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A RESEARCH ARTICLE ON EVALUATION OF ANTI-OBESITY ACTIVITY OF HORDEUM VULGAREGRAINS IN ALBINO RATS

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ABSTRACT

Obesity is abnormal or excessive fat accumulation that presents risk to health. The body mass index (BMI) of a person is 25-30 kg/m^2 indicates overweight and above 30 kg/m^2 represents obesity. The successful management of obesity is possible through lifestyle changes in diet and physical activity. *Hordeum vulgare* is traditionally used as weight losing remedy so, present study selected grains of *Hordeum vulgare* plant for evaluation of anti-obesity activity by using high fat diet induced, anti-psychotic drug induced obesity in rats.

KEYWORDS

Obesity, BMI, *Hordeum vulgare* and Anti-psychotic drug.

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INTRODUCTION[1,2]

Obesity is abnormal or excessive fat accumulation that presents risk to health. The body mass index (BMI) of a person is 25-30 kg/m^2 indicates overweight and above 30kg/m^2 represents obesity. World Health Organization (WHO) assigns obesity as global epidemic. WHO's latest study indicate that globally in 2005, approximately 1.6 billion adults (15+) were overweight and at least 400 million adults were obese. Further WHO projects that by 2015 approximately 2.3 billion people will be overweight and more than 700 million will be obese.

Once it was considered that obesity was only in high income countries. But now a day, it has spread dramatically in medium and low income countries[3].

Recently, there has been increasing interest in the use of medicinal plants. The use of medicinal plants in modern medicine suffers from the fact that though hundreds of plants are used in the world to prevent or to cure diseases. Recently search for appropriate anti-obesity agent has been focused on plants used in traditional medicine because of leads provided by natural products that may be better treatment than currently used.

There are reports that *Hordeum vulgare* is traditionally used as weight losing remedy so, present study selected grains of *Hordeum vulgare* plant for evaluation of anti-obesity activity by using high fat diet induced, anti-psychotic drug induced obesity in rats.

MATERIALS AND METHODS [3-6]

Collection and Authentication of Plant Material

The grains of *Hordeumvulgare* was collected and authentified by Prof. P. Sunitha, MSc., M.Phil., (PhD), MHRM; Head of Botany Department ; Yousfguda; Hyderabad.

Preparation of extract[7]

The air-dried *Hordeumvulgare* seeds (500 g) were powdered and extracted with hydro alcoholic solvent (1:1) in soxhlet apparatus. The extract was evaporated and lyophilized to get dry powder. The yield of extract was 29.43%w/w.

Investigation of Preliminary Qualitative Phytochemical Analysis

Phytochemical analysis of hydro alcoholic extract of *Hordeumvulgarem* (HEHV) was carried out by using the standard procedures. Alkaloids, carbohydrates, flavonoids, glycosides, lactones, phytosterols, proteins, Saponins and triterpenoids were qualitatively analyzed.

EXPERIMENTAL ANIMALS[8]

Female Swiss albino rats of 4 weeks old and weighing around 80-90g were used. These animals were housed in separate cages under 12-12 h day light cycle, 25±3°C temperature and 55-65% humidity condition. The animals had free access to food (commercial rat feed pellets) and water. The animals were kept fasted 2 h before and 2 h after drug administration. The experimental protocol was approved by Institutional Animals Ethics Committee (IAEC) of BIOGENE LABORATORIES, HYDERABAD (A.P) and animal care was taken as per the guidelines of Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA). The protocol number is CPCSEA/IAEC/EXP/29/409/2013/EXP/68.

In vivo studies

Acute toxicity studies

The acute toxicity was performed as per WHO guideline and the Organization of Economic Co-operation and Development (OECD) guideline for testing of chemicals (no.420) using albino mice of either sex prior to the evaluation of anti-urolithiatic activity. The EHV was tested using graded doses (500, 1000, 2000 and 5000 mg/kg) in mice. Furthermore, the general behaviour of mice was recorded continuously for 12 h and daily for a further 2 weeks for any eventual mortality. The EHV did not show mortality, or any remarkable symptoms of toxicity and/or any significant changes in general behavior in mice.

Effect of HEHV in high fat diet induced obesity

High fat diet composition

Harlan teklad formula was used in the preparation of high fat diet food for this model. According to Harlan and teklad formula, the composition of high fat diet food is in this Table No.2.

The K.cal of HFD was 583 Kcal/100 g.

The experimental Female Swiss albino rats of 4 weeks old and weighing around 80-90g were taken. After induction of high fat diet for one week, these animals were divided into 5 Groups based upon their body weights at around 100 to 110g. Group I serve as normal diet and were receive only vehicle (distilled water, 1ml/kg b.w) for 40 days. Group II receives high fat diet only. Group III receives hydro alcoholic extract of *H. vulgare* (250 mg/kg p.o.) along with high fat diet for 40 days. Group IV receives hydro alcoholic extract of *H. Vulgare* (500

mg/kg p.o.) along with high fat diet for 40 days. Group V receives standard drug atorvastin (10 mg/kg p.o.) along with high fat diet.

On 39th day the body temperature of animals were measured. On 41 day, the animals were used for the study of various biochemical parameters. Blood was collected by orbital plexus of rat under ether anaesthesia and centrifuged using centrifuge at 2000 rpm for 30 min to get serum.

Group I

The animals were feed with normal diet and the distilled water will be supplied and serve as negative control.

Group II

These animals receives only high-fat diet for six weeks.

Group III

250 mg/kg body/day extract compound along with high-fat diet for six weeks orally.

Group IV

500 mg/kg body/day extract compound along with high-fat diet for six weeks orally.

Group V

Standard group receive Atorvastatin (10mg/kg, p.o.) along with high fat diet.

Effect of HEHV in sulpiride induced obesity[9,10]

The experimental Female Swiss albino rats of 4 weeks old and weighing around 80-90g were taken. After induction of sulpiride for one week, these animals were divided into 5 Groups based upon their body weight. Group I serve as normal diet and were receive only vehicle (distilled water, 1ml/kg b.w) for 28 days. Group II receives sulpiride only. Group III were receive hydro alcoholic extract of *H. Vulgare* (250 mg/kg p.o.) along with sulpiride for 28 days. Group IV animals were receive hydro alcoholic extract of H. Vulgare (500 mg/kg p.o.) along with sulpiride for 28 days. Group V animals were receiving standard drug atorvastin (10 mg/kg p.o.) along with sulpiride.

On 29th day, the animals were used for the study of various biochemical parameters. Blood was collected by orbital plexus of rat under ether anaesthesia and centrifuged using centrifuge at 2000 rpm for 30 min to get serum.

Group I

The animals were fed with normal diet and the distilled water was supplied and serves as negative control.

Group II

These animals received sulpiride along with normal diet.

Group III

The animals received extract (250mg/kg) before 2 hrs administration of sulpiride.

Group IV

The animals received extract (500mg/kg) before 2 hrs administration of sulpiride.

Group V

The animals received Atorvastatin (10mg/kg, p.o.) along with sulpiride.

Parameters studied

Body weight: The body weight (g) was recorded once in a week for 40 days in high fat diet induced model and for 28 days in sulpiride induced model in each group.

Food intake: The food intake (g) was recorded once in a week for 40 days in high fat diet induced model and for 28 days in sulpiride induced model in each group.

Fat pad weights: At the end of experiment of the experiment rats were sacrificed, parametrial adipose tissue was dissected and weighed.

Biochemical parameters[11-13]

At end of treatment period blood samples were collected from all the groups of the animals through the orbital sinus without the use of anti-coagulant. The blood sample was Centrifuged using centrifuge at 2000 rpm for 30 min to get serum for study of various biochemical parameters.

The biochemical parameters were estimated as per the standard procedure prescribed by the manufacturer's instruction manual provided in the kit. (Coral clinical systems, Verna Goa, India) using Semi Autoanalyser (ARTOS).

Estimation of cholesterol

Cholesterol esterase hydrolyses esterified cholesterols to free cholesterol. The free cholesterol

is oxidized to form hydrogen peroxide which further reacts with phenol and 4-amino antipyrine by the catalytic action of peroxidise to form a red coloured quinine imine dye complex. Intensity of the colour formed is directly proportional to the amount of cholesterol present in the sample.

Cholesterol Esterase

Cholesterol Esters $+H_2O \longrightarrow$ Cholesterol + Fatty acids.

Cholesterol oxidase

Cholesterol + $O_2 \longrightarrow$ Cholestenone + H_2O.

H_2O_2 + 4aminoantipyrine + Phenol $\longrightarrow$ Red Quinoneimine dye + H_2O.

Pipette out 1.0ml of working reagent into a clean dry test tube and add 0.01ml of sample to it mix well and incubate at 37^0 C for 5 min and aspirate.

Estimation of Triglycerides

Triglycerides are first hydrolyzed by lipoprotein lipase to glycerol and free fatty acids. Glycerol is then phosphorylated by adenosine-5¢-triphosphate (ATP) forming glycerol-1-phosphate (G-1-P) and adenosine-5¢-diphosphate (ADP) in the reaction catalyzed by glycerol kinase (GK). G-1-P is then oxidized by glycerol phosphate oxidase (GPO) to dihydroxy acetone phosphate (DAP) and hydrogen peroxide (H2O2). Peroxidase (POD) catalyzes the coupling of H2O2 with 4-aminoantipyrine (4-AAP) and sodium N-ethyl-N-(3-sulfopropyl) manisidine (ESPA) to produce a quinoneimine dye

Triglycerides $\longrightarrow$ Glycerol + Fatty acids

GK: Glycerol + ATP $\longrightarrow$ G-1-P + ADP

GPO: G-1-P + $O_2 \longrightarrow$ DAP + H_2O_2

POD: H2O2 + 4-AAP + ESPA $\longrightarrow$ Quinoneimine dye + H_2O.

HDL

The apoB containing lipoproteins in the specimen are reacted with a blocking reagent that renders them non-reactive with the enzymatic cholesterol reagent under conditions of the assay. The apoB containing lipoproteins are thus effectively excluded from the assay and only HDL-chol is detected under the assay conditions. The method uses sulfated alpha-cyclodextrin in the presence of Mg+2, which forms complexes with apoB containing lipoproteins, and polyethylene glycol-coupled cholesteryl esterase and cholesterol oxidase for the HDL-cholesterol measurement.

ApoB containing lipoproteins + α-cyclodextrin + Mg+2 + dextran SO4 $\longrightarrow$ > soluble non-reactive complexes with apoB-containing lipoproteins.

PEG-cholesteryl esterase

HDL-cholesteryl esters $\longrightarrow$ HDL-unesterified cholesterol + fatty acid.

PEG-cholesterol oxidase

Unesterifiedchol + O2 $\longrightarrow$ cholestenone + H2O2.

H2O2 + 5-aminophenazone + N-ethyl-N-(3-methylphenyl)-N'_succinyl ethylene diamine+ H2O + H+ peroxidase $\longrightarrow$ >qunoneimine dye + H2O.

Estimation of LDL

Using data obtained above the low density lipoprotein cholesterol levels were calculated using empirical formula of friedewaid

Serum Low density lipoprotein =total cholesterol-High density lipoprotein – Triglycerides/5

Histopathological studies

The rats were sacrificed and the liver was carefully dissected, cleaned of extraneous tissue and fixed in 10% formalin for at least 24 h. Then the extract at a dose of 100 mg/kg b.w. The paraffin sections were prepared (automatic tissue processor, autotechnique) and cut into 5mm thick sections, using a rotary microtome. The sections were stained with haemotoxylin-eosin dye and studied for histopathological changes.

Statistical Analysis

The values are expressed as Mean±SEM. The data was analysed by using one way ANOVA followed by Dunnet's test using Graph pad prism software. Statistical significance was set at $P \leq 0.05$.

RESULTS

The results are showed in the Table No.1 to 11 and represented in the figures.

Table No.1: List of materials and source

S.No	Name of the materials	source
1	Chem...kit for Serum LDL estimation	Coral clinical systems
2	Chem...kit for Serum HDL estimation	Coral clinical systems
3	Chem...kit for Serum Triglycerides estimation	Coral clinical systems
4	Chem...kit for Glucose estimation	Coral clinical systems
5	Chem...kit for cholesterol estimation	Coral clinical systems

Table No.2: High Fat Diet Composition

S.No	Composition	g/100g
1	Corn starch	10
2	Sugar	10
3	Lard	40
4	Vitamine mixture	1
5	Mineral mixture	4
6	Casein	20
7	Cellulose	5
8	Soyabean oil	7
9	Methionine	3

Table No.3: Qualitative Phytochemical Analyses

S.No	Test	MECM
1	Alkaloids	–
2	Carbohydrates	+
3	Flavonoids	–
4	Glycosides	+
5	Lactones	-
6	Phytosterols	+
7	Proteins	–
8	Saponins	–
9	Triterpenoids	–

(+)Indicates positive result (–) Indicates negative result

In, preliminary phyto chemical studies of extracts of *Hordeumvulgare*confirmed the strong presence of desired phyto chemicals in hydro alcoholic extract. Hence for the further studies hydro alcoholic extract of *Hordeumvulgare* (HEHV) have been selected.

Anti-obesity activity

Table No.4: Effect of HEHV on body weights in high fat diet induced obese albino rats

S.No	Weeks	Normal	Positive control	HEHV (250mg/kg)	HEHV (500mg/kg)	Standard drug Atorvastatin (10mg/kg)
1	0	105.28±1.64	108.07±1.9	108.24±1.8**	108.1±1.79**	109.03±1.58**
2	1	115.10±2.82	146.54±2.93	136.16±2.9*	133.25±3.56*	122.91±2.55*
3	2	128.55±2.4	182.55±3.38	175.28±3.7*	162.53±2.56*	143.02±3.32**
4	3	141.40±2.72	216.12±3.7	188.29±3.38*	177.07±2.98*	158.77±2.27**
5	4	150.31±2.57	234.44±3.92	197.44±3.68*	186.25±3.45*	169.17±2.48**
6	5	162.85±2.6	262±3.86	217.44±2.94*	189.39±3.59*	178.625±2.72**
7	6	174.7±2.73	283.14±3.66	224.66±3.43*	186.44±3.52**	181.44±2.52**

Table No.5: Effect of HEHV on food intake in high fat diet induced obese albino rats

Groups	0 Week	1 Week	2 Week	3Week	4 Week	5 Week	6 Week
Group i	10.2±1.28	12.7±2.43	14.7±2.67	17.2±2.69	19.3±2.87	23.5±2.74	21.7±2.44
Group ii	10.4±1.52	14.03±4.3	17.8±4.65	21.9±4.38	25.8±5.21	28.8±4.34	30.6±4.78
Group iii	9.8±1.74**	13.7±3.56*	16.4±3.87*	18.4±3.65**	24.4±3.53*	26.4±3.65*	28.4±3.88*
Group iv	10.7±1.49**	12.5±3.71**	15.9±3.29*	17.6±3.76**	22.3±3.47*	23.5±3.54**	24.9±3.59*
Group v	9.7±1.738**	12.2±2.98**	15.8±4.02*	18.3±3.85*	21.1±3.77**	22.9±3.69**	23.3±3.83**

Table No.6: Effect of HEHV on biochemical parameters and in high fat diet induced obese albino rats

S.No	Biochemical parameter	normal	Positive control	HEHV (250mg/kg)	HEHV (500mg/kg)	Standard drug Atorvastatin (10mg/kg)
1	Total cholesterol	43.85±1.46	136.65±3.77	79.35±2.39*	67.41±2.29**	58.69±3.32**
2	Triglycerides	82.06±1.6	186.64±2.91	107.02±3.7*	90.06±2.54*	81.68±2.41**
3	HDL	36.53±1.82	18.45±2.23	27.24±3. 21*	43.01±3.16**	47.81±3.37**
4	LDL	47.80±1.41	119.64±3.39	69.95±2.86*	48.19±3.41**	42.99±3.36**
5	Glucose	105.95±1.09	182.71±2.41	136.90±3.98*	117.21±1.85**	107.10±2.49**

Table No.7: Effect of HEHV on fat pad weights in high fat diet induced obese rats

S.No	Groups	Fat pad weight
1	Normal control	0.22± 0.003
2	Positive control	0.68±0.005
3	HEHV (250mg/kg)	0.62±0.01*
4	HEHV (500mg/kg)	0.49±0.006**
5	Atorvastatin (10mg/kg)	0.43±0.008**

Table No.8: Effect of HEHV on body weights in sulpiride induced obese albino rats

S.No	Weeks	Normal	Positive Control	HEHV (250mg/kg)	HEHV (500mg/kg)	Standard drug Atorvastatin (10mg/kg)
1	0	108.21±1.51	108.42±1.49	108.52±1.54*	108.57±1.51**	108.66±1.55**
2	1	114.37±1.32	131.61±3.51	129.54±3.4*	125.28±3.38*	123.75±3.32*
3	2	126.86±2.4	156.37±3.55	148.79±2.27*	135.50±3.55*	131.84±2.21**
4	3	138.12±1.87	186.23±2.45	167.69±3.24*	159.59±2.22**	147.27±2.45**
5	4	149.73±1.98	227.03±3.79	185.49±3.32*	176.73±2.42**	164.30±2.44**

Table No.9: Effect of HEHV on food intake in sulpiride induced obese albino rats

S.No	Groups	0 week	1 week	2 week	3 week	4 week
1	group i	10.8±1.28	12.7±2.43	14.1±3.67	16.3±2.69	20.3±2.87
2	group ii	10.3±1.52	13.4±3.3.	16.5±3.65	20.43±3.38	25.8±4.21
3	group iii	10.9±1.74*	12.9±3.56*	16.1±2.87*	17.4±3.27*	23.4±4.53*
4	group iv	9.7±1.49**	12.5±3.71*	14.6±3.51*	16.36±3.59*	22.8±2.97**
5	group v	10.4±1.73**	12.6±2.98**	14.3±3.25*	16.02±4.5*	22.1±2.77**

Table No.10: Effect of HEHV on biochemical parameters in sulpiride induced obese albino rats

S.No	Biochemical parameter	Normal	Positive control	HEHV (250mg/kg)	HEHV (500mg/kg)	Standard drug Atorvastatin (10mg/kg)
1	Total cholesterol	43.85±1.59	76.19±2.81	50.73±3.39*	44.52±3.39**	43.37±2.47**
2	Triglycerides	75.67±1.0	173.58±2.67	113.04±4.76*	88.03±3.72**	80.53±3.60**
3	HDL	35.02±1.48	28.77±3.78	30.74±3.55*	37.60±2.40*	39.67±3.56**
4	LDL	43.69±1.66	101.64±3.88	62.14±3.78**	47.38±3.77**	41.81±3.52**
5	Glucose	98.05±1.46	162.10±2.5	133.67±2.89*	117.49±4.1**	104.71±4.72**

Table No.11: Effect of HEHV on fat pad weights in sulpiride induced obese rats

S.No	Groups	Fat pad weight
1	Normal control	0.24±0.01
2	Positive control	0.73±0.01
3	HEHV (250mg/kg)	0.67±0.006*
4	HEHV (500mg/kg)	0.56±0.001**
5	Atorvastatin (10mg/kg)	0.47±0.005**

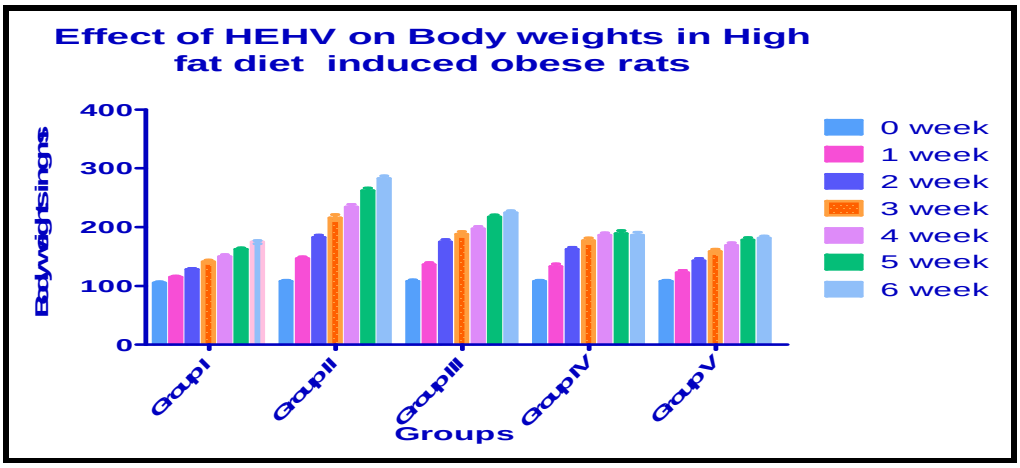

Figure No.1: Effect of HEHV on body weights of high fat diet Induced obese rats

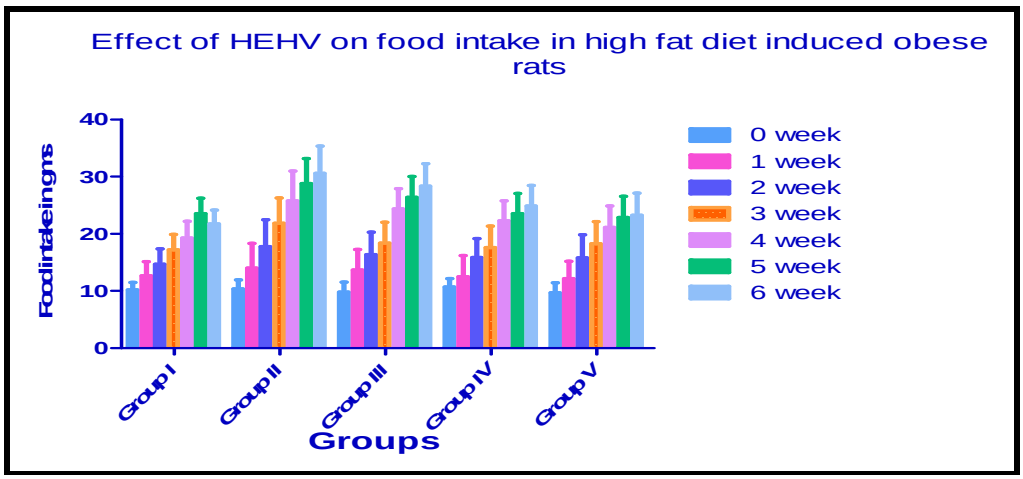

Figure No.2: Effect of HEHV on food intake in high fat diet Induced obese albino rats

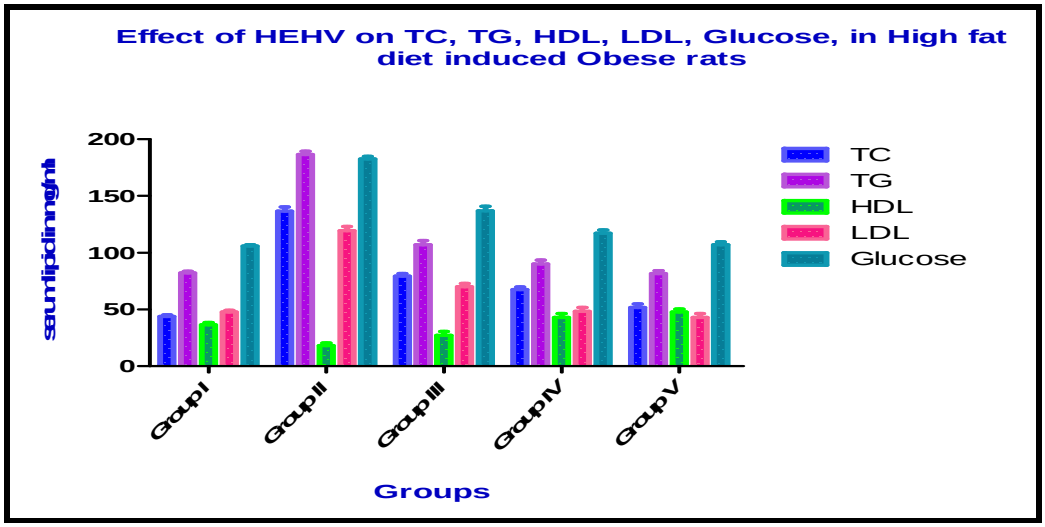

Figure No.3: Effect of HEHV on biochemical parameters and in high fat diet Induced obese rats

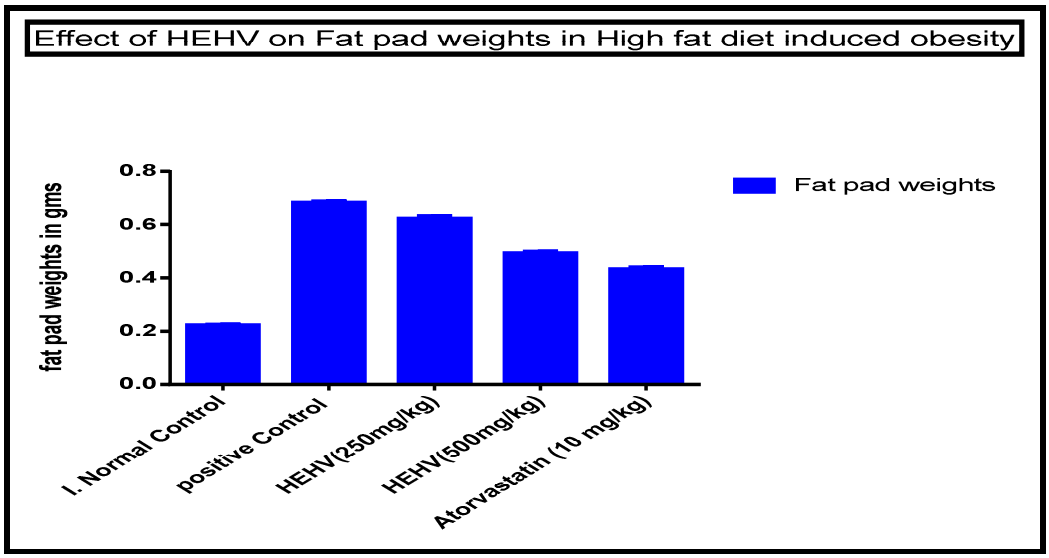

Figure No.4: Effect of HEHV on fat pad weights in high fat diet Induced obese rats

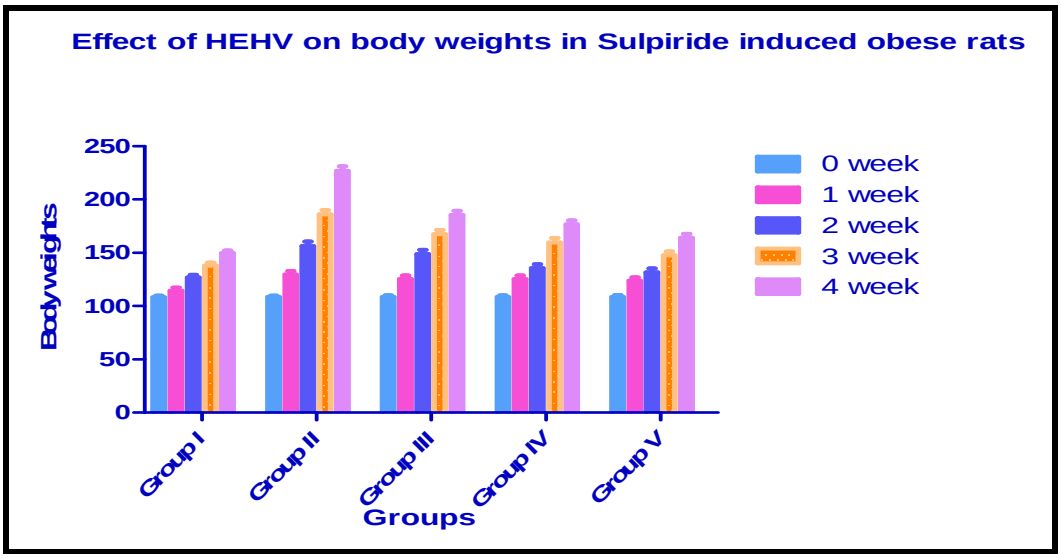

Figure No.5: Effect of HEHV on body weights in sulpiride Induced obese rats

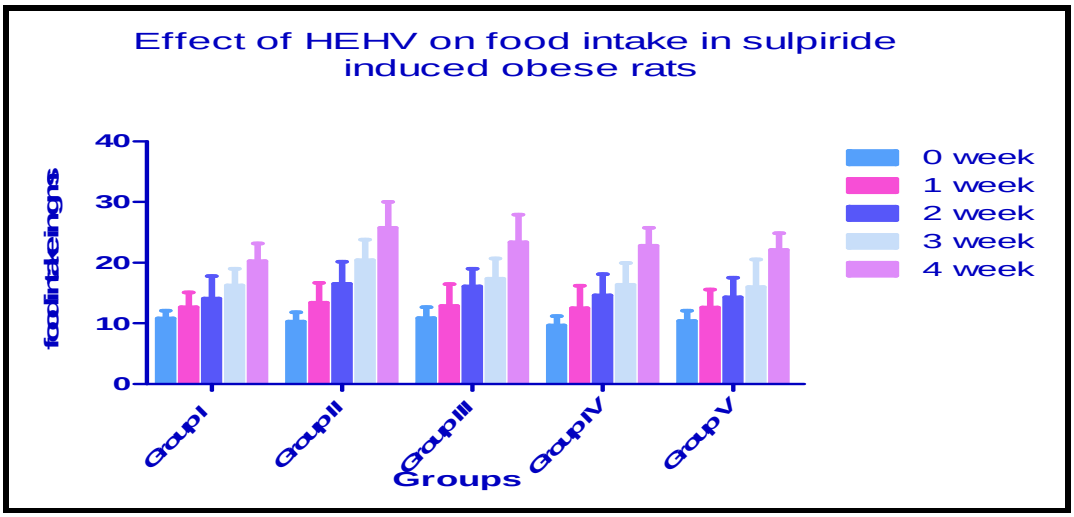

Figure No.6: Effect of HEHV on food intake in sulpiride induced obese albino rats

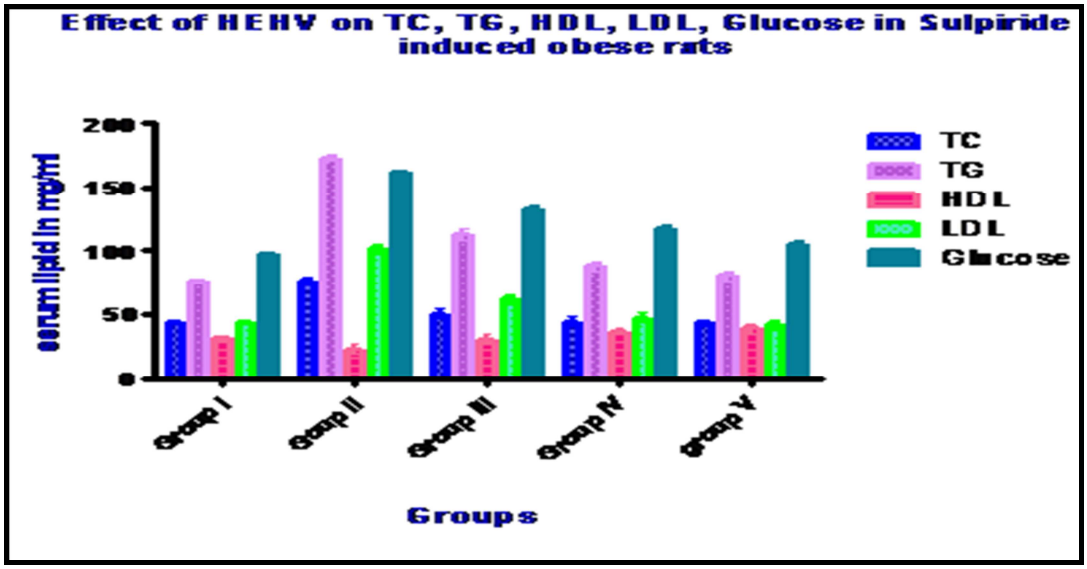

Figure No.7: Effect of HEHV on biochemical parameters in sulpiride induced obese rats

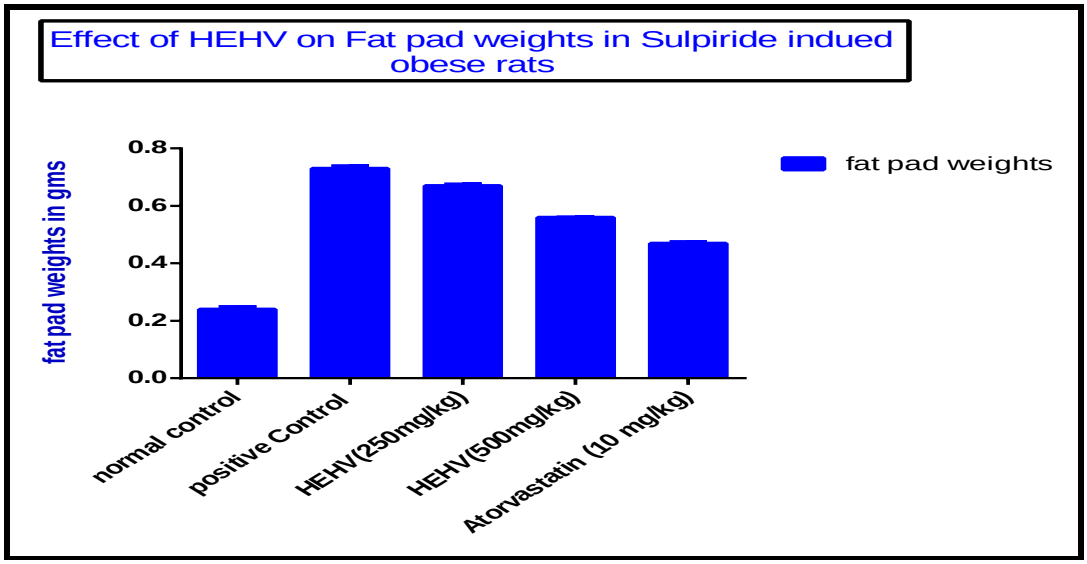

Figure No.8: Effect of HEHV on fat pad weights in sulpiride induced obese rats

CONCLUSION

The anti-obesity activity of Hydro alcoholic Extract of *Hordeum vulgare* grains was confirmed by the following measuresin high fat diet and sulpiride induced obese rats:

Reduced food intake
Reduced the body weight
Reduced serum glucose levels
Reduced LDL levels
Reduced Triglycerides
Reduced cholesterol levels
Reduced fad pad weights
Increased HDL levels

From the above, it may be concluded that the plant possesses anti-obesity property. When taken along with diet, the plant is shown to reduce obesity. Further investigations detailing about the chemical constituents responsible for the activity in the hydro alcoholic extract are welcomed and the plant may be further explored for its potential in treatment of obesity.

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CONFLICT OF INTEREST

We declare that we have no conflict of interest.

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