.06 \pm 0.23 ^a

Results are expressed as Mean \pm Standard deviation (SD) of five animals per group. Means with $p \leq 0.05$ indicated by similar superscripted letters in the same column are considered not significantly different by ANOVA and post ANOVA (Tukeys) test.

Table 3: Effects of Oral Administration of *Triticum aestivum* Aqueous in Rats on Liver Function Test

Treatment Group	Liver Function Test Analytes					
	AST (IU/L)	ALT (IU/L)	ALP (IU/L)	GGT (IU/L)	TP (g/L)	ALB (g/L)
Normal Control	415 \pm 191 ^a	185 \pm 44 ^a	283 \pm 142 ^a	3.5 \pm 2.1 ^{ab}	70 \pm 16 ^a	34 \pm 8 ^a
High Non-Therapeutic Aqueous Extract Doses (mg/kg body weight) of <i>Triticum Aestivum</i>						
300	639 \pm 394 ^a	228 \pm 159 ^a	249 \pm 127 ^a	4.6 \pm 2.3 ^{ab}	76 \pm 7 ^a	30 \pm 5 ^a
448.14	449 \pm 211 ^a	113 \pm 29 ^a	227 \pm 130 ^a	1.4 \pm 1.2 ^b	70 \pm 3 ^a	30 \pm 9 ^a
669.4	546 \pm 179 ^a	224 \pm 181 ^a	234 \pm 158 ^a	8.3 \pm 3.7 ^a	69 \pm 3 ^a	28 \pm 3 ^a
1000	759 \pm 197 ^a	160 \pm 56 ^a	162 \pm 81 ^a	7.6 \pm 4.0 ^a	70 \pm 5 ^a	25 \pm 3 ^a

Results are expressed as Mean \pm Standard deviation (SD) of five animals per group. Means with $p \leq 0.05$ indicated by similar superscripted letters in the same column are considered not significantly different by ANOVA and post ANOVA (Tukeys) test.

Table 4: Effects of Oral Administration of *Triticum aestivum* Aqueous Extracts in Rats on Cardiac, Pancreatic and Renal Function Tests

Treatment Group	Cardiac		Pancreatic		Renal Function Test Analytes			
	LDH (IU/L)	CK (UI/L)	AMYLASE (IU/L)	LIPASE (IU/L)	UREA (Mmol/L)	CREATININE (μ mol/L)	SODIUM (Mmol/L)	POTASSIUM (Mmol/L)
Normal control	1517 \pm 814 ^a	909 \pm 447 ^a	1346 \pm 409 ^a	16 \pm 1.0 ^a	11 \pm 2 ^a	106 \pm 19 ^a	156 \pm 1 ^a	22 \pm 8 ^a
High Non-Therapeutic Aqueous Extract Doses (mg/kg Body Weight) of <i>Triticum Aestivum</i>								
300	1990 \pm 621 ^a	1335 \pm 1103 ^a	1318 \pm 511 ^a	15 \pm 0.8 ^a	12 \pm 1 ^a	86 \pm 10 ^a	158 \pm 5 ^a	11 \pm 3 ^b
448.14	1344 \pm 223 ^a	2587 \pm 1103 ^a	1268 \pm 304 ^a	16 \pm 2.6 ^a	11 \pm 3 ^a	79 \pm 19 ^a	153 \pm 3 ^a	9 \pm 1 ^b
669.4	1478 \pm 580 ^a	2928 \pm 1333 ^a	1228 \pm 410 ^a	14 \pm 0.9 ^a	11 \pm 1 ^a	85 \pm 24 ^a	154 \pm 6 ^a	10 \pm 3 ^b
1000	1674 \pm 230 ^a	1923 \pm 1510 ^a	901 \pm 206 ^a	15 \pm 0.5 ^a	10 \pm 2 ^a	74 \pm 12 ^a	150 \pm 3 ^a	9 \pm 1 ^b

Results are expressed as Mean \pm Standard deviation (SD) of five animals per group. Means with $p \leq 0.05$ indicated by similar superscripted letters in the same column are considered not significantly different by ANOVA and post ANOVA (Tukeys) test.

Table 5: Effects of Oral Administration of High Non-Therapeutic Aqueous Extract Doses of *Triticum aestivum* in Rats Daily for 28 Days on Haematological Parameters

Treatment Group	RBC Indices		PLT ($\times 10^3/\mu\text{L}$)	WBC ($\times 10^9/\text{L}$)	Differential leucocytes count ($\times 10^9/\text{L}$)				
	RBC ($\times 10^6/\mu\text{L}$)	Hb (g/dL)			Neutrophils	Lymphocytes	Monocytes	Eosinophils	Basophils
Normal control	6.1 \pm 1.1 ^a	12.2 \pm 0.8 ^b	632 \pm 217 ^a	4.0 \pm 1.7 ^a	0.9 \pm 0.7 ^a	1.9 \pm 1.1 ^a	0.6 \pm 0.7 ^a	0.3 \pm 0.2 ^a	0.4 \pm 0.7 ^a
High non-therapeutic aqueous extract doses (mg/kg body weight) of <i>Triticum aestivum</i>									
300	7.1 \pm 0.4 ^a	13.5 \pm 0.4 ^{ab}	569 \pm 295 ^a	6.1 \pm 4.5 ^a	0.6 \pm 0.4 ^a	3.8 \pm 3.6 ^a	0.7 \pm 0.9 ^a	0.5 \pm 0.9 ^a	0.4 \pm 0.5 ^a
448.14	7.4 \pm 0.5 ^a	14.3 \pm 1.2 ^a	789 \pm 158 ^a	4.7 \pm 3.1 ^a	0.9 \pm 0.7 ^a	2.4 \pm 2.1 ^a	0.4 \pm 0.4 ^a	0.5 \pm 0.5 ^a	0.4 \pm 0.5 ^a
669.4	6.7 \pm 0.2 ^a	13.2 \pm 1.0 ^{ab}	746 \pm 113 ^a	5.3 \pm 2.8 ^a	1.6 \pm 1.3 ^a	2.8 \pm 2.6 ^a	0.5 \pm 0.5 ^a	0.2 \pm 0.3 ^a	0.3 \pm 0.4 ^a
1000	6.6 \pm 1.0 ^a	12.9 \pm 1.3 ^{ab}	787 \pm 208 ^a	4.3 \pm 2.4 ^a	0.8 \pm 0.3 ^a	1.9 \pm 1.2 ^a	0.9 \pm 0.8 ^a	0.3 \pm 0.4 ^a	0.5 \pm 0.6 ^a

Results are expressed as Mean \pm Standard deviation (SD) of five animals per group. Means with $p \leq 0.05$ indicated by similar superscripted letters in the same column are considered not significantly different by ANOVA and post ANOVA (Tukeys) test.

Phytochemical Analysis

Qualitative phytochemical screening established high presence of flavonoids, terpenoids, saponins and cardiac glycosides, moderate presence of tannins and alkaloids (mayers test) and mild presence of steroids, phylobatannins and alkaloids (hagers test). Anthraquinones were absent. The phytochemical composition has been reported by Yogesha et al., 2013. Among them, alkaloids are used as therapeutic agent for their analgesic and anti-bacterial properties, flavonoids and saponins are useful for anti-inflammatory, anti-bacterial and antioxidant activities, cardiac glycosides are useful for ulcer and diabetic treatment, terpenoids and tannins are used as anti-microbial and anti-diarrheal agent.

CONCLUSIONS

The results obtained from this study show glycemia lowering effects of the *Triticum aestivum* aqueous extracts which at doses higher than 50mg/kg body weight compares to that of glibenclamide. The results obtained in evaluation of chronic toxicity of *Triticum aestivum* aqueous extracts oral administered at high non-therapeutic doses, indicated that the *Triticum aestivum* aqueous extracts does not contribute to any toxicity effects post chronic consumption. In conclusion, aqueous extracts of *Triticum aestivum* at therapeutic doses has high antihyperglycaemic ability and is nontoxic. The phytochemicals present in the aqueous extract of *Triticum aestivum* has proven therapeutic properties and contribute to the total therapeutic effects experienced with *Triticum aestivum*. Hence, the aqueous extracts of *Triticum aestivum* is safe and valuable in control and management of glycemia and its free radical scavenging property is paramount in preventing diabetic associated complications and anti-diabetic therapeutic drugs toxicities. While the synergistic use (WHO 2013) of *Triticum aestivum* with conventional antidiabetic drugs could be more beneficial to the diabetic patient, its use as a monotherapy for diabetes should be recommended after carrying out extensive translational research involving human beings.

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