ejpmr, 2016,3(6), 396-401

SJIF Impact Factor 3.628

EUROPEAN JOURNAL OF PHARMACEUTICAL AND MEDICAL RESEARCH

www.ejpmr.com

Research Article

ISSN 2394-3211

EJPMR

EFFECT OF HYDRO-ALCOHOLIC POLY HERBAL FORMULATION ON LIPID PROFILES WITH ATHEROGENIC DIET FED ANIMAL MODEL OF OBESITY IN WISTAR RATS

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Article Received on 05/04/2016 **Article Revised on 25/04/2016** **Article Accepted on 15/05/2016**

ABSTRACT

Present study planned on anti-obesity in response to an atherogenic diet and to estimate the effect of *Cyperus Rotundus* Linn, *Terminalia Arjuna* & *Cinnamomum Zeylanicum* Nees, on serum lipid profiles, renal, hepatic function analysis in wistar rats. Obesity associated with Cardio vascular abnormalities is rapidly increasing throughout the world. Atherogenic or high fat diets were known to induce cardiovascular diseases, and several active compounds were tested to protect/prevent the risk of cardiovascular diseases. Obesity was induced by feeding rats with an atherogenic diet over a period of 60 days. Animals were divided into 6 groups, control, atherogenic diet, and 3 dose levels of hot infusion extract of herbal formulations of the hydro-alcoholic extract of herbal formulation containing *Cyperous rotundus*, *Terminalia arjuna* and *Cinnamomum zeylanicum* was administered orally in dose of 480mg/kg, p.o (high dose), 360mg/kg p.o (mid dose), 120mg/kg p.o (low dose) was administered daily for a treated group over a period of 60 days. Body weight, locomotor activity, organ and fat pad weights, lipid profile and renal function (Urea, Uric acid, Creatinine) ALT, AST activities were analyzed. Since the treatment with *Cyperus rotundus* poly herbal formulation significantly normalized the lipid profile. Plasma Adiponectin, Serum ALT, AST, urea, uric acid, and creatinine. Atherogenic diet induced obesity associated with a disturbed lipid profile, renal and liver implications for the progress of obesity related problems were examined, followed by the treatment with *Cyperus rotundus* poly herbal formulation improved obesity and its associated metabolic problems in different levels.

KEYWORDS: *Cyperus rotundus* poly herbal formulation, Atherogenic diet, Lipid Profile, Renal Profile, Anti-obesity.

INTRODUCTION

Atherosclerosis is a complex multifactorial disease, which develops in the arterial wall in response to various stimuli and results in excessive inflammatory and fibro proliferative reactions. Cardiovascular disease (CVD) due to atherosclerosis is the leading cause of morbidity and mortality in westernised countries.

This high incidence of death in low-income countries therefore made the study of lipid profile in the general population important in this society. Study had equally shown rise in the level of serum total cholesterol (TC) particularly in urban setting in Asian countries.[1] Atherosclerosis affects many important biochemical and/or physiological processes in the body. These include loss of ability of intima endothelium to secrete nitric oxide (NO) which is a vasoprotective gas against vascular inflammation.

The percentage prevalence of obesity in Indian is still less in comparison with the global context, but being the second-most populated country in the world, urgent attention is required toward this issue; otherwise, India would be one of the first nations from the developing countries in Asia to be put on the obesity map.[2]

High fat diet (atherogenic diet) is commonly used to induce atherosclerosis[3, 4, and 5], oxidative stress[6, 7], hyperlipemia and obesity [8, 9, and 10] in rodent animal models.

These are all similar pathogenesis found in humans when intoxicated with high fat diet. Three organs highly exposed to oxidative stress are the heart (based on circulation), the liver (the site of metabolism) and the kidney (site for excretion). These organs are often studied in order to access the degree of oxidative stress/damage in animals.[8, 9, 11, 12, 13, 14]

A number of drugs are available than can lower the serum lipid. their use can be limited by adverse effects like hepatitis etc. at present ,herbal anti hyperlipidemic drugs have gained importance to compensate the setbacks of modern allopathic drugs in this category.

Numerous plants like Achyranthus aspera, Bauhinia purpurea, Cinnamomum tamala, Cissus quadrangularis, Commiphora mukul, Garcinia cambogia, etc.[15]

Available literature shows that the herb *Cyperous Rotundus Linn*, has various degrees of anti hyperlipidemic, anti obesity and hypertensive activities along with many other beneficial pharmacological properties.[16]

The herb *Terminalia Arjuna*, has cardiovascular, anti inflammatory, anti diabetic, anti oxidant, anti atherosclerosis and much more pharmacological effects.[17]

Cinamomum Zeylanicum shows effect on blood pressure, lipid and glycemic control, anti oxidant and etc.[18] Hence, it is envisaged to undertake to study effects of hydro-alcoholic extracts of tubers of *Cyperous Rotundus, Terminalia Arjuna* bark and *Cinamomum Zeylanicum* bark on lipid profiles with atherogenic diet fed animal model of obesity in wistar rats

MATERIALS AND METHODS

Plant extract: *Cyperus rotundus* tubers powder was obtained from Dr. shrikar Hiremath,prof. in Sanskrit ayurveda mahavidyalay, Bijapur. *Terminalia arjuna* bark and *Cinnamomum zeylanicum* powder obtained from Amruth kesari depot in mamulpet, Bangalore.

Dose selection: in the present study three doses of the hydro-alcoholic extract of herbal formulation containing ***Cyperous rotundus*** tubers, ***Terminalia arjuna*** bark and ***Cinnamomum zeylanicum*** bark ,were selected one being the lower dose (120mg/kg), mid dose (360mg/kg) and higher dose (480mg/kg).[16] The doses of extracts were administered per oral route in morning and evening through the study period.

Experimental design

Atherogenic diet induced obesity: experimental study was carried out using adult male wister rats weighing between 150-200g.

All experimental animals' were maintained under standard lab conditions. The approval of institutional animal ethics committee (IAEC/NCP/87/14) was taken prior to the commencement of the study.

Composition of the atherogenic diet: 1% cholesterol (s d fine-chem limited), 0.5% cholic acid (Loba Cheme), 5% lard oil (open market).the diets were provided in addition to normal pellet chow.[19]

The parameters studied were: Body weight: the body weight (gm) was recorded on day 1 and then on alternative days during the study period using an electronic balance.

Locomotor activity: the locomotor activity was recorded on day 60 using open field behavior test apparatus and 30 minutes after the administration of hydro- alcoholic extract of poly herbal formulation to the treatment groups. the apparatus consisted of a circular wooden area of 75 cm diameter and wall with height of 25 cm. open field test was performed by placing the rat in the center circle and recording the ambulatory activity, the frequency of rearing and grooming for a 5 min test period.

Organ and fat pad weights: the animals were sacrificed by cervical dislocation and then different fat pads (mesenteric, left and right perirenal and uterine fat pads) were removed and weighed.

Biochemical markers like, LDH, CK-MB, CK-NAC, AST, ALT, Urea, Uric acid, Creatinine were estimated.

RESULTS

Body Weight

Table 1: **Effect of hydro-alcoholic extract of poly herbal formulation of *Cyperus rotundus* on body weight.**

Groups	control	Atherogenic diet	AD + Low Dose (120mg/kg)	AD + Mid dose (360mg/kg)	AD + High dose (480mg/kg)
% increased in body weight	35.487±0.540	89.220±9.297 a ***	78.345 ±1.47 b**	52.846±1.03 b***	41.564±0.949 b***

Results are expressed as mean ± SEM. (n=6)
a = compared with vehicle control.
b = compared with Atherogenic diet.
AD = atherogenic diet
Data was analyzed by One way ANOVA followed by Dunnett's multiple comparison tests. *P<0.05, **P<0.01, ***P<0.001, ns=non significant.
Atherogenic diet group showed a significant (P<0.001) increase in body weight (g)When compared to control group.

Poly herbal extract, low dose level (120mg/kg) showed a decrease in body weight (g)
Less significantly (P<0.01) when compared to atherogenic diet group.

poly herbal formulation Mid dose level (360mg/kg) showed a significant decrease

(P<0.001) in body weight when compared to Atherogenic diet group

Poly herbal formulation High dose level (480mg/kg) showed a significant decrease (P<0.001) in body weight when compared to atherogenic diet.

Biochemical Estimations

Table 2: Effect of poly herbal formulation on biochemical estimation.

Groups	Total cholesterol (mg/dl)	Triglyceride (mg/dl)	HDL (mg/dl)	LDL (mg/dl)
control	139.833 ± 2.272	84.283 ± 2.706	41.283± 0.837	125.616 ± 1.976
Atherogenic diet	168.333 ± 0.760a***	99.1 ± 1.201a***	33.45 ± 1.070a*	156.133 ± 0.872a***
AD+ Low Dose (120mg/kg)	163.5 ± 0.428b***	94.25 ± 0.189b***	37.733± 0.248b**	140.433 ± 1.059b**
AD+ Mid dose (360mg/kg)	155.833 ± 0.477b***	87.866 ± 0.393b ns	40.95 ± 0.279b ns	120.433 ± 0.829b***
AD+ High dose (480mg/kg)	151.666 ± 0.494b***	84.25 ± 0.297b ns	42.483± 0.098b ns	113.05± 0.575b***

Results are expressed as mean ± SEM. (n=6)
a=compared with vehicle control.
b=compared with Atherogenic diet.
AD= atherogenic diet
Data was analyzed by One way ANOVA followed by Dunnett's multiple comparison tests. *P<0.05, **P<0.01, ***P<0.001, ns=non significant.
Atherogenic diet group showed a significant (p<0.001) increase in serum total cholesterol, triglyceride LDL level when compared to control group.

Atherogenic diet group showed a significant (p<0.05) decrease in serum HDL level when compared to control group.
poly herbal formulation extract low dose level (120mg/kg) showed a reduce in serum total cholesterol, triglyceride, LDL significantly and significant increase in HDL when compared to atherogenic diet group (p<0.001)

Poly herbal formulation extract mid dose level (360mg/kg) showed significant decrease in LDL and total cholesterol (p<0.001) and non significant decrease in triglyceride when compared with atherogenic diet.

Poly herbal formulation extract mid dose level (360mg/kg) shows increase in HDL which is non significant when compared with atherogenic diet.

Poly herbal formulation extract, high dose level (480mg/kg) shows significant decrease in LDL and total cholesterol (p<0.001) and non significant decrease in triglyceride when compared with atherogenic diet.
Poly herbal formulation extract high dose level (480mg/kg) showed increase in HDL which is non significant when compared with atherogenic diet.

DISCUSSION

High-fat diet up-regulates either insulin resistance or triglycerides, which is assumed to be related to the expression of peroxisome proliferator-activated receptor (PPAR)-α and PPAR-γ. The beneficial effects of vitamin E on insulin resistance are well known; however, it is not clear if vitamin E with a high-fat diet alters the expression of PPAR-α and PPAR-γ. When their studies investigated the effects of d-α-tocopherol supplementation on insulin sensitivity, blood lipid profiles, lipid peroxidation, and the expression of PPAR-α and PPAR-γ in a high-fat (HF) diet-fed male C57BL/6J model of insulin resistance.

The animals were given a regular diet (CON; 10% fat), a HF diet containing 45% fat, or a HF diet plus d-α-tocopherol (HF-E) for a period of 20 weeks. The results showed that the HF diet induced insulin resistance and altered the lipid profile, specifically the triglyceride (TG) and total cholesterol (TC) levels ($P < 0.05$).

In this animal model, supplementation with d-α-tocopherol improved insulin resistance as well as the serum levels of TG and very-low-density lipoprotein-cholesterol (VLDL-C) ($P < 0.05$). Moreover, the treatment decreased the levels of malondialdehyde (MDA) in the serum and liver while increasing hepatic PPAR-α expression and decreasing PPAR-γ expression. In conclusion, the oral administration of d-α-tocopherol with a high-fat diet had positive effects on insulin resistance, lipid profiles, and oxidative stress through the expression of PPAR-α and PPAR-γ in a high-fat diet-fed male mouse.[20]

A weight loss supplement containing Cissus quadrangularis and other ingredients including, Glycine angustifolia, Avena sativa and Spinacia oleracea was evaluated in a 6-week trial in female Wister rats fed on

cafeteria and atherogenic diets. Polyherbal Formulation (PHF) was prepared and evaluated for physicochemical parameters. Female Wistar rats were fed cafeteria diet (highly palatable, energy rich animal diet that includes a variety of human snack foods) and atherogenic diet for 6-week. Polyherbal formulation was administered in a dose of 400 mg/kg, p.o., once daily to the drug treatment groups.

The effect of Polyherbal formulation was recorded on the parameters like body weight, food and water intake, behavioral activity and various biochemical parameters like serum glucose, total cholesterol and triglyceride levels. Significant reduction in body weight, behavioral activity and serum glucose levels after treatment was observed with Polyherbal formulation in cafeteria diet and atherogenic diet fed rats.

Treatment with Polyherbal formulation also significantly decreased total cholesterol and triglyceride in rats fed with atherogenic diet. Polyherbal formulation had no adverse effect on behavioral parameter. The Polyherbal formulation helped reduce body weight by approx 20-25 % in animal fed on cafeteria and atherogenic diets.[21]

Previous reports on Piper species showed that they are potent free radical scavengers, with strong antioxidant activity against copper induced lower density lipoprotein oxidation, hence inhibiting the formation of foam cells and development of atherosclerosis.[3, 6, 7]

In a case controlled study in rabbits fed on high cholestrol diet and administred *T.arjuna* bark powder 250mg/kg twice daily was carried out recently to determine its hypolipidaemic effect.it was found that the rabbits receiving terminalia arjuna had a marked reduction in total cholestrol than control rabbits.[22]

The administration of *C.rotundus* extract restored the age associated change in serum lipids(total cholestrol, LDL cholestrol, DL cholestrol, triglycerides and VLDL triglyceride level) to the level of young control rats.in young rats treatment of *C.rotundus* significantly increased HDL cholestrol level.[23] In a pilot study carried out on 30 obese people who were administerd the powderd tubers of *C.rotundus* for 90 days, showed reduction in weight along with a decrease in serum cholestrol and triglycerides.[24]

C.zeylanicum bark extracts were found to be potent and consists of cinnamtanin B1 was identified as the potential active compound responsible for these effects.the beneficial effects of CZ *in-vivo* includes : attenuation of weight loss associated with diabetes, reduction of fasting blood glucose, reducing LDL and increasing HDL cholestrol, increasing circulating insulin levels [18]

Obesity has become a leading global health problem owing to its strong association with a high incidence of diseases. Induction of rat obesity carried out using high fat diet (HFD) and to estimate oxidative stress markers in their liver, heart and kidney tissues in order to shed the light on the effect of obesity on these organs. In this research data showed that feeding HFD diet significantly increased final body weight and induced a state of dyslipideamia, a significant increase MDA and PCO levels in the hepatic, heart and renal tissues of obese rats, as well as a significant decrease in the activity of GST, GPx and PON 1 enzymes. On the other hand CAT enzyme activity showed significant decrease only in renal tissues of obese rats with non significant difference in hepatic and heart tissues.

GSH levels showed significant decrease in both renal and hepatic tissues of obese animals and significant increase in their heart tissues. Correlation studies in obese animals showed a negative correlation between MDA and PCO tissue levels and the activities of GPx, GST and PON1 in all tissues and also with CAT enzyme activity in renal tissues. Also a negative correlation was detected between MDA & PCO tissues levels and GSH levels in both hepatic and renal tissues.

While positive correlation was found between them and GSH levels in heart tissues. High fat diet-induced obesity is accompanied by increased hepatic, heart, and renal tissues oxidative stress, which is characterized by reduction in the antioxidant enzymes activities and glutathione levels, that correlate with the increase in MDA and PCO levels in most tissues. This may probably contribute to the additional progression of obesity related problems.[25]

In the present study, the anti obesity and cardio vascular properties of hydro-alcoholic extracts of poly herbal formulation containing *Cyperus rotundus, Terminalia arjuna & Cinnamomum zeylanicum* was studied using atherogenic diet fed animal model of obesity as they have been reported to bear close resemblance to human obesity.

Same inferences were correlated in this present study; the hydro-alcoholic extract has a definite influence in body fat metabolism. It is well known that hyperlipedemia is the leading risk factor for atherosclerosis.

Epidemiological investigation revealed a positive correlation between the severe degree of atherosclerosis and the concentration of plasma cholesterol as well as LDL.[26] The animals treated with hydro-alcoholic extract of poly herbal formulation decrease in the lipid component such as TGs, TC, LDL and a significant increase in the lipid component HDL (table 1 & 2)

The increased blood levels of total cholesterol, LDL, VLDL as well as lowered levels of HDL in atherogenic diet rat have been identified in the development of hypercholestremia, which is one of the risk factors for

CAD [27], Abnormal renal function is mainly associated with diabetic nephropathy.

The pathophysiology involves glucose that binds irreversibly to proteins in the kidney circulation to form advanced glycosylation end products that can form complexes that contribute to renal damage by stimulation of fibrotic growth factors [28] Atherogenic diet induces alteration of renal lipid metabolism by an imbalance between lipogenesis and lipolysis in the kidney, as well as systemic metabolic abnormalities and subsequent renal lipid accumulation leading to renal injury.[29,30,31]

CONCLUTION In the present study hydro-alcoholic extracts of poly herbal formulation containing *Cyperous rotundus*, *Terminalia arjuna*, and *Cinnamomum zeylanicum* ,were very effective in controlling the atherogenic diet fed obesity, hence it is counteracted on lipid profiles and established its anti obesity therapeutic responses

ACKNOWLEDGMENT
The authors are thankful to P.G. Department of Pharmacology, Dr Nargund Research Foundation. Bangalore, India for providing infrastructural facility during this study

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