ANTIDIABETIC ACTIVITY OF HORDEUM VULGARE USING STREPTOZOTOCIN INDUCED DIABETIC RATS

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Abstract:

Diabetes mellitus is a difficult metabolic disorder that has seriously impact the human health and quality of life. Medicinal plants are being used to control diabetes However, they are not entirely effective and no one has ever been reported to have fully recovered from diabetes. Many plants have been used for the management of diabetes mellitus in various traditional systems of medicine worldwide as they are a great source of biological constituents and many of them are known to be effective against diabetes. Medicinal plants with anti hyperglycemic activities are being more desired, owing to lesser side effects and low cost. In this present study, for the antidiabetic effect the albino rats were divided into 6 groups, each consisting of 6 animals. Diabetes was induced by a single i.p. injection of streptozotocin at a dose of 45mg/kg body weight. Standard drug, glibenclamide and ethanolic extract of Hordeum Vulgare (EEHV) at doses 100mg/kg and 200mg/kg body weight was fed to the rats and it was continued till the end of the study. The blood glucose levels were estimated on day 0, 7, and 14 day. The standard drug and the extract were fed from day 4 onwards. The antidiabetic property of the extract has shown increasing trend with increase in dose and there was a gradual decrease in blood glucose levels with increased period of exposure to the test drug. Results obtained in this study substantiate the anti-diabetic activity of EEHV leaves.

Keywords: Antidiabetic, Hordeum Vulgare, Streptozotocin

Introduction:

Diabetes mellitus (DM) is a metabolic disorder that affects people of all age groups and from all walks of life. There is an estimated 150 million people worldwide suffering from diabetes[1], which is almost 5 times more than the estimated number 10 years ago. Management of diabetes without any side effects is still a challenge in the medical field, as presently available drugs for diabetes have one or more adverse effects [2]. Since the existing drugs for the treatment of DM do not satisfy our need completely, the search for new drugs continues. In recent years, herbal remedies for the unsolved medical problems have been gaining importance in the research field. Although many researchers have studied the anti-diabetic activity of *Hordeum vulgare*, no satisfactory study was conducted to investigate its efficacy in streptozotocin induced diabetic rats or to explore how this drug acts as an anti-diabetic agent. Thus, this study was undertaken to explore the efficacy of anti-

diabetic activity of *Hordeum vulgare* in diabetic rats. The possible mechanism by which this drug may act is discussed in this study.

Hordeum vulgare is a grass belonging to the family *Poaceae*, the tribe *Triticeae* and the genus *Hordeum*. It is now the fourth most important cereal crop of the world after wheat, corn and rice. Historically, barley was the major food grain in many parts of the world. This grain was used as a staple food in the Near East several thousand years ago and was the chief form of nourishment of Greeks in Homeric times. The increased interest in barley as a human food ingredient results from studies which have shown barley to be an excellent source of dietary fibre and, in particular, ß-glucan. Barley kernels contain complex carbohydrates (mainly starch), have a low fat content and are moderately well-balanced in terms of protein to meet amino acid requirements, as well as minerals, vitamins (particularly vitamin E) and antioxidant polyphenols. In addition to its use in the production of malt for brewing, barley is used as whole-grain, pearled, raw-grain flour, whole roasted-grains mature barley flour and roasted-grain flour for the production of breakfast cereals, stews, soups, pastas and noodles, as a coffee substitute and in porridges, sauces and baked products (including bread and flat bread)

MATERIALS AND METHODS:

COLLECTION AND IDENTIFICATION OF PLANTS MATERIAL:

2 kg of the stem of *Hordeum vulgare* leaf was procured from the Thirumala forest in Andhra Pradesh State, India, in the months of June and July 2018. The leaf of *Hordeum vulgare was* cleaned and dried in 15 days. Leaf of *Hordeum vulgare* grind the powder using the laboratory Hammer mill. Powdered samples were stored desiccators until required for extraction.

PREPARATION OF EXTRACTS

Preparation of extracts from *Hordeum vulgare* leaf:

The powdered materials of *Hordeum vulgare* leaf was extracted individually with methanol using soxhlet apparatus, for 18 hours. The extract were concentrated using rotary evaporator till free from the solvents and obtained yield was respectively 15g/kg respectively. The remain crude material was macerated with distilled water.

PRELIMINARY PHYTOCHEMICAL ANALYSIS:

Chemical Constituent	Name Of The Test	Observation
Carbohydrates	Molisch's test, Fehling's test	+ve
Amino acids	Ninhydrin test	-ve
Proteins	Biuret test	-ve
Fats and oils	Spot test	-ve
Alkaloids	Mayer's test, Dragendorff's test	+ve
Terpenoids	Liebermann-Burchard reaction	+ve
Flavonoids	Shinoda test	+ve

Tannins and Phenolic compounds	Ferric chloride test	+ve
Cardiac glycosides	Keller-Killiani test	+ve

ANIMALS

Wistar Albino rats of either sex weighing between 100-

200 g were used for this purpose. The animals were housed in polypropylenecages and maintained at 24 ± 2 o under 12h light dark cycle and

were fed adlibitum with standard pellet diet and had free access to water maintenance and use of animals as per the experiment was approved by the institutional Animal Ethics Committee(014/IAEC/NCPA/PHD/2018-19).

EXPERIMENTAL PROCEDURE:

Preparation of STREPTOZOTOCIN (STZ) induced diabetic rats

The diabetic animals[3] were prepared by administration of single dose of streptozotocin (45 mg/kg B.wt.i.p) dissolved in freshly prepared 0.01 M citrate buffer P^H 4.5. After 72 hrs rats with marked hyperglycemia (blood glucose ≥ 200 mg/dl) were selected. The stable hyperglycemic condition was exhibited in after one week of STZ treatment[4], then after that sample treatment was performed [5, 6]

Experiment procedure for acute treatment of *EEHV* extracts in diabetic rats.

The diabetic rats were divided into five groups with 6 animals each group

Groups	**Treatment**
Group I	Normal rats
Group II	Tween 80
Group III	Glibenclamide (10 mg/kg B.wt.)
Group IV	Diabetic rats (STZ 45 mg/kg B.Wt.)
Group V	EEHV (100 mg/kg B.Wt.)
Group VI	EEHV (200 mg/kg B.Wt.)

Blood samples were collected at 1hr, 2hr, 4hr, 6hr and 8hr time intervals for estimation of glucose levels[7] using glucose peroxidase method.

Experiment procedure for chronic treatment of *EEHV* extracts in diabetic rats.

The diabetic rats were divided into five groups with 6 animals each group.

Groups	**Treatment**
Group I	Normal rats
Group II	Tween 80
Group III	Glibenclamide (10 mg/kg B.wt.)
Group IV	Diabetic rats (STZ 45 mg/kg B.Wt.)

Group V EEHV (100 mg/kg B.Wt.)

Group VI EEHV (200 mg/kg B.Wt.)

At end of 7th, 14th day blood was collected by puncture of retro orbital plexus[8]. The blood glucose was estimated by using glucose peroxidase method.

STATISTICAL ANALYSIS

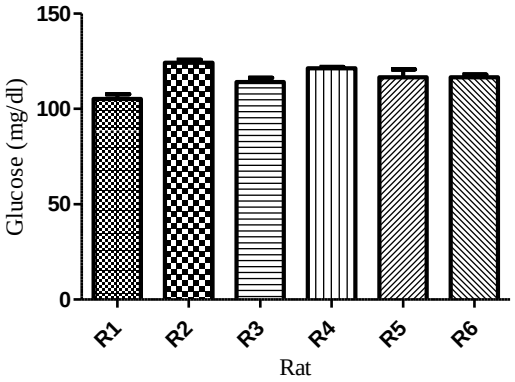
Data obtained from this work were analyzed statistically using Student's t-test and ANOVA (One-way) followed by a post-test (Tukey-Kramer multiple comparison test). Differences between means were considered significant at 0.1% and 5% level of significance i.e $p \leq 0.001$ and 0.05.

RESULTS :

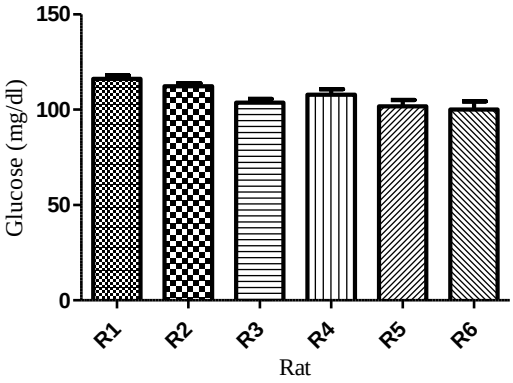
Effect of acute treatment of EEHV on Blood glucose levels in STZ rats

Group	Treatment	Blood glucose levels (mg/dl)					
		0 min	**1 hr**	**2 hr**	**4 hr**	**6 hr**	**8 hr**
I	Normal Rats	110.16± 3.7	112.5 ± 3.45	114.8± 3.21	117.6± 2.3	121 ± 2.64	121.8 ± 2.49
II	Tween 80	98.8 ± 3.7	102.6 ± 3.24	105.5 ± 3.87	107.6 ± 2.7	111.6 ± 1.3	115.5 ± 1.8
III	STZ (45 mg/kg B.wt.)	255 ± 8.2	245.5 ± 7.2	249.8 ± 5.3	251.6 ± 7.3	250 ± 5.6	249.6 ± 6.7
IV	EEHV (100 mg/kg B.wt.)	248.6 ± 9.3	234.3 ± 7.1	224.5 ± 6.6	211 ± 7.4	201.3 ± 8.4 (19.05%)	202.5 ± 18.3
V	EEHV (200 mg/kg B.wt.)	264.6 ± 5	236.6 ± 4.5	223.8 ± 6.2	201.5 ± 4.7	182.1 ± 4.3 (31.25%)	187.3 ± 4.5

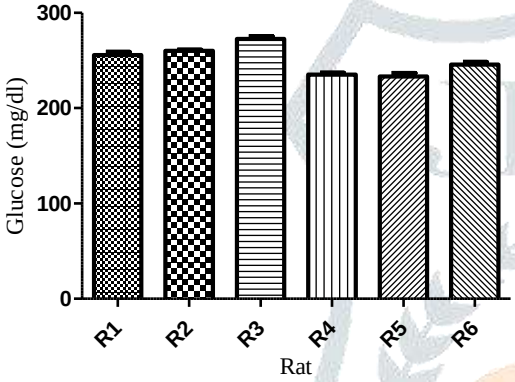
Blood glucose (mg/dl) levels in normal rats



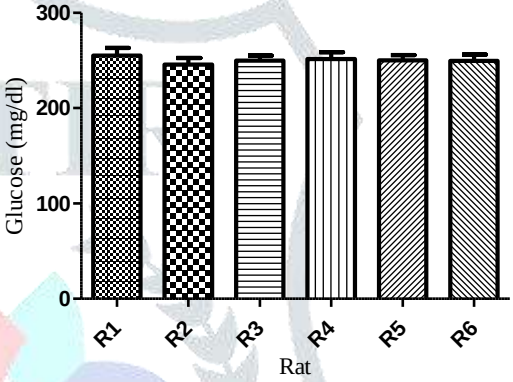
Blood glucose (mg/dl) levels in tween 80 treated rats



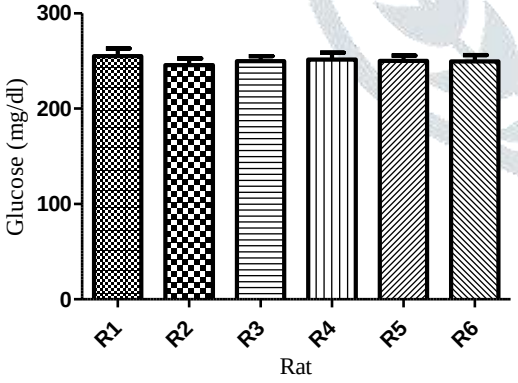
Blood glucose (mg/dl) levels in Streptozotocin treated rats



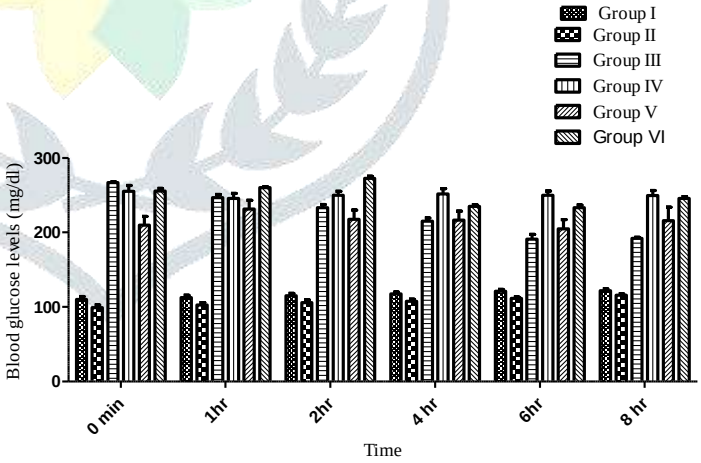
Effect of EEHV (100 mg/kg B.wt.) on Blood glucose levels in STZ rats



Effect of EEHV (200 mg/kg B.wt.) on Blood glucose levels in STZ rats



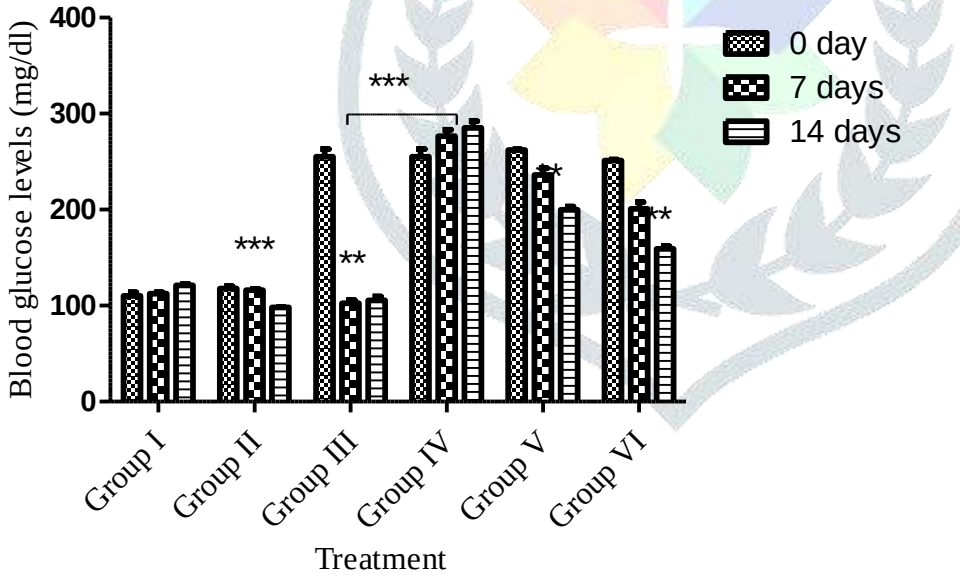
Effect of acute treatment of EEHV on Blood glucose levels in STZ rats



Effect of chronic treatment of EEHV on Blood glucose levels in STZ rats

Group	Treatment	Blood glucose levels (mg/dl)		
		0 min	7 days	14 days
I	Normal Rats	110.16± 3.7	112.5 ± 3.45	114.8± 3.21
II	Tween 80	98.8 ± 3.7	102.6 ± 3.24	105.5 ± 3.87
III	STZ (45 mg/kg B.wt.)	255 ± 8.2	265+1.2	282±2.1
IV	EEHV (100 mg/kg B.wt.)	265+2.4	236± 7.2 (14.3%)	199.8±3.3 (29.9%)
V	EEHV (200 mg/kg B.wt.)	267±2.1	200.7±7.1 (27.2%)	160.3±2.4 (44.1%)

Effect of chronic treatmnet of EEHV on blood glucose levels in STZ induced diabetes rats



Acute treatment

At the dose of 100 mg/kg bd.wt. EEHV was exhibited 19.05 % reduction in blood glucose at the 5th hr of treatment in diabetic rats[9]

At the dose of 200 mg/kg bd.wt. EEHV was exhibited 31.25% reduction in blood glucose at the 5th hr of treatment in diabetic rats[10]

Chronic treatment

At the dose of 100 mg/kg bd.wt. EEHV was exhibited 27.2 % reduction in blood glucose at the end of 14th day of treatment in diabetic rats

At the dose of 200 mg/kg bd.wt. *EEHV* was exhibited 44.1% reduction in blood glucose at the end of 14th day of treatment in diabetic rats.

DISCUSSION:

Diabetes mellitus is a metabolic disorder characterized by elevated blood glucose levels resulting due to deficiency in insulin release, insulin action, or both. Many mechanisms are involved in anti diabetic and hypoglycemic activity. One of these might involve the modulating insulin secretion or insulin action, or related with extrapancreatic and pancreatic effects. Other mechanisms may involve an antioxidant system or may include enhancement of cell glucose metabolism or activation of enzyme system via generation of cyclic AMP or phospholipid derived messenger.

The STZ induced diabetic rat is one of the animal models of human diabetes mellitus. Diabetes arises from irreversible destruction of pancreatic β cells, causing reduction of insulin secretion. The observed increased blood glucose level in the study is in agreement with reports by several workers that STZ induced diabetes mellitus leads to increased blood glucose. It has been reported that STZ at lower doses produce partial destruction of pancreatic β cells with permanent diabetes condition and there is possibility of many surviving β cells. Since a low dose of STZ (45mg/kg body wt. i.p.) was chosen for this study there might have been many surviving β cells, capable of undergoing regeneration[11].

Glibenclamide, a standard hypoglycemic agent was taken for comparison of the glucose lowering effectiveness of the ethanolic extract of *Hordeum Vulgare*. For the estimation of blood glucose level glucose peroxidase method was used.

Albino rats have been used for experimental models of hyperglycemia. They are the commonest laboratory animals suitable for experimental work because of their small size, greater sensitivity to most drugs, easy breeding and resemblance to human nutritionally[12].

The study shows that the ethanolic extract of *Hordeum Vulgare* has antidiabetic potential even though it is lesser than the standard drug, glibenclamide. In the present study, glibenclamide, a member of sulphonylurea is used as the standard drug. It has been proposed that sulphonylurea produce their hypoglycemic effect primarily through increased release of insulin in pancreatic β cells. Thus any plant secondary metabolite or chemical constituent which is capable of affecting the insulin secretion from pancreatic β cells will be a good mimicker of sulphonylureas[13].

The ethanolic extract of *Hordeum Vulgare* was able to significantly lower the blood glucose level. Therefore, the extract might have been able to potentiate the release of insulin from pancreatic islets similar to that of results observed after glibenclamide administration. Also there is a possibility that there might be the presence of some constituents with insulin like action which directly lowered the blood glucose level independent of insulin secretion.

Hordeum Vulgare reveals that wide range of phytochemical constituents have been isolated from the plant like alkaloids, flavonoids, tannins, phenol, glycosides. Plants which contain active principles like glycosides, alkaloids, flavonoids are claimed to possess antidiabetic activity.

CONCLUSION:

The present study shows that the methanol extract of *Hordeum Vulgare* has potential antidiabetic action in STZ induced diabetic rats and the effect was found to be more similar to the reference drug glibenclamide.

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