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Pharmacological Investigation of Anti-obesity Effect of *Cyperus Rotundus*

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Authors' contributions

This work was carried out in collaboration between both authors. Both authors read and approved the final manuscript.

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Original Research Article

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ABSTRACT

According to WHO, obesity is a not unusual place fitness ailment of lipid and carbohydrate metabolism that consequences from immoderate fats accumulation in adipose tissue, liver, skeletal muscle etc. This research was focused on the development and Investigation of Anti-Obesity effect of *Cyperus Rotundus* in obese rats. The plant- Nagarmotha was collected from Moradabad region and authenticated by the botanist. Firstly, the calibration curve was plotted. Animals were obtained from the animal house maintained at Faculty of Pharmacy, IFTM University, Moradabad, IN. Animals were free to access pellet chow diet and water *ad libitum*. They were kept as per CPCSEA Guidelines for one week and kept on fasting for 24 hours prior sacrificing the rodents. The rats were divided into 6 groups, each containing 6 animals. It includes Group 1: Normal Diet Control, Group 2: High caloric Diet control (HCD), Group 3: HCD + Orlistat (5 mg/kg), Group 4: HCD + EEN (100 mg/kg), Group 5: HCD+ EEN (200 mg/kg and Group 6: HCD + EEN (300 mg/kg). Each rat was evaluated after the continuous treatments for different parameters such as body weight & food intake, Total Body Electrical Conductivity (TOBEC), blood glucose levels, oral glucose tolerance test, histopathological studies for adipose tissue and liver, RNA Extraction and Semi-Quantitative PCR Analysis and liver and kidney functions test. In results, Ethanolic Extract of Nagarmotha (EEN) at all the three doses- 100mg/kg, 200mg/kg and 300mg/kg significantly exhibited lipid lowering effects when compared to standard group treated with Orlistat and control or high calories diet animals, in all the parameters. The effects were observed in dose-dependent manner, in

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ascending order with increase in dose. In conclusion, it may be said that EEN is a potential anti-lipidemic agent. This research suggests to characterize and isolate the main moiety responsible for its pharmacological activity (as anti-obesity) and confirm the mode of action- at which receptor subtype binding occurs.

Keywords: Cyperus Rotundus; Nagarmotha, anti-obesity; orlistat; high calorie diet; oral glucose tolerance test.

1. INTRODUCTION

According to WHO, obesity is a not unusual place fitness ailment of lipid and carbohydrate metabolism that consequences from immoderate fats accumulation in adipose tissue, liver, skeletal muscle etc. The incidence of weight problems is escalating the world over slicing throughout age businesses, intercourse and ethnicity. In each the evolved and growing international locations the proportion of obese and overweight humans has expanded dramatically in latest a long time basically because of expanded consumption of excessive caloric eating regimen, sedentary lifestyles patterns and decreased bodily interest. Obesity is a critical, persistent, prevalent, relapsing ailment of twenty first century and is one of the main reasons of preventable deaths. At an alarming fee the superiority of obese and weight problems is growing international affecting each growing and evolved international locations. The hassle of weight problems seems to be growing immensely in kids of this generation, the real fitness results may also most effectively come to be absolutely obvious withinside the future. Nagarmotha *(Cyperus rotundus)* normally referred to as Nagarmotha is determined all through India. It belongs to the own circle of relatives Cyperaceous. The genus call Cyperus is derived from Cypeiros, which became the historic Greek call for the genus, rotundus is Latin phrase for spherical and refers back to the tuber [1]. The own circle of relatives contains approximately 104 genera and extra than 5000 species world-wide, despite the fact that wide variety range significantly because of differing taxonomic principles of man or woman researchers. The biggest genus is Carex with approximately 2000 species world-wide, observed through *Cyperus* with approximately 550 species [2]. It is a pestiferous perennial weed with darkish inexperienced glabrous culms, springing up from underground tubers. It is honestly a discipline weed recognized in all of the Southern States as nut grass. The plant produces rhizomes, tubers, basal bulbs and fibrous roots beneath neath floor and rosettes of leaves, scapes and umbels above floor [3].

2. TAXONOMY [4]

Kingdom	:	Plantae
Subkingdom	:	Tracheobionta
Super division	:	Spermatophyta
Division	:	Magnoliophyta
Class	:	Liliopsida
Subclass	:	Commelinidae
Order (Cyperales)	:	Poales
Family	:	*Cyperacae*
Genus	:	*Cyperus*
Species	:	*Rotund*

Fig. 1. Nagarmotha (*Cyprus Rotundus)*

3. MATERIALS AND METHODS

3.1 Experimental Requirements

Blood sample, DNPH, oxaloacetate, EEN, NaOH, SGOT, SGPT, ALT & AST Assay Kit, carboxy methyl cellulose (CMC), Accu-Chek glucometer, Male Sprague-Dawley rats, Pellet chow diet, distilled water, micropipette, EM-SCAN, spectrophotometer, Nagarmotha *(Cyperus rotundus).*

3.2 Construction of Calibration Curve

In the dimension of each serum AST and ALT, most effective pyruvate is used as the usual. Theoretically speaking, oxalocetate have to be used as the usual for AST assay and pyruvate as the usual for ALT assay. 1 unit/L of AST or ALT is described because the liberation of 1m mol of pyruvate according to minute at 37 zero incubation according to lit of serum. Pipette-out one kind volumes of pyruvate, (0.1, 0.2, 0.3, 0.4ml) in accurately categorised take a look at tubes. The very last extent in every respective tube became adjusted to at least 1.0ml through addition of corresponding extent of ALT/AST substrate. Then 0.2ml of water became brought to every take a look at tube and became observed through 1.0ml of DNPH. The tubes have been left for 20 min at room temperature and 10ml of 0.4M NaOH became brought to every take a look at tube [5]. Constructed a calibration curve, through plotting the corresponding absorbance of requirements with concentrations.

3.3 Animals and Diets

Male Sprague-Dawley rats, everyday eating regimen and excessive caloric eating regimen have been acquired from National Centre for Laboratory Animal Sciences (NCLAS), National Institute of Nutrition (NIN), Hyderabad, India. Normal eating regimen contained pellet chow of popular composition containing all of the encouraged macro and micronutrients (56% carbohydrate, 18.5% protein, 8% Fat, 12% fiber and good enough tiers of minerals and vitamins). HCD contained 29.5% beef tallow, 22.0% casein, 23.0% starch, 17.9% cellulose, 4.0% L-cystine, 0.3% choline chloride, 1.8% vitamin mixture (AIN-93 ViX) and 1.5% salt mixture [6]. During the direction of the experimental duration (20 weeks), rats have been fed with both everyday eating regimen or freshly organized HCD (15g/rat/day) [7] as noted beneathneath and water advert libitum. Experimental animals have been maintained beneathneath popular laboratory situations (temperature: 22±2ºC; humidity: 60%). Rats first of all weighing 180-200g have been randomly divided in to 6 businesses of six every (n= 6). After induction of weight problems, to check the interest of EEN, rats have been handled with kind doses of EEN (one hundred, 2 hundred, or three hundred mg kg/ b. wt), suspended in 0.5% carboxy methyl cellulose (CMC), for 42 days (15^{th} to 20^{th} week), the use of an intragastric tube. All experimental protocols have been observed as according to institutional animal moral committee suggestions [8,9].

3.4 Experimental Design [10]

Group 1: Normal Diet Control (Normal Diet)
Group 2: High caloric Diet control (HCD)
Group 3: HCD + Orlistat (5 mg kg^{-1} b.wt)
Group 4: HCD + EEN (100 mg kg^{-1} b.wt)
Group 5: HCD+ EEN (200 mg kg^{-1} b.wt)
Group 6: HCD + EEN (300 mg kg^{-1} b.wt)

3.5 Composition of High Caloric Diet

Rats are given pellet chow diet for better nutritional nourishment and convenient to ingest. The following table concerns the ingredients in the same-

Table 1. Ingredients in pellet chow diet [11]

Ingredients	Weight in %
Beef tallow	29.5%
Casein	22.0%
Starch	23.0%
Cellulose	17.9%
L-Cystine	4.0%
Choline chloride	0.3%
Vitamin mixture (AIN-93 Vix)	1.8%
Salt mixture	1.5%

3.6 B. Evaluation

3.6.1 Determination of body weight and food intake

Throughout the experimental duration the burden benefit of rats became monitored weekly and the meals consumption became monitored each 2nd day. The common weight of the animals in a set is represented withinside the consequences. Initial weight approach weight of the animals on the stop of 14th week and very last weight approach, the burden of the animals on the stop of 20 weeks [12]. Total quantity of meals ate up through a rat became measured as: Consumption of feed = Total quantity of feed given to rat - left over feed.

3.6.2 Body composition by Total Body Electrical Conductivity (TOBEC)

Total Body Electrical Conductivity of experimental animals became assessed on the stop of test through Total Body Electrical Conductivity (TOBEC) the use of small animal frame composition evaluation system (EM-SCAN, Model SA-3000 Multi detector, Delhi). Lean frame mass, fats-unfastened, mass and overall frame fats, fats possibilities have been calculated as according to manufacturer's protocol [13].

3.6.3 Estimation of fasting blood glucose

Rats have been fasted in a single day and blood became drawn through unfashionable orbital puncture approach. Blood glucose tiers have been measured through dextrose (glucose oxidase peroxidase approach) with a fundamental contact Accu-Chek glucometer (Johnson and Baker, 1998) [14].

3.7 Oral Glucose Tolerance Test (OGTT)

To take a look at glucose tolerance, OGTT became achieved. At the stop of the test, after in a single day fasting, glucose became administered per oral at a dose of 2g/kg b.wt to rats and blood samples have been amassed from supra orbital sinus at 0, 30, 60 and a hundred and twenty mins and glucose stage became expected [15].

3.8 Histopathological Examination of Adipose and Liver Tissue

Adipose and liver tissues from all businesses of rats have been eliminated and stored in 10% formalin buffer answer. A small piece of tissue became sectioned with microtome, constant on slides and stained the use of haematoxilin and eosin (H&E) staining techniques and determined beneathneath optical microscope [16].

3.9 RNA Extraction and Semi-Quantitative PCR Analysis

Total RNA became remoted from the adipose tissue of rats through the use of tri reagent (Sigma-Aldrich, DELHI) in keeping with manufacturer's protocol, and opposite transcribed to gain cDNA the use of DNA synthesis kit (Applied Biosystems, Foster City, DELHI). 20ng of cDNA became used for semi-quantitative PCR with particular primers. The primer units used for goal genes are indexed in desk [17,18].

3.10 Liver and Renal Function Tests

Biochemical parameters consisting of SGOT, SGPT and ALP, overall proteins, albumins, overall bilirubin, serum creatinine and urea tiers have been studied on top of things and handled businesses. Liver markers like SGPT, SGOT ALP and renal characteristic [19,20]checks Like creatinine and urea tiers have been determined to be little decreasing in handled businesses as compared to govern organization, in which as overall proteins, albumin tiers and overall bilirubins have been nearly equal in each the businesses [21,22]. Administration of EEN had confirmed powerful antioxidant and defensive character, that's supported through the enhancements of biochemical parameters.

4. RESULTS AND DISCUSSION

4.1 Effect on Body Organs Test

Firstly, the pharmacological effect of EEN was tested on liver and kidney function tests. Two different tables were used to demonstrate the same as discussed further.

4.1.1 Effect of EEN on liver function tests

The following table depicts the role of EEN on SGOP, SGPT, ALP, Total Proteins, Albumins and Total Bilirubin-

Table 2. Depiction of effect of EEN on liver function test

Parameters	Control Group	Treated Group (EEN)		
		100mg/kg	200mg/kg	300mg/kg
SGOT (U/L)	22.6±4.2**	19.6±4.3*	17.2±1.3**	15.4±3.2**
SGPT (U/L)	34.2±1.63**	25.6±4.2***	22.4±2.0**	20.3±5.3**
ALP (U/L)	250.6±4.65***	215±4.6**	197±4.4**	182±2.4**
Total proteins (g/dL)	9.2±0.48**	7.8±0.44**	7.1±0.31***	6.2±0.12*
Albumins (g/dL)	5.3±0.32**	4.8±0.32***	4.2±0.41**	3.7±0.22**
Total Bilirubin (mg/dL)	0.92±0.12***	0.98±0.89***	1.21±0.27**	1.49±0.42**

Results are expressed as mean ± SD & SEM. The statistical analysis was carried out by using one-way analysis (ANOVA)

EEN was found liver protective action as it significantly decreased SGOT, SGPT, ALP, Total proteins and Albumins in all the doses when compared to control group. But liver protective action was dose-dependent. Total Bilirubin was observed as increased in the treatment group but it was minimum in control group. SGOT was observed as 22.6±4.2** U/L, 34.2±1.63** U/L, and 19.6±4.3* U/L and 25.6±4.2*** U/L in control and treated group (100mg/kg) respectively.

4.1.2 Effect of EEN on renal function tests

The following table concerns with the effect of EEN on renal function test-

Table 3. Depiction of effect of EEN on renal function tests

Observation	Control Group	Treated Group (EEN)		
		100mg/kg	200mg/kg	300mg/kg
Serum creatinine (mg/dL)	0.96±0.05**	0.94±0.05***	0.88±0.03**	0.81±0.04*
Urea (mg/dL)	46.6±4.82**	43.6±4.8***	39.5±3.7**	36.2±3.6***

Results are expressed as mean ± SD & SEM. The statistical analysis was carried out by using one- way analysis (ANOVA)

EEN significantly favored renal function in terms of serum creatinine and urea when tested. The serum creatinine (mg/dl) was found as 0.96±0.05** in control and as 0.94±0.05***, 0.88±0.03** and 0.81±0.04* in 100mg/kg, 200mg/kg and 300mg/kg treated groups respectively. Similarly, action on urea was observed. It significantly lowered the urea level in kidney at all the doses.

4.2 Effect of EEN on Body Composition and Food Intake

Consumption of HCD for 20 weeks produced a good-sized growth in frame weight (46.8±8.3g), overall fats (75.9±9.4g), fats % (16.1±3.8), and fats unfastened mass (173.1±2.8g) in HCD manipulate organization while as compared to everyday manipulate organization of rats whose frame weight, overall fats, fats % and fats unfastened mass have been 36.7±11.6g, 26.9±4.8g, 7.3±1.2% and 153.4±6.6g respectively. Oral management of EEN (100, 200, 300mg/kg b. wt.) for 42 days (from fifteenth to twentieth week) notably decreased frame weight and frame composition in a dose dependant way. Among the 3 doses administered, EEN at a dose of three hundred mg kg/b.wt, confirmed full-size (p < zero.05) healing impact. At 300mgmg/kg b. w. of EEN, the frame weight, overall fats, fats % and fats unfastened mass have been 387.1±6.7g, 40.2±3.3g, 10.97±2.6% and 155±4.9g respectively.

4.3 Effect of EEN on Plasma Lipid Profile

The tiers of overall ldl cholesterol, FFAs, TGs, Phospholipids (PLs), HDL, LDL and VLDL have been measured in plasma. The concentrations of overall cholesterol, FFAs, TGs, PLs, LDL and VLDL have been markedly increased whilst HDL stage reduced in experimental overweight rats while as compared to everyday manipulate rats. Oral management of EEN has reversed those changes in a dose dependant way, the maximum profound impact being stated at a dose of three hundred mg kg-1b.wt.

4.4 Effect of EEN on Blood Glucose, Insulin and Insulin Resistance

The tiers of plasma glucose, insulin and insulin resistance on top of things and experimental

overweight rats are proven. There became a full-size ($p<0.05$) elevation in plasma glucose, insulin and insulin resistance in HCD manipulate overweight rats while as compared to everyday manipulate rats. Oral management of various doses of EEN ought to carry those modifications to close to normalcy in a dose-based way, the maximum full-size impact being determined at 300mg/kg b. wt. The consequences are proven in desk.

Table 4. Effect of EEN on plasma glucose, insulin and insulin resistance in normal and experimental obese rats

Groups	Glucose (mg.dl-1)	Insulin (µU.ml-1)	Insulin Resistance
Control	81.9±4.3	5.5±0.7	3.3±0.07
HCD Control	160.9±12.2a***	14.8±1.5a*	5.1±1.6a*
HCD +Orlistat	128.5±13.1b***	7.8±1.2b*	3.1±0.8b*
HCD+EEN (100 mg.kg-1 bw)	137.4±10.6b**	12.4±1.9b*	4.4±1.2b*
HCD + EEN (200 mg.kg-1 b w)	132.1 ± 2.6b**	9.5±0.8b*	3.8±1.2b*
HCD+EEN (300 mg.kg-1 b w)	129.7±15.3b**	8.1±0.7b*	3.2±0.6b*

*Values mean ± S.D & SEM, n=6. Values are statistically significant at *p <0.05. a* Significantly different from normal control. b* Significantly different from HCD control*

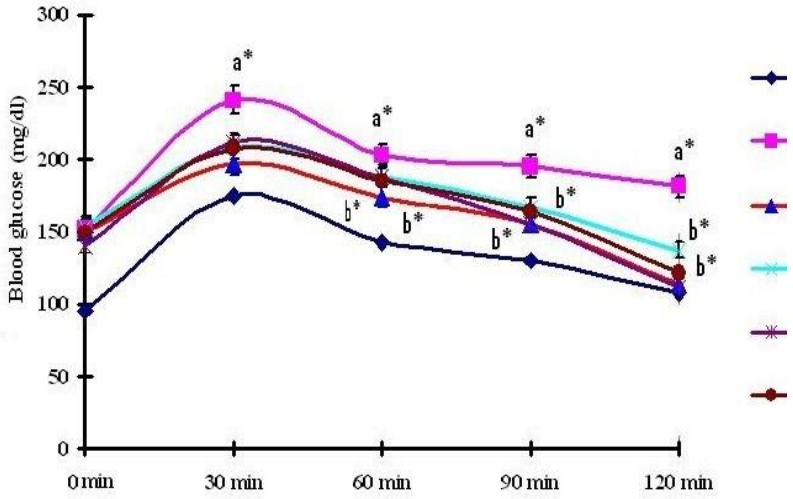

Fig. 2. Effect of *Cyperus rotundus* on glucose tolerance

*Values are mean ± S.D & SEM, n = 6. Values are statistically significant at *p < 0.05.a* Significantly different from normal control; b* Significantly different from HCD control*

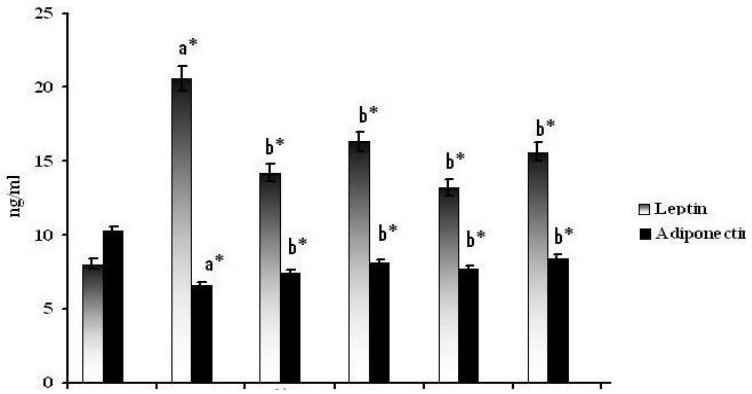

Fig. 3. Effect of EEN on leptin and adiponectin levels

*Values are mean ± S.D & SEM, n=6. Values are statistically significant at *p <0.05. a* Significantly different from normal control. b* Significantly different from HCD control*

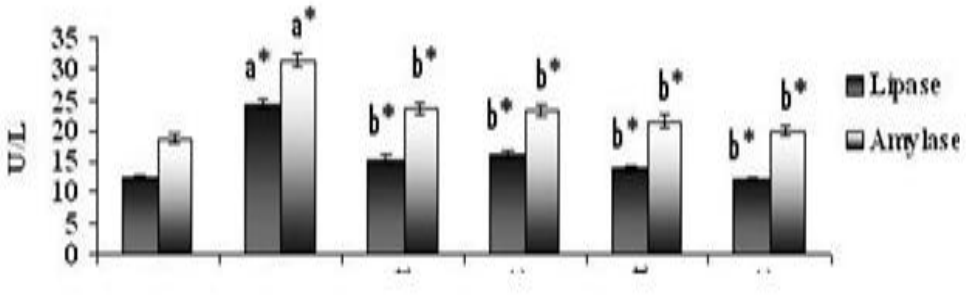

Fig. 4. Effect of EEN on amylase and lipase enzymes

*Values are mean ± S.D & SEM, n=6. Values are statistically significant at *p <0.05. a* Significantly different from normal control. b*Significantly different from HCD control*

4.5 Effect of Oral Glucose Tolerance Test

Depicts the consequences of oral glucose tolerance take a look at achieved on manipulate and experimental overweight rats. In the everyday manipulate organization of rats, blood glucose stage reached its most fee at 60 min after glucose load and declined to close to basal stage at a hundred and twenty min, while, in HCD-brought about overweight rats, the height growth in blood glucose stage became observed even after 60 min and remained excessive over the following 60 min. Administration of EEN (three hundred mg/kg b wt.) or orlistat to overweight rats elicited a full-size lower in blood glucose stage at 60 min and past while as compared with HCD manipulate rats.

4.6 Leptin and Adiponectin Levels

Leptin and adiponectin are primary adipocyte of adipose tissue. explains the tiers of leptin and adiponectin on top of things and experimental overweight rats. There became a marked elevation in leptin and reduce in adiponectin tiers in HCD fed overweight rats over their everyday manipulate rats. Interestingly, remedy with EEN (300mg/kg b. wt.) or orlistat has considerably ($p<0.05$) restored their tiers to normalcy.

4.7 Assay of Amylase and Lipase

The sports of amylase and pancreatic lipase of everyday and experimental overweight rats are exemplified in discern four nine. There became a fold growth withinside the sports of lipase and amylase in HCD fed manipulate rats while as compared to everyday manipulate organization of rats. Administration of EEN has added down their sports in a dose dependent way. A full-size ($p <$ zero.05) discount of their sports became stated at three hundred mg kg/ b. wt. of EEN.

4.8 Effect of EEN on Fecal Lipid Content

Fecal mat became amassed from the rectum and moist weight of the feces became measured. Decreased fecal depend weights have been observed in HCD+EEN administered businesses while as compared to HFD organization. Further, the extracted lipids from dry feces have been analyzed to discover the metabolic destiny of unabsorbed trigycerides. Increased excretion of fecal tirglycerides became determined in orlistat and EEN handled HCD businesses, indicating that EEN may intervene in lipid absorption and transportation.

4.9 Effect EEN on Fat Pads and Adipose Tissue

Adipose tissue is a dynamic organ the mass of which modifications in the course of lifetime in reaction to metabolic necessities of the animal, and for this reason, performs an crucial position in electricity balance. Extensive morphological modifications in fats pad deposition have been determined in retroperitoneal and epididymal tissues among NC, HCD and EEN handled businesses. The weight of retroperitoneal, epididymal adipose tissues have been markedly expanded in HCD fed organization. However, management of EEN decreased in a dose-based way the buildup of fats pads and adipose tissue weight.

Table 5. Effect of EEB on fecal lipids of normal and HCD fed obese rats

Parameters	NC	HCD	Orlistat	EEB(100)	EEN(200)	EEN(300)
Fecal mat weight (g)						
Initial weight	1.2 ± 0.02*	1.8 ± 0.02**	1.8 ± 0.02**	1.8 ± 0.36*	1.8 ± 0.4**	1.8 ± 1.30*
Final weight	1.8 ± 0.34**	2.4 ± 0.021*	1.2 ± 0.52**	1.5 ± 0.46**	1.4 ± 0.022*	1.3 ± 0.9*
Fecal lipids (mg/g)						
Initial level	11.0 ± 3.25	16.0 ± 1.32*	16.5 ± 1.2**	15.2 ± 1.6*	17.4 ± 0.51*	17.0 ± 0.02**
Final level	10.8 ± 0.18*	16.3 ± 1.3**	19.4 ± 0.2*	16.7 ± 2.4**	18.2 ± 2.1*	6.8 ± 1.6*

*Values mean ± S.D & SEM, n=6. Values are statistically significant at *p <0.05. a* Significantly different from normal control. b* Significantly different from HCD control*

Dissection of rats was done to isolate and weight the different organs. The following figure depicts the dissection of rats-

Fig. 5. Picture showing dissection of SD rat

Table 6. Effect of EEN on organ weights

Group	Liver	Kidney	Spleen	Testis
NC	9.5 ± 0.8**	1.9 ± 0.1*	0.4 ± 0.01**	2.9 ± 0.1**
HCD	13.6 ± 0.7**	4 ± 0.2*	0.6 ± 0.02***	1.7 ± 0.2**
ORL	10.3 ± 0.2*	1.9 ± 0.3**	0.4 ± 0.03**	2.7 ± 0.3***
EEN (100)	13.1 ± 0.9***	2.4 ± 0.1**	0.6 ± 0.07**	1.9 ± 0.2**
EEN (200)	12.2 ± 0.3**	2.2 ± 0.1**	0.5 ± 0.01**	2.2 ± 0.4***
EEN (300)	11.1 ± 0.2***	2.0 ± 0.2**	0.4 ± 0.02***	2.6± 0.3**

4.10 Effect of EEN on Organ Weights

After the stop of the experimental duration, rats have been anesthetized with isoflorane and sacrificed, weighed all organs consisting of liver, kidney, spleen and testis (fig 6). The liver and kidney weights of HCD-fed rats expanded notably which have been considerably (p < zero.05) and dose dependently decreased through EEN remedy (one hundred,2 hundred and 300mg/kg b.wt). Whereas spleen and testis of EEN handled businesses did now no longer display any full-size variant as compared to HCD manipulate.

4.11 Histopathology Studies

To in addition verify the anti weight problems interest of EEN on liver and adipose tissue, we completed histopathology research to

understand the deposition of fats, droplets and structure of tissue.

4.11.1 Liver histopathology examination

The Hematoxylin and Eosin-stained liver microtome sections have been determined beneath neath microscope. HCD fed manipulate organization rats confirmed better accumulation of lipid droplets, lack of nucleus, inflammatory cells and excessive swelling of hepatocytes indicating steatosis. However, EEN handled businesses confirmed, reduced lipid accumulation, lesser harm and close to everyday hepatocytes. The consequences are proven.

4.12 Procedure for isolation of Total RNA from Tissue Samples [23]

Step-1: *Sample preparation*

Tissue: Tissue samples were homogenized (Adipose/liver) in tri-reagent (1ml/50-100 mg of tissue) in a polytron or another appropriate homogenizer.

↓

Note: After homogenized the tissue samples in tri reagent (TR), samples can be stored at- 70^0 C for up to one month.

↓

Step-2: Added 0.2ml of chloroform/ml of TR used and covered the sample tightly. Shaken vigorously for 15 Sec and allowed standing for 2-15 min at room temp

↓

Centrifuged the resulting mix at 12000xg for 15 min at 4^0C

Step3: The aqueous phage was transformed to a fresh tube and added 0.5 ml isopropanol/ml of Tri reagent used in sample preparation and mix allowed the sample to stand for 5-10 min at room temp

↓

Centrifuged at 12000xg for 10 min at 4^0C. The RNA precipitate was formed as pellet on the side and bottom of the tube.

↓

Step-4: Supernatant was removed and washed the RNA pellet by adding 1ml of 75% ethanol/ml of TR used in sample preparation step, vortexed the sample and then centrifuged at 7500xg for 5min at 4^0C

↓

Step-5: RNA pellet was briefly dried for 5-10 min by air drying or under a vacuum

↓

Step-6: RNA pellet was dissolved in DEPC H2O and kept in ice for 2 min and quantitated after giving short spin

4.13 Liver Histopathology

The following figure depicts the histopathology- anatomical configuration of liver.

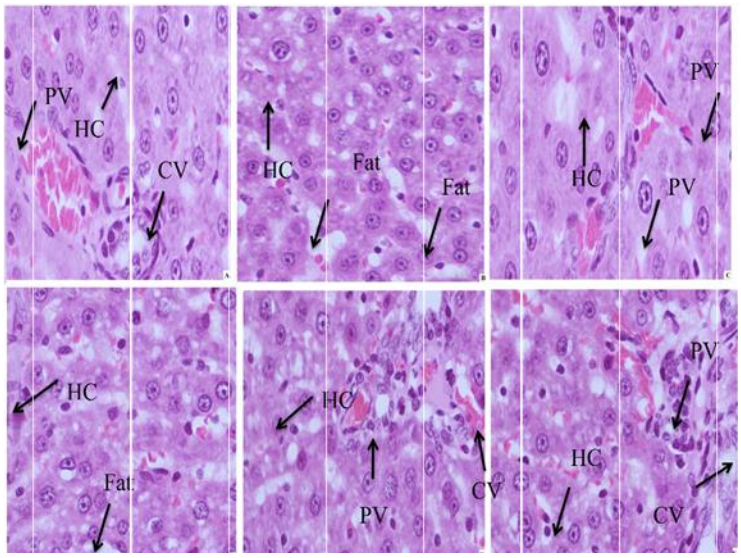

Fig. 6. Liver histopathology

NC: Normal control group b) HCD: high calorie diet c) Orlistatd) EEB: Ethanolic extract of Cyperus rotundus100 mg /kg /b.wt e) 200 mg /kg /b.wt f) 300 mg /kg b. wt.)

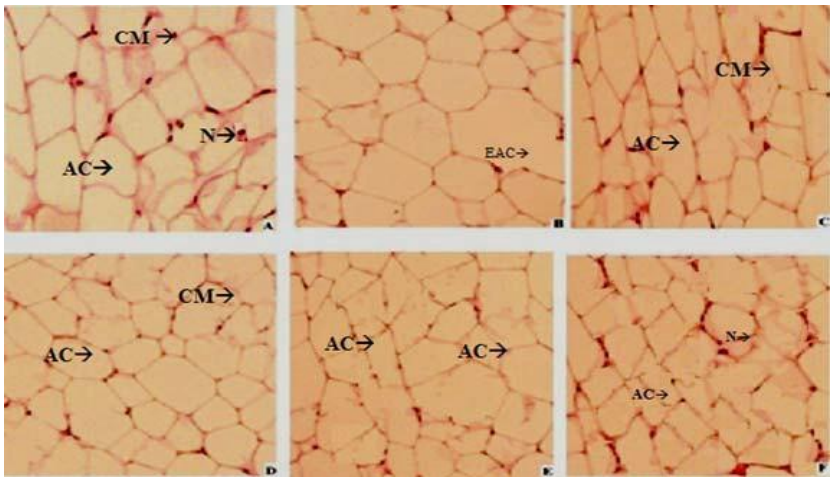

Fig. 7. Histopathology slides of adipose tissue

NC: Normal control group, b) HCD: high calorie diet, c) Orlistat, d) EEN: Ethanolic extract of Nagarmotha 100 mg /kg /b.wt, e) 200 mg /kg /b.wt, f)300 mg /kg /b.wt) CM - Cytoplasmic Membrane, N-Nucleus, AC-Adipose cell, EAC- Enlarged Adipose cell

Liver histopathology confirms recovering potential of EEN when compared to control and high calorie diet group of animals. As, it can clearly be seen that it increases the cell nourishment and their growth in terms of building blocks for liver. It majorly depleted the fat globules and concentrations in the Liver and thus kept liver non-fatty after all at all the doses.

4.14 Adipose Tissue Histopathology

To examine the energetic position of EEN we have tested the Hematoxylin and Eosin-stained adipose tissue microtome sections beneath neath mild microscope at 10 X. The length of adipocytes enlarged notably and extra fats deposits have been observed in HCD fed businesses while as compared to normal manipulate organization. Administration of EEB (one hundred, 2 hundred, 300mg/Kg frame weight) has extensively decreased the dimensions of adipocytes and normalized the structure of adipocytes in a dose dependant way.

4.15 Histopathology of Adipose Tissue

The following figure demonstrates the anatomical configurations after the treatment of EEN and compared with control and standard group.

4.16 Effect of EEN on Some Genes involved in Adipogenesis

The consequences from the semi-quantitative PCR evaluation of the adipose tissue of rats ate up HCD confirmed reputedly up regulated expression of FAS, PPARγ, SREBP-1c(Fig.four.14).However, remedy with EEN (one hundred,2 hundred and three hundred mg/kg b.wt) had dose dependently reversed the up expression of genes in HCD- brought about overweight businesses.

Our consequences imply that EEN at a dose of 300mg/kg b.wt ought to extensively alter adipogenesis and decrease fats accumulation in HCD-fed rats, like that of orlistat, the usual anti weight problems drug .

The mRNA change fold was found identical when compared with standard group treated with Orlistat. The effect was minimum at the dose 100mg/kg but was observed in ascending order with dose and optimum effect was seen in highest dose treatment. High fat diet group showed mRNA fold change approx. 2.5 which was highest among all the groups. EEN treated group (300mh/kg) significantly lowered by up to 1.4 (approx.).

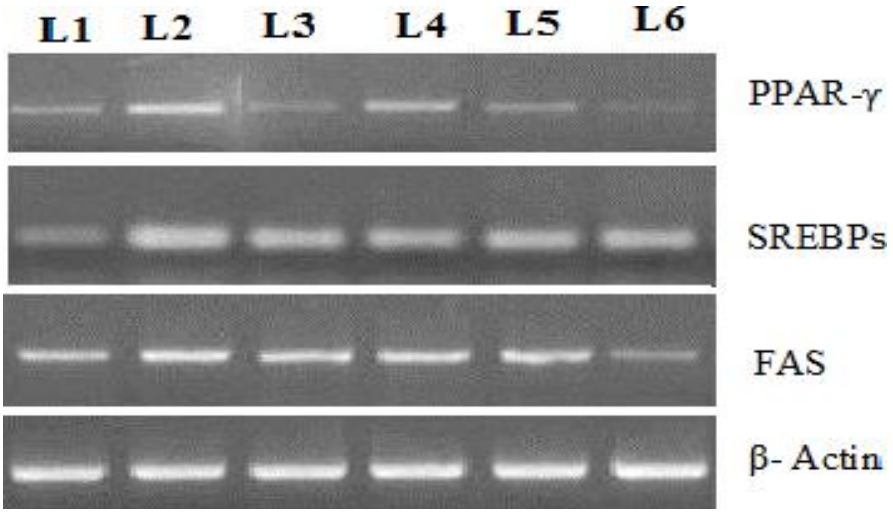

Fig. 8. Expression of semi-quantitative PCR products

L1: Normal Diet Control *L2: High Caloric Diet (HCD) Control*
L3: HCD+ Orlistat 5mg/kg b. wt. *L4 : HCD+EEB 100 mg/kg b. wt.*
L5 : HCD+EEN 200 mg/kg b. wt. *L6 : HCD+EEB 300 mg/kg b. wt.*

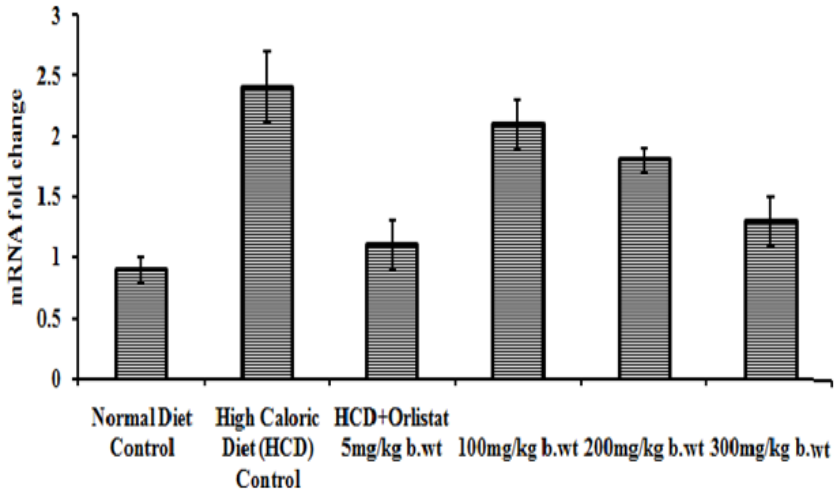

Fig 9. (a)Effect of ethanolic extract of *Cyperus rotundus* (EEN) expression in control and experimental rats on PPAR-γ

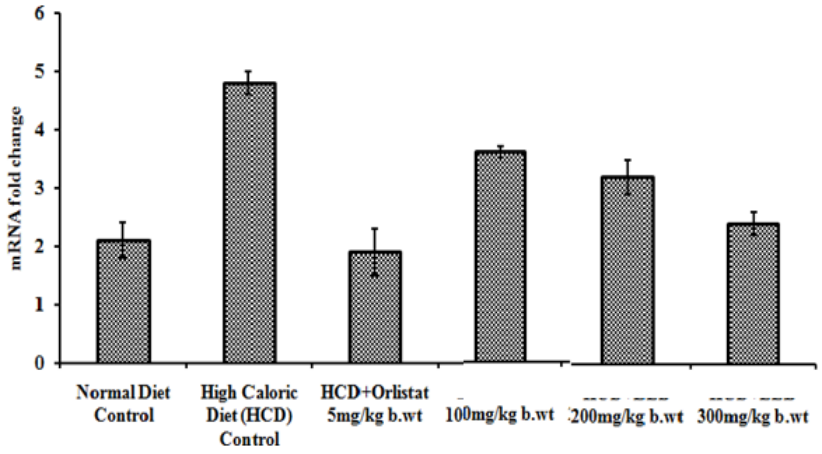

Fig 9(b). Effect of ethanolic extract of *Cyperus rotundus purpruea* (EEN) expression in control and experimental rats on SREBPs

It significantly exhibited effects in terms of SREBP's and anti-obesity potential when compared to control and standard group.

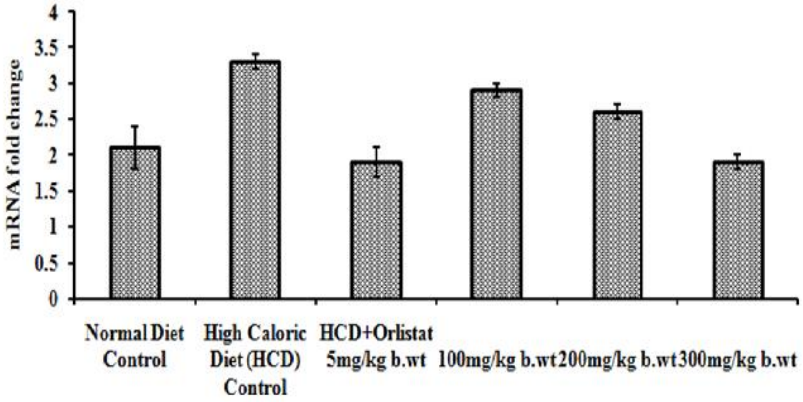

Fig 9 (c) Effect of ethanolic extract of *Cyperus rotundus purpurea* (EEN expression in control and experimental rats) on β-actin

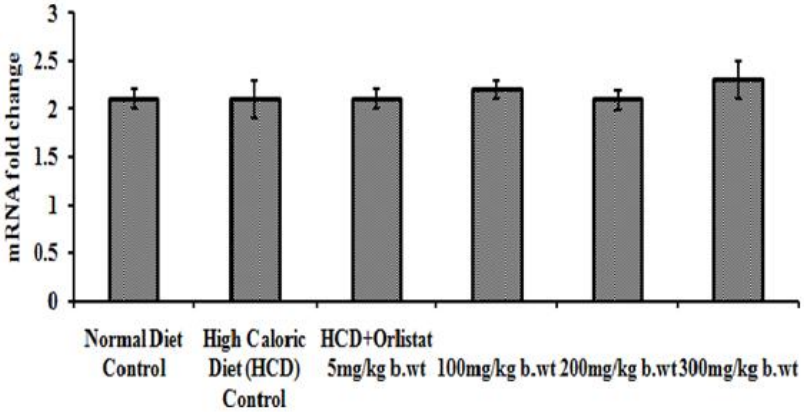

Fig 9(d). Effect of ethanolic extract of *Cyperus rotundus purpurea* (EEN) expression in control and experimental rats) on FAS

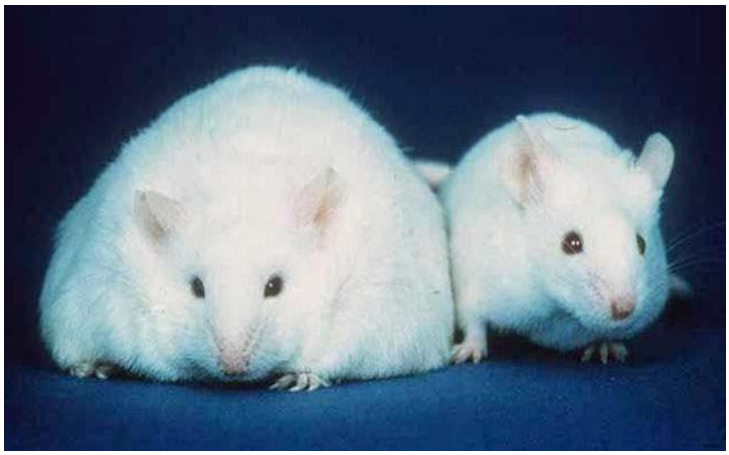

Fig. 10. Normal and obese rats

The effect on Beta-actin was found identical when compared with standard group treated with Orlistat. The effect was minimum at the dose 100mg/kg but was observed in ascending order with dose and maximum effect was seen in highest dose treatment. High fat diet group showed mRNA fold change approx. 3.5 which was highest among all the groups. EEN treated group (300mh/kg) significantly lowered by up to 2 (approx.).

5. VALUES ARE EXPRESSED IN MEAN &SEM

Effect on FAS was found impressive of EEN treated animals when compared with standard group treated with Orlistat. The effect was minimum at the dose 100mg/kg but was observed in ascending order with dose and optimum effect was seen in highest dose treatment. High fat diet group showed mRNA fold change approx. 2.1 and EEN treated group (300mh/kg) significantly increased by up to 2.4 (highest).

The increased prevalence of weight problems around the world has required the search for effective treatment options. Although there is a growing interest in natural remedies around the world, the lack of enough systematic research and clinical data on flora and herbs is limiting their use [24]. As a result, more detailed natural research on relevant animal models is required. The development of a closed animal model for weight problems research that can closely mimic the typical metabolic processes of human weight disorders could be quite difficult. Initial phytochemical evaluation of Cyprus rotundus was done using popular methodologies in the current study. Saponins, steroids, carbohydrates, polyphenols, tannins, triterpenoids, flavonoids, and alkaloids were quantified using one-of-a-kind solvent extracts (hexane, ethyl acetate).

Generally, elevation of SGOT, SGPT, ALP, creatinine and bilirubin are determined in liver and kidney harm [25] through any poisonous substance or beneath neath ailment situation, however very wholesome and defensive consequences have been determined withinside the gift paintings while EEN became administered. Based at the consequences determined in our look at, we finish that Ethanolic extract of *Cyprus rotundus* is more secure and non poisonous and will be properly used for anti-hyperlipidemic and anti-weight problems healing purposes.

HCD considerably expanded liver overall ldl cholesterol (TC), triglycerides (TG), unfastened fattyacids, (FFA), and MDA however reduced the sports of SOD, CAT which have been in the end reversed through the management of EEN in a dose based way and maximum profound interest became proven through EEB at a dose of three hundred mg / kgb. wt. Similarly, HCD-brought about changes in plasma lipid profiles have been additionally alleviated through EEN management.

Furthermore, histopathological exam of adipose tissue discovered that HCD brought on expansion of adipocyte with extra fats drops. Administration of EEN at the side of HCD ended in decreased length of adipocytes. Histopathological examinations of liver sections have virtually validated the advent of everyday hepatocytes with decreased lipid droplets in HCD+EEN handled businesses.

In addition, to verify the anti-obesity effect of EEN the m-RNA expression of sure genes was evaluated concerned in weight problems via semi quantitative RT-PCR research. HCD has expanded the expression of PPARγ, SREBP-1c, FAS and, β-actin. Administration of EEN has dose dependently decreased the expression of the stated genes.

6. CONCLUSION

In conclusion, primarily based totally on acute toxicity research we gift that EEN is non poisonous and secure as much as 3000 mg/ kg b. wt in SD rats. Treatment with EEN has dose dependently and considerably alleviated HCD brought about weight problems, hyperlipidemia, as supported through physiological, biochemical, histological and molecular research. The presence of phytoconstituents discovered through LC-MS evaluation may also play a distinguished position in healing interest of EEN. This looks at demonstrates the antihyperlipidemic and anti-weight problems cappotential of EEN and gives clinical validation and foundation to broaden anti-weight problems capsules.

Therefore, in all the parameters EEN significantly modulated the anti-obesity potential in rat model. It also enhanced glucose tolerability, renal and liver functions that it beneficial to normal body function and growth. It can be used in the treatment of obesity in human being after successful reports in clinical trials.

This research suggests to isolate the concerning constituent responsible for this activity that can be incorporated in the suitable dosage forms to demonstrate better tolerability and pharmacological potential.

DISCLAIMER

The products used for this research are commonly and predominantly use products in our area of research and country. There is absolutely no conflict of interest between the authors and producers of the products because we do not intend to use these products as an avenue for any litigation but for the advancement of knowledge. Also, the research was not funded by the producing company rather it was funded by personal efforts of the authors.

CONSENT

It is not applicable.

ETHICAL APPROVAL

As per international standard or university standard written ethical approval has been collected and preserved by the author(s).

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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