International Journal of Pharmacy and Analytical Research (IJPAR)

ISSN: 2320-

IJPAR |Vol.12 | Issue 3 | July - Sept -2023
www.ijpar.com

Research article ***Analytical research***

Anti-Hyperglycemic and Antioxidant Actvity of Wheat Grass Juice

Mohammed Tauqueer Shaikh[1], Dr.Mehraj A. Pathan[2], Dr.M.R.N. Shaikh[3], Dr Amjad Khan A. Pathan,

[1]*Research Scholar, JJT, University, Rajasthan, India*
[2]*Associated Professor, JJT University, Rajasthan, India*
[3]*Principal, MET'S IOP, College of D Pharmacy, Nashik, M.S.*

***Corresponding Author: Mohammed Tauqueer Shaikh**
Published on: 10.08.2023

ABSTRACT

Diabetes is a common medical problem in India, which has become yet more in most of India. Diabetes is occurs when your blood glucose is too high. The Streptozotocin which is used in the above experiment is the islets of pancreas which helps in increase of the insulin level which simultaneously shows the decrease in the Blood glucose level. This study is aimed to show the antihyperglaemic and antioxidant activity of the wheatgrass on the Human body.

Keywords: Streptozotocin, anti-insulin, hyperglycemia

INTRODUCTION

Suitable area for the cultivation of wheatgrass was selected. As no fertilizer was suggested, only natural soil used for sowing. About 100 g of wheat seeds (grains) of good quality were soaked in water for 12 hours and allowed for sprouting. The seeds were sown after sprouting in the selected field for seven days by spreading on the bed covered with soil. Water was sprinkled (not poured) on fertile soil for seven days. During the cultivation procedure the field area was not over exposed to the sun light. On the eighth day, wheatgrass found to have length of about 6 to 7 inches. Wheatgrass was cut at root level and pluck. Juice was then made from collected wheatgrass by using juicer (with little addition of water for lubrication purpose not as a vehicle).Wheatgrass may also be grown in water or soil with utmost care.

Wistar rats of either sex are used, weighing between 150 – 200 g. The animals were housed under standard laboratory conditions, maintained on natural light and dark cycle and free access to food and water. Animals were acclimatized to laboratory conditions before the experimentation. All experiments were carried out between 0900 and 1500 h. The experimental protocols were approved by the Institutional Ethics Committee and conducted according the Indian National Science Academy Guidelines for the use and care of experimental animals [1].

Streptozotocin (STZ) was originally identified in the late 1950s as an antibiotic and was discovered in a strain of the soil microbe Streptomyces achromogenes. In the mid-1960s, STZ was found to be selectively toxic to the beta cells of the pancreatic islets and thus it is used in animal model of diabetes and as a medical treatment for cancers of the beta cells [2].

MATERIAL AND METHOD

Wheatgrass was cultivated in the garden area of MET's Institute of D. Pharmacy, Nashik. The fresh grass (7-8 days old from the day of sprouting) was collected. Herbarium of wheatgrass was prepared for authentication purpose.

Mr. Dr. P. G. Diwakar, joint director, Botanical Survey of India, Koregaon Road, Pune, did the confirmation of the plant. The authentification of plant was done by comparing the morphological features (leaf arrangement, fruit and seed morphology). The herbarium of the plant has been deposited at botanical survey of India, Pune. The voucher no. of specimen being SMRN – 1 and the reference no of letter was BSI/WC/TECH/2010/829.

Fig 1: Herbarium of Wheatgrass (*Triticum aestivum* L.)

PRELIMINARY PHARMACOGNOSTIC CHARACTERISTICS

Macroscopy

Grass of Wheat berries
Botanical name: *Triticum aestivum* L.
Family: Poaceae
Description: Wheatgrass is young grass shoots of wheat berry. In appearance, wheatgrass is like any other grass. Culms are simple, hollow or pithy, glabrous, 1.2 m tall. Leaves flat, narrow, 20-38 cm long, 1.3 cm broad. Spikes long, slender, dorsally, compressed, somewhat flattened; rachis tough, not separating from spikelet's; 2-5 flowered, relatively far apart from stem, slightly overlapping, nearly erect, pressed closed to rachis; glumes keeled in upper half, firm, glabrous, shorter than lemmas; lemmas awned or awnless, less than 1.3 cm long; palea as long as lemma, remaining entire at maturity, caryopsis free threshing, soft or hard, red or white (hexaploid).
Taste: Acrid
Shape: Longitudinal
Color: bright green/ dark green
Odour: slight.

Loss on Drying

Accurately weighed quantity of sample was taken in a tared glass bottle and initial weight was taken. The sample was heated at 105^0C in an oven and weighed. This procedure was repeated until a constant weight was obtained. The moisture content of the sample was calculated with reference to air-dried drug and the results are in (Table 3).
Loss on Drying = Loss in weight x 100/W
W= Weight of the crude drug in grams

Extractive Values

The extractive values for various solvents of air-dried sample were evaluated.
i) Water-soluble extractives.
ii) Alcohol soluble extractives.

Water-soluble extractives

Macerated 5gm of the air dried, coarsely powdered drug with 100ml of chloroform water I.P. in a conical flask for 24 hours, shaked frequently during six hours and allowed to stand for 18 hours. Filtered and evaporated 25% of the filtrate to dryness in a tared bottomed shallow dish dried at 105^0c and calculated the percentage of water soluble extractive with reference to the air dried drug.

Alcohol soluble extractives

Macerated 5gm of the air dried, coarsely powdered drug with 100ml of alcohol in a conical flask for 24 hours, shacked frequently during six hours and allowed to stand for
18 hours. Filtered rapidly taking precaution against loss of alcohol, evaporated 25% of the filtrate to dryness in a tared bottomed shallow dish dried at 105^0c and calculated the percentage of alcohol soluble extractive with reference to the air dried drug.

Table 1: Results of Physicochemical Evaluation

Sl.No.	Physical Constants	*Triticum aestivum* L.
1.	Microscopic Characteristics	
	• Nature	Grass
	• Colour	Bright green/ Dark green
	• Odour	Characteristic
	• Taste	Acrid

3.	Loss on Drying (%w/w)	21.1%
4.	Extractive Values (% w/w)	
	• Water-soluble Extractives	26.4%
	• Alcohol soluble Extractive	9.1%

Phytochemical investigation

- Cultivation, collection and preparation of Wheatgrass juice.
- Qualitative chemical tests.
- Chromatographic studies

High Performance Thin Layer Chromatography (HPTLC)

RESULTS

Table 2: Physical Characteristics of wheatgrass (*Triticum aestivum* L.) juice

Sl. No.	Name of the Extract	Nature	Colour	Odour	Taste	Quantity in gms (for 2.5 kg of wheatgrass)	Percentage yield
1	Wheatgrass juice	Liquid juice	Dark green	Characteristic	Acrid	1.86 kg	74.4

Table 3: Results of Phytochemical Investigation of Wheatgrass Juice

S.No.	Name of the Test	Wheatgrass juice
1.	**Test for carbohydrates**	
	a) Molisch's test	+
	b) Fehling's test	+
	c) Benedict's test	+
2.	**Test for proteins**	
	a) Biuret test	+
	b) Xantho protein test	+
	c) Millons test	+
3.	**Test for Amino acids**	
	a) Ninhydrin test	+
4.	**Test for Alkaloids**	
	b) Dragendroff's test	+
	c) Mayer's test	+
	d) Hager's test	+
	e) Wagner's test	+
5.	**Test for sterols**	
	a) Salkowski test	-
	b) Liebermann reaction	-
6.	**Test for phenolics & tannins**	
	a) Ferric chloride test	-
	b) Lead acetate test	-
	c) Dil. HNO_3 test	-
7.	**Tests fixed oils and fats** a) Saponification test	-
	b) Stain test	-
8.	**Test for triterpenoids**	
	a) Liebermann Burchard's Test	-
	b) Salkowski test	-
9.	**Test for glycosides**	

	a)	Keller-Killiani Test	-
	b)	Baljet's Test	-
10.	**Test for saponins**		
	a)	Haemolytic test	+
	b)	Foam test	+

(+) = Positive, (-) = Negative

PHARMACOLOGICAL EVALUATION

Animals

Wistar rats of either sex are used, weighing between 150 – 200 g. The animals were housed under standard laboratory conditions, maintained on natural light and dark cycle and free access to food and water. Animals were acclimatized to laboratory conditions before the experimentation. All experiments were carried out between 0900 and 1500 h. The experimental protocols were approved by the Institutional Ethics Committee and conducted according the Indian National Science Academy Guidelines for the use and care of experimental animals.

Drugs

Streptozotocin (Loba chemie, Mumbai), Gliclazide (Panacea Biotec), Glucose estimation kit (Span diagnostics Ltd., Surat, India) were employed.

Evaluation of Anti-hyperglycemic activity

The Pharmacological evaluation of both herbs is carried out by using wistar albino male rats which weighing around 250gm, the study comprised of five groups in which each group comprised of 5 animals.

Ad libitum diet for a month prior to the study with the provision of 12X12 dark and light cycles. Diabetes was induced by i.p. injection of Streptozotocin 60mg/kg body weight. The mortality rate was high at first instance killing 9 out of first 12 animals.

Then orally our drug has been fed (per oral route) and as a reference standard Gliclazide (8mg) is used then by drawing blood which used for checking blood glucose level.

Blood samples were drawn from the tail tip of the rat at weekly intervals till the end of study (i.e. 2 weeks). Fasting blood glucose estimation was done on day 1, 7, and 14 of the study. Blood glucose estimation can be done by onetouch electronic glucometer using glucose test strips.

Statistical analysis

One specific group of rats was assigned to one specific drug treatment condition and each group comprised of six rats (n=6). The data was analyzed using one-way ANOVA followed by Dunnett's test. In all the tests the criterion for the statistical significance was $p < 0.05$.

Table 4: Effect of wheatgrass juice on blood glucose in rats

Treatment	Blood glucose level (mg/dl) (1)	Blood glucose level (mg/dl) (2)	Blood glucose level (mg/dl) (3)	Blood glucose level (mg/dl) (4)	Blood glucose level (mg/dl) (5)
Vehicle	85.0	90.0	82.0	88.0	80.0
STZ	225.0	235.0	240.0	243.0	255.0
Extract (50 mg)	145.0	155.0	162.0	160.0	148.0
Extract (100mg)	120.0	100.0	115.0	120.0	125.0
Gliclazide (8.0 mg)	90.0	110.0	105.0	95.0	100.0

Treatment	Blood glucose level in mg/dl (Mean)
Vehicle	85.0 ± 1.84
STZ	239.6 ± 4.91*
Extract (50)	154.0 ± 3.3#
Extract (100)	116.0 ± 4.3#
Gliclazide	100.0 ± 3.56#

- *Blood Glucose level: $F_{4,20} = 275.2$, $P = 0.0001$, $P < 0.05$)*
- *(*) $P < 0.05$ compared to Vehicle treated group and (#) $P < 0.05$ compared to STZ treated group.*
- *(1), (2), (3), (4), (5) are nothing but the codes for the animals from each group.*
- *Both in Anti-hyperglycemic study and Liver Glycogen estimation, same animal groups were used.*

Number of values	5	5	5	5	5
Minimum	80	225	145	100	90
25% Percentile	81	230	147	108	93
Median	85	240	155	120	100
75% Percentile	89	249	161	123	108
Maximum	90	255	162	125	110
Mean	85	240	154	116	100
Std. Deviation	4.1	11	7.4	9.6	7.9
Std. Error	1.8	4.9	3.3	4.3	3.5
Lower 95% CI of mean	80	226	145	104	90
Upper 95% CI of mean	90	253	163	128	110
Sum	425	1198	770	580	500

Analytical Method

Blood Glucose Level (Statistical Analysis-1)

P value	0.5041
P value summary	Ns
Do the variances differ significant (P<0.05)	No

ANOVA Table	SS	Df	MS
Treatment (between columns)	76557	4	19139
Residual (within columns)	1389	20	69
Total	77946	24	

Mean			Significant? P<		95% CI of
Dunnett's Multiple Comparison Test	Diff.	Q	0.05?	Summay	diff
CN (VEHICLE) vs ALLOXAN 150 MG	-155	29	Yes	***	-169 to -141
CN (VEHICLE) vs EXTRACT 50 MG	-69	13	Yes	----	-83 to -55
CN (VEHICLE) vs EXTRACT 100 MG	-31	5.9	Yes	***	-45 to -17
CN (VEHICLE) vs GLICLAZIDE 8 MG	-15	2.8	Yes	*	-29 to -1.0

Blood Glucose Level (Statistical Analysis-2

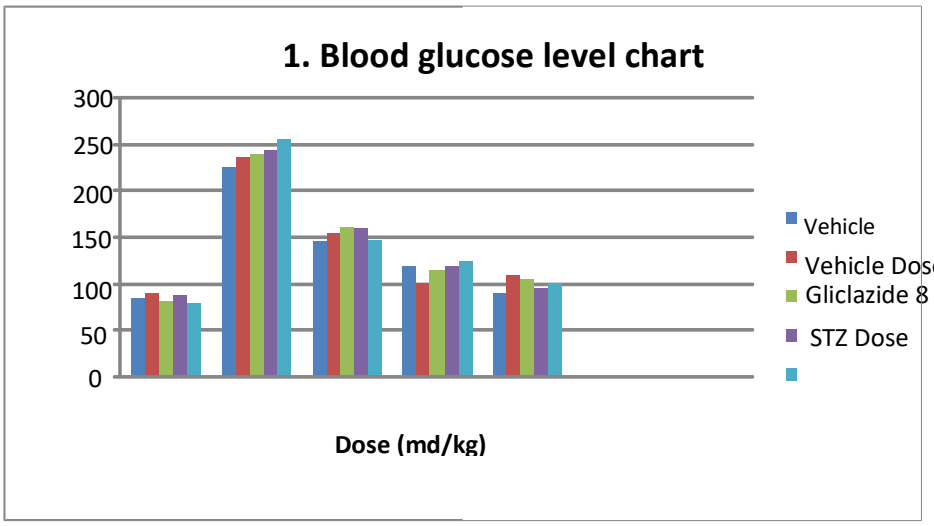

Fig 2: Blood glucose level chart

Evaluation of Antioxidant activity [5,6]

Antioxidants have defined as the substance those in small quantities, able to prevent or greatly retard oxidation of easily oxidisable material such as fats. Antioxidants may exert their effect by different mechanisms such as suppressing the formation of active species by reducing hyperoxides (ROO) and H_2O_2 and also by sequestering metal ions scavenging active free radicals, repair and/or clearing damage. Mechanisms of radical scavenging activity of antioxidants and their pros an cons are well available

Various antioxidant activity methods have been used to monitor and compare the antioxidant activity of foods. These analytical methods measure the radical scavenging activity of antioxidants against free radicals like DPPH, the hydroxyl radical or the peroxyl radical (ROO), superoxide anion radical O_2. The various methods used to measure the antioxidant activity of various extracts compounds can give varying results depending on the specificity of the free radical being used as reactant. The following different reactants are being used to determine the activity.

1. Malondialdehyde (MDA)
2. Thiobarbituric acid-reactive substances(TBARS)
3. 2,2-aznobis(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS)
4. 2,2-azobis(2-amidinopropane)(AAPH)

A) DPPH scavenging activity

Procedure

Antiradical activity of extract was performed by DPPH model stock. Solution of DPPH (1.3mg/ml) in methanol was prepared. 75ml of stock solution of DPPH was added in 3ml of methanol and absorbance at 516nm was taken. The various concentrations of test sample (25, 50, 75, 100, 200 □g/ml) were prepared. In all diluted Solutions, 75ml of stock solution of DPPH was added than absorbance was recorded at 516nm and Ec_{50} was calculated against methanol as a blank.

$$\% \text{ inhibition} = \frac{(\text{O.D of standard} - \text{O.D of test}) \times 100}{\text{O.D of standard}}$$

Table 5: Observation for antioxidant activity in terms of DPPH method

	Concentration(µg/ml)					
	Absorbance					
Wheatgrass juice	**25**	**50**	**75**	**100**	**200**	**500**
	0.8164	0.7099	0.6410	0.5488	0.4243	0.2772
	0.8170	0.7104	0.6419	0.5494	0.4242	0.2778
	0.8171	0.7101	0.6412	0.5491	0.4235	0.2789
Ascorbic acid	**5.0**	**10.0**	**15.0**	**20.0**	**25.0**	**--**
	0.7360	0.5720	0.4234	0.2280	0.0701	--
	0.7370	0.5715	0.4240	0.2286	0.0707	--
	0.7367	0.5719	0.4245	0.2281	0.0708	--

Drug	**% Scavenging (mean ± SEM)**					
	25µg/ml	**50µg/ml**	**75µg/ml**	**100µg/ml**	**200µg/ml**	**500µg/ml**
WG Juice	6.44±0.05	18.66±0.025	26.55±0.12	37.11±0.21	51.44±0.16	68.17±0.10
Ascorbic acid	15.64± 0.09 (5µg/ml)	34.51±0.11 (10µg/ml)	51.45± 0.04 (15µg/ml)	73.87±0.01 (20µg/ml)	91.93±0.03 (25µg/ml)	--

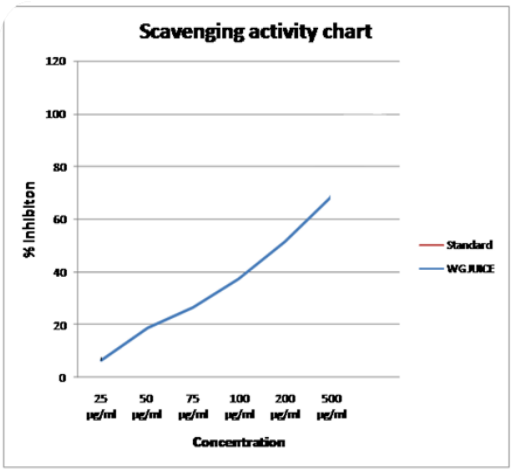

Fig 3: Graph of % inhibition of Antioxidant activity (DPPH model)

Reducing Power Determination

The reducing power of the test sample was determined by the method stated by Dorman and Hiltunen. The reaction mixture containing varying concentrations of test sample and standard (5-25 µg/ml) in 1ml of distilled water, phosphate buffer (2.5 ml, 0.2 M, pH 6.6) and potassium ferricyanide [$K_3Fe(CN)_6$] (2.5ml, 1% w/v) was incubated at 50°C for 20 min. A portion (2.5 ml) of trichloroacetic acid (10% w/v) was added to the mixture, which was then centrifuged for 20 min at 1000*g*. The upper layer solution (2.5 ml) was mixed with 2.5 ml of distilled water $FeCl_3$ (0.5 ml, 0.1%), and the absorbance was measured at 700 nm in the spectrophotometer. Higher the absorbance of the reaction indicated greater the reducing potential.

Observations of Reducing Power assay

Table 6: Reducing Power assay

Samples	Concentration(µg/ml)					
Wheatgrass Juice (conc.)	**25**	**50**	**75**	**100**	**200**	**500**
Absorbance	**0.024**	**0.036**	**0.057**	**0.093**	**0.169**	**0.341**
	5	**10**	**15**	**20**	**25**	**--**
Ascorbic acid	**0.046**	**0.17**	**0.232**	**0.484**	**0.812**	**--**

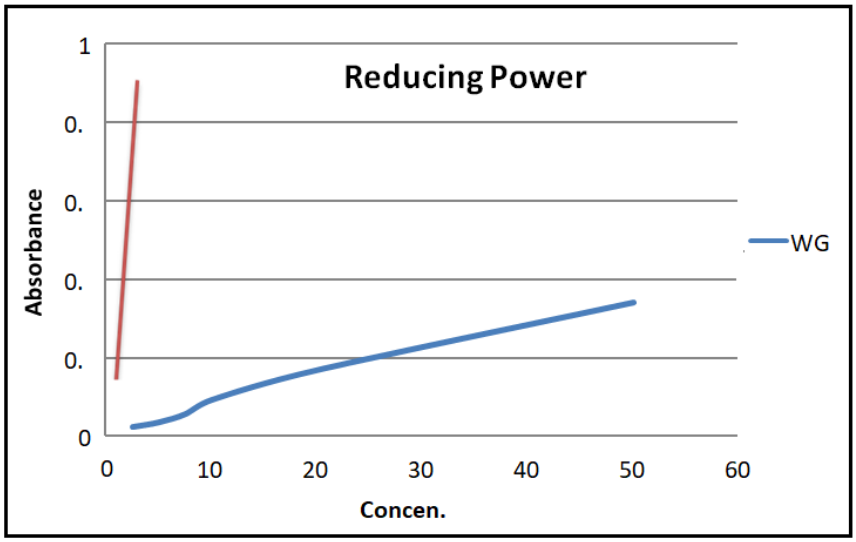

Fig 4: Graph of reducing power assay

CONCLUSION

The site of action of Alloxan for diabetogenesis is the islet cell membrane. After i.v. injection the binding of Alloxan to its site of action is completed within a few minutes. The histological and most biochemical changes observed later than 5min after i.v. Alloxan injection are secondary changes and are not due to a direct Alloxan effect.

ACKNOWLEDGMENT

We are very thankful to our principal, Management of JMCT Institute of Pharmacy Nashik, for providing required facilities to carry out this research work.

REFERENCES

1. Duke JA. Triticum aestivum L. handbook of energy crops 1983.
2. Kothari S, Jain AK, Mehta SC, Tonpay SD. Effect of fresh Triticum aestivum grass juice on lipid profile of normal rats. Indian J Pharmacol. 2008 Oct;40(5):235-6. doi: 10.4103/0253-7613.44157. PMID: 20040964; PMCID: PMC2792628.
3. Stanely Mainzen Prince P, Menon VP. Hypoglycaemic and hypolipidaemic action of alcohol extract of Tinospora cordifolia roots in chemical induced diabetes in rats. Phytother Res. 2003 Apr;17(4):410-3. doi: 10.1002/ptr.1130. PMID: 12722152.
4. Dr. Nayampally S. Green gold. Wheatgrass Ther (A Sanjivani). 2005.
5. Antia BS, Okon JE, Okon PA. Hypoglycemic activity of aqueous leaf extract of Persea americana Mill., (National Seminar on Emerging Trends in Ethnopharmacology. Karnataka; 2005; 37(5). DOI:10.4103/0253-7613.16858
6. The Hippocrates Health Institute. Wheatgraas- the complete food. MT: The Essentialist. June 25 2008.
7. Goel RK, Mahajan MP, Kulkarni SK. Evaluation of anti-hyperglycemic activity of some novel monocyclic beta lactams. J Pharm Pharm Sci. 2004 Apr 21;7(1):80-3. PMID: 15144739.