RESEARCH ARTICLE

Antidiabetic activity and Safety evaluation of *Triticum aestivum* (wheatgrass) extract in Alloxan-induced diabetic rats model

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ABSTRACT

Triticum aestivum (wheatgrasses) extract was studied for antidiabetic activity using alloxan-induced diabetes mellitus in rats. *Triticum aestivum* at 50, 100, and 200 mg/kg showed significant antidiabetic activity; it effected profound reductions in blood glucose over 28 days, especially the higher doses of 100 and 200 mg/kg, which were more effective than Glibenclamide. At 2000 mg/kg, the extract did not lower the blood glucose of normoglycemic rats- an indication of its safety and lack of hypoglycemic responses under non-diabetic conditions. It would appear that the antidiabetic activity of *Triticum aestivum* is through the protection and regeneration of beta cells that enhance insulin secretion, which in turn increases the utilization of glucose and normalizes carbohydrate, fat, and protein metabolism. This gradual lowering of blood glucose levels brought on by *Triticum aestivum* is clinically highly desired. The elicited antidepressant effect of *Triticum aestivum* was very likely due to its antioxidant effects and beta-cell regeneration activity. Thus, *Triticum aestivum* can meet the demand for a nontoxic, safe alternative to orthodox antidiabetic agents. *Triticum aestivum*, antidiabetic activity, alloxan-induced diabetes, insulin secretion, beta-cell regeneration, glucose metabolism, safety, and Glibenclamide.

Keywords: *Triticum aestivum*, wheatgrass, antidiabetic activity, alloxan-induced diabetes, insulin secretion, beta-cell regeneration, glucose metabolism, safety, Glibenclamide.

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INTRODUCTION

Diabetes mellitus is a major lifestyle disorder in the present world. It is a chronic carbohydrate, fat and protein metabolism disorder characterized by increased fasting and postprandial blood sugar levels. More than 220 million people across the world have diabetes. In 2005, an estimated 1.1 million people have died from diabetes. Almost 80% of diabetes deaths occur in low- and middle-income countries. Nearly half of diabetes deaths occur in people under the age of 70 years; 55% of diabetes deaths are in women. WHO projects that diabetes deaths will double between 2005 and 2030. The global prevalence of diabetes is estimated to increase from 4% in 1995 to 5.4% by the year 2030.1

Diabetes and its complications have a significant economic impact on individuals, families, health systems, and countries. Diabetes mellitus is a complex metabolic disorder resulting from either insulin insufficiency or insulin dysfunction. Type 1 diabetes (insulin-dependent) is caused by insulin insufficiency due to a lack of functional beta cells. Therefore, patients suffering from this depend on an exogenous insulin source. In contrast, patients who have Type II diabetes (insulin-independent) are unable to respond to insulin and can be treated with dietary changes, exercise, and medication. Type 2 diabetes is the more common form of diabetes, constituting 90% of the diabetic population. The experimental evidence suggests that free radicals' involvement in the pathogenesis of diabetes2 and, more importantly, in the development of diabetic complications.3-5 Free radicals can damage cellular molecules, DNA, proteins, and lipids, leading to altered cellular functions. Many recent studies revealed that antioxidants capable of neutralizing free radicals are effective in preventing experimentally induced diabetes in animal models6,7 and reducing the severity of diabetic complications.5 The abnormalities produced in lipids and proteins are the major contributing factors for the development of diabetic complications like neuropathy, retinopathy, and nephropathy.

Long before the use of insulin became common, indigenous remedies were used for the treatment of diabetes mellitus and

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hyperlipidemia. There has been an increasing demand from patients for the use of natural products with antidiabetic and antihyperlipidemic activity. This is large because insulin cannot be used orally, and insulin injections are associated with the risk of hypoglycemia and impairment of hepatic and other body functions. The undesirable side effects and contraindications of synthetic drugs and the fact that they are not suitable for use during pregnancy have made scientists look towards hypoglycemic agents of plant origin.8 Many herbs and plant products have been shown to have antihyperglycemic and antihyperlipidemic action.9-11 In the last few years, there has been exponential growth in the field of herbal medicine, and these drugs are gaining popularity both in developing and developed countries because of their natural origin and fewer side effects. Many traditional medicines in use are derived from medicinal plants, minerals, and organic matter.12

The medicines of the modern era, i.e., allopathic medication, though very potent and provide immediate relief, are potentially toxic and costly, give no definite answer in chronic conditions, provide only symptomatic relief, and do not arrest the underlying disease process. Due to these reasons, people worldwide are looking to various alternative systems of medicine, especially herbal medicines, which are considered nontoxic, cost effective, and effective compared to allopathic drugs and may provide some answers in chronic disease. Therefore, for this research project, an herbal plant, Triticum aestivum (wheatgrass), was selected to study its effect on peptic ulceration and *in the treatment of diabetes mellitus, a major crippling disease in the world leading to huge economic losses.*

Triticum aestivum Linn. Commonly known as wheatgrass, it belongs to the family Poaceae. It has been claimed to be useful for anticancer activity,[13-23] immunopotency activity[24-25,] antioxidant activity,[26-30] anti-thalassemic activity,[31-34] cardioprotective and hepatoprotective activity,[35-39] anti-inflammatory and antiarthritic activity.[40-41] Based on multipurpose activities and observed shortcomings/limitations of earlier published reports, we explored the different forms extracts of Triticum aestivum for antidiabetic, anticancer, and antiulcer potential.

MATERIALS AND METHODS

Materials

Collection and authentication of plant

Adequate quantities of wheat grain were soaked overnight in water in a glass bowl. The next day, the soaked wheat grains were spread on the soil's surface and filled in plastic trays. Care was taken so that the grains did not touch one another. A thin layer of soil was sprinkled on the wheat grains, and then the tray was covered with a newspaper to provide darkness, which helped the sprouting. The tray was kept on a covered balcony. The next day, the tray was uncovered to spray on some water and was covered again with the newspaper. The previous step was repeated every day until sprouting took place, after which the tray was left uncovered and watered every day for ten days. On the 10th day, the wheatgrass was harvested by cutting it with a scissor about 1/2" above the soil's surface. This collection was done from November to December 2013-2014. A taxonomist confirmed and authenticated the botanical identity of the plant.

Animal

The present study was conducted in Wistar rats. After getting permission from the institutional animal ethical committee (IAEC), animals were procured from Shree Pharm, Bhandara. They were caged in wired meshed cages and kept in the animal house of the department for seven days to adapt to the new environment before subjecting them to the experiment. Animals were fed on a pallet diet. Animals were maintained in ideal conditions of temperature 25-26^{0}C, relative humidity 50-70%, and light and dark cycles of 12 hours each. Animals used in this study were albino rats of the Wistar strain, weighing between 120-250 g of either sex. Rats were used to investigate the antidiabetic activity, antiulcer activity, and histopathology of the pancreas in the antidiabetic study, and the stomach in the antiulcer research was studied.

Preparation of plant extract

The collected Triticum aestivum (wheatgrass) was carefully cleaned, dried under shade, powdered with an electric mixture, and stored in a separate airtight container until it was used to prepare the extract. 200gm of dried wheatgrass powder was macerated separately in 70% ethanol overnight. Then, it was packed in the percolator tube and was extracted using 70% ethanol, yielding an extract that was brownish-black semi-solid; from 200 g of dried powder, 9 g extract was yielded. The extract was separated in an airtight container and kept inside the refrigerator.

Induction of diabetes

The chemical method was employed to study the hypoglycemic activity. Alloxan monohydrate was used to induce diabetes. Animals used were Wistar rats of either sex. The random blood sugar was estimated with the help of a glucometer. Following an overnight fast, animals were injected intraperitoneal with freshly prepared alloxan monohydrate 2% solution dissolved in 0.9% sodium chloride in a dose of 150 mg/kg body weight. Following injection, animals were carefully observed for the first 24 hours for any evidence of adverse effects like allergic reactions, behavioral changes, and convulsions. No untoward response was observed in any animal. Since alloxan is capable of producing fatal hypoglycemia as a result of massive pancreatic insulin release, animals were treated with 30 percent glucose solution orally at different time intervals after six hours of alloxan induction, and 5 percent glucose solution was kept in bottles in their cages for the next 24 hrs to prevent hypoglycemia. Random blood glucose (RBS) was recorded daily for one week. Animals developed stable hyperglycemia after 4-5 days. Only those animals with blood glucose >250 mg/dl were selected for the study. Later, they were divided into six groups of 6 animals each.

Collection of the blood and estimation of serum glucose

Blood was withdrawn from the retro-orbital sinus under ether inhalation anaesthesia, and glucose levels were estimated at intervals of 0^{th},1^{st},3^{rd},7^{th}, 14^{th}, and 28^{th} day, using a ContourTS glucometer manufactured by Bayer Healthcare LLC, USA

Ethical clearance

The Institutional Animal Ethics Committee (IAEC) approved the experimental protocol.

Experimental Design for Antidiabetic Study

Animals were divided into six groups of six animals each. Doses (50,100,200 mg/kg orally) for the study. Feed and water were provided ad libitum to the animals. Animals were divided for the antidiabetic study in the following manner:

Group I (NC): Control vehicle only (1 ml of 2 % gum acacia).

Group II (DC): Diabetic control alloxan (150 mg/kg body wt) treated only once

Group III (DE1): Diabetic + Triticum aestivum 50 mg/kg body wt extract treated

Group IV (DE2): Diabetic + Triticum aestivum 100 mg/kg body wt extract treated

Group V (DE3): Diabetic + Triticum aestivum 200 mg/kg body wt extract treated

Group VI (DG): Diabetic + Glibenclamide 5 mg/kg body weight).

The study was conducted for a total of 28 days in rats.

In rats, random blood glucose levels were determined on days 0, 1, 3, 7, 14, and 28.

Histopathological Studies

Pancreatic tissues from all groups were subjected to histopathological studies. The whole pancreas from each animal was removed 28 days after sacrificing the animal under anaesthesia. Pancreatic tissue was collected in 10% formalin solution and immediately processed by the paraffin technique. Sections of 5 μm thickness were cut and stained by haematoxylin and eosin (H and E) for histological examination.[42]

Acute Toxicity study

An acute oral toxicity study for the test extract of the plant was carried out using OECD/OCED guideline 425. The test procedure minimizes the number of animals required to estimate the oral acute toxicity. The test also allows the observation of signs of toxicity and can also be used to identify chemicals that are likely to have low toxicity.[43] Healthy, young male Wistar rats weighing 120-200 grams were taken, and the animals were divided into six groups of six animals each. All groups were given hydroalcoholic extract *Triticum aestivum* in graded doses of 100, 200, 400, 800, 1000, and 2000 mg/kg body weight. A single dose of an extract of Triticum aestivum was administered orally after overnight fasting. The animals were observed continuously for 30 min, 1 hrs, 24 hours, and then occasionally for a further 14^{th} day for any toxic effect of extract [44-47].

Limit test at 2000 mg/kg.

The drug was administered in a dose of 2000 mg/kg of body weight orally to one animal. This first test animal survived. Then, five other animals were dosed sequentially; six were tested. Animals were observed individually for tremors, clonic convulsions, tonic extensions, catatonia, spasticity, opisthotonus, loss of righting reflex, ataxia, sedation, muscle relaxation, arching and rolling, ptosis, lacrimation, diarrhoea, salivation, writhing, respiration and skin color. Further photo actometer was used to observe any change in motor activity, whether it is increased or decreased during the first 30 min after dosing, periodically during the first 24 hrs (with special attention given during the first 4 hrs), and daily after that, for a total of 14 days. No animal died. Therefore, the LD_{50}

Table 1: Signs in different groups when observed for 24 hours.

Signs	*Gp1 (100*)*	*Gp 2 (200*)*	*Gp 3 (400*)*	*Gp 4 (800*)*	*Gp 5 (1000*)*	*Gp 6 (2000*)*
Tremors	Ab	Ab	Ab	Ab	Ab	Ab
convulsions	Ab	Ab	Ab	Ab	Ab	Ab
Gait	N	N	N	N	N	N
Spasticity	Ab	Ab	Ab	Ab	Ab	Ab
Opisthotonus	Ab	Ab	Ab	Ab	Ab	Ab
Loss of righting reflex	No	No	No	No	No	No
Ataxia	NM	NM	NM	NM	NM	NM
Sedation	NS	NS	NS	NS	NS	NS
Muscle relaxation	NT	NT	NT	NT	NT	NT
Ptosis	Ab	Ab	Ab	Ab	Ab	Ab
Lacrimation	No	No	No	No	No	No
Diarrhea	NBR	NBR	NBR	NBR	NBR	NBR
Writhing	No	No	No	No	No	No
Respiration	N	N	N	N	N	N

Gp: Group *mg/ml Ab: Absent NM: Normal Movement NS: Normal Sleep,
NT: Normal Tone NBR: Normal Bowel Reflex N: Normal

is greater than 2000 mg/kg.[43] An investigation with 1/40th, 1/20th, 1/10 to, and 1/5 to of 2000 mg/kg, i.e., 50, 100, 200, and 400 mg, was done in pre-screening; hence this dose was used in final screening.

RESULTS

Acute Toxicity Studies

The hydroalcoholic extract of Triticum aestivum (HETA) was administered in graded doses of 100, 200, 400, 800, 1000 & 2000 mg/kg and was given to six groups with six rats in each group. The animals were observed for signs such as tremors, clonic convulsions, tonic extensions, catatonia, spasticity, opisthotonos, ataxia, ptosis, lacrimation, diarrhea, and salivation. The rats did not show any adverse effect mentioned above, even at the 2000mg/kg dose, ten times the study dose. All reflexes were found normal, as shown in Table 1.

Antidiabetic activity

Table 2-A shows the mean values of all readings at baseline, after alloxan administration, on the 1st, 3rd, 7th, 14th, and 28th day in all six groups. Table 2-B shows statistical calculation with t-test (t-value) and p-value after comparing (a) distill water with alloxan diabetic control, (b) alloxan diabetic control with extract-treated groups, and (c) standard ranitidine with extract-treated groups. All six groups had no significant difference in the Baseline Blood Sugar Level. In group I (normal), there was no significant change in the Blood Sugar level over 28 days. In groups II, III, IV, V, and VI, after administration of alloxan, there was a significant increase in Blood Sugar level after 48 hr. In group II (Diabetic control), there was a progressive increase in blood sugar level for 1st seven days, and then there was a slight decrease in the Blood Sugar level from day 14 onwards. In groups treated with HETA, there was a decrease

Table 2A: Effect of hydroalcoholic extract of *Triticum aestivum* (HETA) on random blood sugar in rats

GR n=6	*Drugs /Dose*	*Blood sugar level (mg/dl) Mean ± SD*						
		Before		*After Drug Administration (ADA)*				
		Base-line	*48hr After alloxan*	*24 hr.*	*72 hr.*	*Seven day*	*14 day*	*28 day*
I	DW 10[2]	132.83 ± 1.47	133.50 ± 1.04	133.33 ± 1.96	133.33 ± 1.96	133 ± 1.41	133.33 ± 2.25	135.5 ± 1.37
II	Alloxan 150[1]	133.83± 1.47	449.16 ± 2.48	467.66±10.11	477.66 ± 7.68	496± 5.51	456.33±3.55	354.83 ± 4.99
III	Alloxan 150[1] + HETA 50[2]	133.66 ± 1.75	423.50 ± 3.01	420.33 ±2.06	383.16± 5.81	333.16 ± 3.06	242± 4.97	169.83 ± 6.70
IV	Alloxan 150[1] + HETA 100[2]	134.33 ± 2.16	426.50 ± 3.08	424.16 ± 3.31	314.33 ± 2.94	284.50±4.37	213.66 ± 2.33	139.33± 3.72
V	Alloxan 150[1] + HETA 200[2]	134.83 ± 1.47	425.50 ± 2.25	361.83 ± 4.91	279.50 ± 6.77	225.83 ±5.15	200 ± 3.68	136.50 ± 4.41
VI	Alloxan 150[1] + gliben 5[2]	133.50 ± 1.87	428.83 ± 2.92	417.83 ± 11.60	326.83± 6.82	261.33±5	169±4.69	142 ± 4.47

GR-Groups DW-Distil water Glib- Glibenclamide

Table 2B: Effect of hydroalcoholic extract of *Triticum aestivum* (HETA) on random blood sugar in rats (t-test, p-value)

GR n=6	*Drugs /Dose*	*Blood sugar level (mg/dl) Mean ± SD*						
		Before		*After Drug Administration (ADA)*				
		Base-line	*48hr After alloxan*	*24 hrs.*	*72 hrs.*	*Seven day*	*14 day*	*28 day*
DW 10[2]	Alloxan 150[1]	0.000 (1.00, NS)	286.83 (0.000, S)	79.49 (0.000, S)	106.32 (0.000, S)	156.21 (0.000, S)	187.88 (0.000, S)	104.59 (0.000, S)
	Alloxan 150[1] + HETA 50[2]	0.178 (0.86,NS)	16.09 (0.000,S)	11.23 (0.000,S)	24.02 (0.000,S)	63.25 (0.000,S)	85.77 (0.000,S)	54.18 (0.000,S)
Alloxan 150[1]	Alloxan 150[1] + HETA 100[2]	0.46 (0.64, NS)	14.02 (0.000, S)	10.01 (0.000, S)	48.61 (0.000, S)	73.63 (0.000, S)	139.58 (0.000, S)	82.11 (0.000, S)
	Alloxan 150[1] + HETA 200[2]	1.17 (0.26, NS)	17.27 (0.000, S)	23.05 (0.000, S)	47.37 (0.000, S)	87.67 (0.000, S)	122.51 (0.000, S)	80.20 (0.000, S)
	Alloxan 150[1] + HETA 50[2]	0.15 (0.87,NS)	3.10 (0.71,NS)	0.52 (0.62,NS)	15.39 (0.000,S)	29.98 (0.000,S)	26.13 (0.000,S)	8.45 (0.000,S)
Alloxan 150[1] + Gliben 5[2]	Alloxan 150[1] + HETA 100[2]	0.71 (0.49,NS)	1.34 (0.20,NS)	1.28 (0.24,NS)	4.12 (0.002,S)	8.53 (0.000,S)	20.87 (0.000,S)	2.91 (0.017,S)
	Alloxan 150[1] + HETA 200[2]	1.37 (0.20,NS)	2.20 (0.05,NS)	10.88 (0.000,S)	12.05 (0.000,S)	12.10 (0.000,S)	12.72 (0.000,S)	3.76 (0.004,S)

GR-Groups **DW-**Distil water **NS-** Non-significant **S-** Significant,

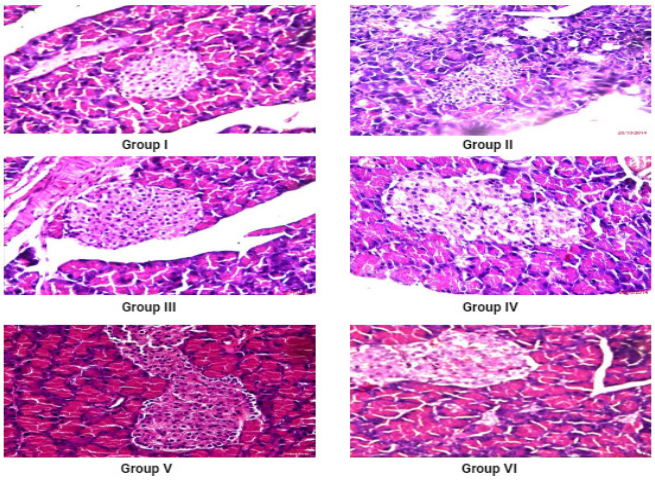

Figure 1: Histopathological studies

in Blood Sugar levels. In group III, a significant reduction in Blood Sugar level was seen on the 7th day, which was highly significant from day 14 onward to the 28th day. In group IV (100 mg), there was a significant decrease in Blood Sugar level on day three, which was highly significant from day seven onwards, which was statistically significant, and blood sugar level came to a non-diabetic level at the end of the study, i.e., on the 28th day. In group V, there was a decrease in Blood Sugar level from day 1, which was statistically significant, and blood sugar level came to a non-diabetic level. In glibenclamide-treated groups, there was a highly significant fall in blood sugar levels on day one and a continuous fall in random blood sugar levels over the next 28th-day period.

Histopathological Studies

Group I photomicrograph showing regular, normal appearance of the nucleus, tightly arranged islet cells were observed in the normal control group. In group II, alloxan caused severe necrotic changes, especially in the center of islets. Nuclear changes, karyolysis, and the disappearance of the nucleus and residue of destroyed cells were visible in some places. Relative size reduction, number of islets especially around the central vessel, and severe reduction of cells were clearly seen. Group III shows the histopathological study of the treated group, which indicated increased volume density of islets and increased number of cells, which may be a sign of regeneration. Group IV photomicrograph of the HETA-treated group (100 mg/kg) showed increased volume and density of islets and increased percentage of cells, as well as an increase in cell diameter, which may be a sign of regeneration. The photomicrograph of group V showed that the size of islets was significantly increased, and the necrosis and atrophy of islets were improved considerably; also, the number and diameter of the cell islets appeared to be regular compared to the diabetic group. Photomicrographs of group V standard (glibenclamide 5mg/kg) treated group rat showing moderate expansion and restoration of normal cellular population size of islets with hyperplasia by Glibenclamide (Figure 1).

Group I: Photomicrograph of the pancreas of normal rat (H&E x 400); Group II: Photomicrograph of the pancreas of rat treated with Alloxan 150 mg/kg body wt (H & E x 400); Group III: Photomicrograph of the pancreas of rat treated with hydroalcoholic extract of *Triticum aestivum* (HETA) at a dose 50 mg/kg (H &E x 400) on 28th day; Group IV: Photomicrograph of the pancreas of rat treated with hydroalcoholic extract of Triticum aestivum (HETA) at dose 100 mg/kg body wt (H &E x 400) on 28th day; Group V: Photomicrograph of the pancreas of rat treated with hydroalcoholic extract of Triticum aestivum (HETA) at dose 200 mg/kg (H &E x 400) on 28th day; Group VI: Photomicrograph of pancreas of rat treated with standard drug Glibenclamide 5 mg/kg (H & E x 400) on 28th day

DISCUSSION

In the present study, Tritium aestivum (wheatgrass) shoot extract has been investigated for its antidiabetic activity in experimentally alloxan-induced diabetic mellitus and antiulcer activity in aspirin-induced ulcer & swimming stress-induced ulcer models in Wistar rats. To explore the mechanism of antidiabetic activity of triticum aestivum, its effect was studied on random blood glucose levels, and we also examined the impact of various dosages on normal rats to look for hypoglycemic effect and histopathology of the beta cells of the pancreas by using rats as an experimental animal. To explore the mechanism of antiulcer activity of triticum aestivum, its effect was studied in an aspirin-induced ulcers model and a swimming stress-induced ulcer model in rats. Despite the availability of several antidiabetic agents, the treatment of diabetes mellitus remains unsatisfactory, especially concerning the complete cure of the disease. Therefore, there is always a constant effort to screen more effective and better drugs.

Further the drugs presently available in modern medicine have toxic potential and are costly, hence cannot be afforded by the poor patient. In search of the least harmful and less expensive alternative, herbal medicine or drugs have become the agents of choice for the treatment of ailments nowadays. Within view and aim, the present study was selected to screen the Triticum aestivum extract for its antidiabetic activity.

In the present experimental work, the antidiabetic activity and histopathology of the beta cells of the pancreas using rats as experimental animals have been evaluated while studying the antihyperglycemic activity of triticum aestivum. In non-diabetic rats, wheatgrass extract administered in daily doses of 50, 100, and 200 mg/kg for 28 days showed no change in the blood glucose level. It suggests extracts of Triticum aestivum do not show a decrease in the normal blood glucose level. Therefore, it does not have hypoglycemic activity, usually the most important and harmful side effect of insulin and sulphonylureas. Extract of Triticum aestivum (wheatgrass) in our study showed a significant decrease in random blood sugar levels in all the study doses (50,100,200mg/dl). Our study agrees with Sheikh et al., 2011.[48] who also reported the blood sugar-lowering properties of Triticum aestivum in alloxan-induced hyperglycaemic rats. However, their research studied the fresh juice of Triticum aestivum (wheatgrass). They

showed that the wheatgrass can reverse the effects of alloxan-induced diabetes significantly. The mechanism they put forward about its hypoglycemic action may be by increasing either the pancreatic secretion of insulin from β-cells of islets of Langerhans or its release from bound form, or the juice may enhance peripheral utilization of glucose or reduce the absorption of glucose from GIT.

In the present study, we treated rats with different dosages of hydroalcoholic extract of Triticum aestivum (wheatgrass). We carried out our study for 28 days (4 weeks) and showed the random blood sugar level reduction in the doses of 50, 100, 200 mg/kg. 100mg/kg and 200mg/kg, there was a significant decrease in random blood glucose levels compared to standard glibenclamide. Histopathological examination of the pancreas has shown an increase in diameter as well as several beta cells, indicating the proliferation with regeneration of beta cells after 28-day treatment with wheatgrass extract in rats. These changes are dose-dependent; maximum proliferation and increase in beta cells were observed orally at 200 mg/kg. In alloxan-induced diabetic rats, the reduction in beta cell number and islet diameter was noticed, indicating the loss of integrity between the cells in the islets. It suggests that the regeneration effect, antioxidant effect supported[49], and the prevention of mucosal damage of beta cells might contribute as an additional mechanism of antidiabetic activity of wheatgrass extract. No such changes were seen in the normal rats, further supporting Triticum aestivum (wheatgrass) activity. The extract of Triticum aestivum (wheatgrass) in our study has shown significant antidiabetic activity supported by histopathological improvement, shown as regeneration of beta cells and diameter. The antidiabetic activity of the extract of Triticum aestivum in the present study appeared to be due to the prevention of oxidative stress and the regeneration of beta cells of islets of Langerhans. This, in turn, might improve insulin secretion from beta cells and increase the peripheral utilization of glucose.

Therefore, as shown by our experimental work, it is inferred that Triticum aestivum (wheatgrass) may be a useful and antidiabetic agent. Since it has not decreased the normoglycemic rat blood glucose level, it will avoid the major side effect of hypoglycemia, which generally occurs with insulin and oral hypoglycemic agents. Because of its significant antidiabetic activity against alloxan-induced diabetes mellitus, more studies and clinical trials will be required before establishing it as an antidiabetic agent for human use. Furthermore, it will be a safe alternative because of the lack of insulin and other antidiabetic drug side effects.

CONCLUSION

The extract of Triticum aestivum did not decrease the blood sugar level even at 2000 mg/kg p.o. in normal glycaemic rats. This suggests its use is safe in diabetes, as it does not produce hypoglycemia. The three doses (50,100, and 200 mg/kg) were selected by trial and error to conduct the present study. The extracts of Triticum aestivum showed significant antidiabetic activity against alloxan-induced diabetes mellitus in rats. This is evident from a decrease in blood sugar levels. This could reach a normal level over 28 days. We compared the antidiabetic effect of Triticum aestivum with Glibenclamide and were found to be more effective than glibenclamide in doses of 100 mg/kg p.o. and 200 mg/kg p.o. An extract of Triticum aestivum (wheatgrass) produced a gradual fall in blood sugar levels, which is desirable in clinical situations. The extract of Triticum aestivum may be responsible for the antidiabetic effect by protecting beta cells and increasing insulin secretion. Our study's regeneration of beta cells in terms of increased number and diameter has further supported the improvements in insulin secretion. The increased secretion of insulin enhances the peripheral utilization of glucose and corrects the metabolisms of carbohydrates, fat, and protein, producing the antidiabetic effect. Thus, in the present study, the significant antidiabetic effect of Triticum aestivum (wheatgrass) in rats may be due to antioxidant properties as well as regeneration of beta cells. Further, Triticum aestivum did not show a decreased blood glucose level in normal rats. Hence, it may be a safer and better alternative than other antidiabetic agents.

REFERENCES

1. Ramachandran A, Snehalatha C, Viswanathan V. Burden of type 2 diabetes and its complications - The Indian scenario. Current Science. 2002; 83: 1471–1476.
2. Matteucci E, Giampietro O. Oxidative stress in families of type 1 diabetic patients. Diabetes Care. 2000; 23(8):1182-1186. Doi: 10.2337/diacare.23.8.1182.
3. Oberley LW. Free radicals and diabetes. Free radical biology and medicine. 1988;5(2):113-124. Doi: 10.1016/0891-5849(88)90036-6.
4. Baynes JW, Thorpe SR. The role of oxidative stress in diabetic complications. Current Opinion in Endocrinology and Diabetes. 1996;3(4):277-284.
5. Lipinski B. Pathophysiology of oxidative stress in diabetes mellitus. Journal of Diabetes and its Complications. 2001;15(4):203-210. Doi: 10.1016/s1056-8727(01)00143-x.
6. Kubisch HM, Wang J, Bray TM, Phillips JP. Targeted overexpression of Cu/Zn superoxide dismutase protects pancreatic beta-cells against oxidative stress. Diabetes. 1997;46(10):1563-1566. doi: 10.2337/diabetes.46.10.1563.
7. Naziroğlu M, Cay M. Protective role of intraperitoneally administered vitamin E and selenium on the antioxidative defense mechanisms in rats with diabetes induced by streptozotocin. Biological trace element research. 2001;79(2):149-59. doi: 10.1385/BTER:79:2:149.
8. Berger W. Incidence of severe side effects during therapy with sulfonylureas and biguanides. Hormone and metabolic research. Supplement series. 1985;15:111-115.
9. Elder C. Ayurveda for diabetes mellitus: a review of the biomedical literature. Alternative Therapies in Health & Medicine. 2004;10(1):44-50.
10. Srinivasan K. Plant foods in the management of diabetes mellitus: spices as beneficial antidiabetic food adjuncts. International journal of food sciences and nutrition. 2005;56(6):399-414. Doi: 10.1080/09637480500512872.
11. Badole S, Patel N, Bodhankar S, Jain B, Bhardwaj S. Antihyperglycemic activity of aqueous extract of leaves of *Cocculus hirsutus* (L.) Diels in alloxan-induced diabetic mice.

Indian Journal of Pharmacology 2006;38(1):49-53. 2006. DOI: 10.4103/0253-7613.19853.
12. Grover JK, Yadav S, Vats V. Medicinal plants of India with antidiabetic potential. Journal of ethnopharmacology. 2002;81(1):81-100. Doi: 10.1016/s0378-8741(02)00059-4.
13. Bonfili L, Amici M, Cecarini V, Cuccioloni M, Tacconi R, Angeletti M, Fioretti E, Keller JN, Eleuteri AM. Wheat sprout extract-induced apoptosis in human cancer cells by proteasomes modulation. Biochimie. 2009;91(9):1131-1144. doi: 10.1016/j.biochi.2009.06.001.
14. Finch CE, Tanzi RE. Genetics of aging. Science. 1997;278(5337):407-411. Doi: 10.1126/science.278.5337.407.
15. Wheat J, Currie G. Herbal Medicine for Cancer Patients: An Evidence Based Review. Internet Journal of Alternative Medicine. 2008; 5(2): 28-30.
16. Mates JM, Sanchez-Jimenez FM. Role of reactive oxygen species in apoptosis: implications for cancer therapy. international journal of biochemistry & cell biology. 2000;32(2):157-170. doi: 10.1016/s1357-2725(99)00088-6.
17. Egner PA, Wang JB, Zhu YR, Zhang BC, Wu Y, Zhang QN, Qian GS, Kuang SY, Gange SJ, Jacobson LP, Helzlsouer KJ, Bailey GS, Groopman JD, Kensler TW. Chlorophyllin intervention reduces aflatoxin-DNA adducts in individuals at high risk for liver cancer. Proceedings of the National Academy of Sciences. 2001;98(25):14601-14606. doi: 10.1073/pnas.251536898.
18. Guo D, Schut HA, Davis CD, Snyderwine EG, Bailey GS, Dashwood RH. Protection By Chlorophyllin And Indole-3-Carbinol Against 2-Amino-1-Methyl-6 Phenylimidazo 4,5-B, Pyridine (Phip)- Induced DNA Adducts And Colonic Aberrant Crypts In The F344 Rat. Carcinogenesis 1995;16(12): 2931-2937.
19. Pratt MM, Reddy AP, Hendricks JD, Pereira C, Kensler TW, Bailey GS. The importance of carcinogen dose in chemoprevention studies: quantitative interrelationships between, dibenzo [a,l]pyrene dose, chlorophyllin dose, target organ DNA adduct biomarkers and final tumor outcome. Carcinogenesis. 2007;28(3):611-624. Doi: 10.1093/carcin/bgl174.
20. Sarkar D, Sharma A, Talukdar G. Chlorophyll and Chlorophyllin as Modifier Of Genotoxic Effects. Mutation Research 1994; 318(3): 239-247. Doi:10.1016/0165-1110(94)90017-5.
21. Bar-Sela G, Tsalic M, Fried G, Goldberg H. Wheat grass juice may improve hematological toxicity related to chemotherapy in breast cancer patients: a pilot study. Nutrition and cancer. 2007;58(1):43-48. Doi: 10.1080/01635580701308083.
22. Peryt B, Szymczyk T, Lesca P. Mechanism of antimutagenicity of wheat sprout extracts. Mutation Research/Fundamental and Molecular Mechanisms of Mutagenesi. 1992;269(2):201-215. doi: 10.1016/0027-5107(92)90201-c.
23. Aydos OS, Avci A, Ozkan T, Karadag A, Gurleyik E, Altinok B *et al*. Antiproliferative, Apoptotic And Antioxidant Activities Of Wheatgrass (*Triticum aestivum* L.) Extract On CML (K562) Cell Line. Turkish Journal of Medical Science 2011; 41(4):657-663. Doi: 10.3906/sag-0912-425.
24. Kay AB. Overview of allergy and allergic diseases: with a view to the future. British medical bulletin. 2000;56(4):843-864. Doi: 10.1258/0007142001903481.
25. Hemalatha R, Karthik M, Narendra Babu K, Dinesh Kumar B. Immunomodulatory Activity of *Triticum aestivum* And Its Effects On Th1/Th2 Cytokines And Nfkb P65 Response. American Journal of Biochemistry and Molecular Biology 2012; 2(1): 19-29. Doi: 10.3923/ajbmb.2012.19.29.
26. Laic N, Dabney BJ, Shaw CR. Inhibition Of In Vitro Metabolic Activation of Carcinogen by Wheat Sprout Extracts. Nutrition and Cancer 1978;1:27-30.
27. Lai CN. Chlorophyll: The Active Factor In Wheat Sprout Extracts Inhibiting The Metabolic Activation Of Carcinogens In Vitro. Nutrition and Cancer 1979;1:19-21.
28. Falcioni G, Fedeli D, Tiano L, Calzuola I, Mancinelli L, Marsili V, Gianfranceschi G. Antioxidant activity of wheat sprouts extract in vitro: inhibition of DNA oxidative damage. Journal of food Science. 2002;67(8):2918-2922. https://doi.org/10.1111/j.1365-2621.2002.tb08838.x
29. Kulkarni SD, Acharya R, Naira G, Reddy AVR. Determination of Elemental Concentration Profiles in Tender Wheatgrass (*Triticum aestivum* L.) Using Instrumental Neutron Activation Analysis. Food Chemistry 2006;4: 699-707. https://doi.org/10.1016/j.foodchem.2005.04.006
30. Siener R, Honow R, Voss S, Seidler A, Hesse A. Oxalate Content Of Cereals and Cereal Products. Journal of Agriculture and Food Chemistry 2006; 54(8):3008-3011. Doi: 10.1021/jf052776v.
31. Marawaha RK, Bansal D, Kaur S, Trehan A. Wheat grass juice reduces transfusion requirement in patients with thalassemia major: a pilot study. Indian Pediatrics. 2004;41(7):716-720.
32. Kelentei, Fekete B, Kun. Influence of Copper Chlorophyllin on Experimental Anemia. Acta Pharmaceutica Hungarica 1958;28: 176-180.
33. Singh K, Pannu MS, Singh P, Singh J. Effect of wheat grass tablets on the frequency of blood transfusions in Thalassemia Major. The Indian Journal of Pediatrics. 2010;77(1):90-91. Doi: 10.1007/s12098-010-0002-8.
34. Mukhopadhyay S, Basak J, Kar M, Mandal S, Mukhopadhyay A (2009) The Role Of Iron Chelation Activity Of Wheatgrass Juice In Patients With Myelodysplastic Syndrome. Journal of Clinical Oncology: 7012-7014. Doi:10.1200/jco.2009.27.15 suppl.7012.
35. Jain G, Argal A, Pathak AK, Singh DK, Kannokia P. Hepatoprotective Activity of Wheatgrass Juice. The Pharmacist 2007;2(1): 29-30.
36. Kamboj JK, Rana SV, Dhawan DK, Vahiphei K. Role of Wheatgrass In Prevention of Carbon Tetrachloride Induced Hepatotoxicity In Rats. Journal of Clinical and Experimental Hepatology 2011;43(2): 267-271. Doi:10.1016/S0973-6889(11)60089-3.
37. Kothari S, Jain AK, Mehta SC, Tonpay SD. Effect of fresh *Triticum aestivum* grass juice on lipid profile of normal rats. Indian journal of pharmacology. 2008;40(5):235-236. Doi: 10.4103/0253-7613.44157.
38. Selamoglu Talas Z, Ozdemir I, Yilmaz I, Gok Y, Orun I. The investigation of the antioxidative properties of the novel synthetic organoselenium compounds in some rat tissues. Experimental Biology and Medicine (Maywood). 2008;233(5):575-579. Doi: 10.3181/0707-RM-191.
39. Sethi J, Yadav M, Dahiya K, Sood S, Singh V, Bhattacharya SB. Antioxidant effect of Triticum aestivium (wheat grass) in high-fat diet-induced oxidative stress in rabbits. Methods and findings in experimental and clinical pharmacology. 2010;32(4):233-5. Doi: 10.1358/mf.2010.32.4.1423889.
40. Balint G, Apathy A, Gaal M, Telekes A, Resetar A, Blazso G, Falkay G, Szende B, Paksy A, Ehrenfeld M, Shoenfeld Y, Hidvegi M. Effect of Avemar--a fermented wheat germ extract--on rheumatoid arthritis. Preliminary data. Clinical and Experimental Rheumatology. 2006;24(3):325-328.

41. Nenonen MT, Helve TA, Rauma AL, Hanninen OO. Uncooked, lactobacilli-rich, vegan food and rheumatoid arthritis. British journal of rheumatology. 1998;37(3):274-81. doi: 10.1093/rheumatology/37.3.274.
42. Luna LC. Manual of histological screening methods of Armed Forces Institute of Pathology. New York: Mc Graw Hill Book Co; 1990;125.
43. OECD. Guideline for Testing of Chemicals 425, Acute oral toxicity (acute toxic class method) December 2001.
44. Sukkur AAA, Samuel AJSJ, Sangeetha S, Vimala AGKA. Chronic Toxicity Investigations with Nilavembu Kudineer in Sprague-Dawley Rats. International Journal of Pharmaceutical Quality Assurance. 2024;15(1):433-442. DOI: 10.25258/ijpqa.15.1.67
45. Johnsymary F, Komala M. Investigation of Antidiabetic Activity of Isolated Molecule from the Bark of Olax scandens. International Journal of Pharmaceutical Quality Assurance. 2023;14(3):481-486. DOI: 10.25258/ijpqa.14.3.04
46. Mane VB, Killedar SG, More HN, Tare HL, Evaluation of acute oral toxicity of the Emblica officinalis Phytosome Formulation in Wistar Rats. International Journal of Drug Delivery Technology. 2022;12(4):1566-1570. DOI: 10.25258/ijddt.12.4.14
47. Alsabah AS, Salim KS, Jasim GA. Assessment of Acute Toxicity of Convolvulus arvensis Methanol Extract. International Journal of Drug Delivery Technology. 2021;11(4):1156-1159. DOI: 10.25258/ijddt.11.4.7
48. Shaikh M, Quazi M, Nandedkar R. Hypoglycemic effect of wheatgrass juice in alloxan induced diabetic rats. Pharma Tutor. 2011;10.
49. Shakya G, Goud C, Pajaniradje S, Rajagopalan R. Research Article; Protective Role of Wheatgrass on Oxidative Stress In Streptozotocin Induced Type 2 Diabetic Rats. International Journal Of Pharmacy And Pharmaceutical Sciences 2012;4(3):415-423.