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Research Article

ANTI-OBESITY AND HYPOGLYCEMIC EFFECT OF ETHANOLIC EXTRACT OF *CROTALARIA JUNCEA* IN HIGH FAT DIET INDUCED HYPERLIPIDEMIC AND HYPERGLYCEMIC RATS

ORUGANTI RAJESH, VENISETTY RAJ KUMAR, PULIGILLA SHANKARAIAH*

Chaitanya College of Pharmacy Education and Research, Warangal, India. Email: drspuligilla@gmail.com

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ABSTRACT

Objective: To study the effect of the ethanolic extract of leaves of *Crotalaria juncea* on lipid profile, body weight and blood glucose levels of high fat induced hyperlipidemic and obese male Albino rats and to compare it with standard Simvastatin.

Method: Ethanolic extract of leaves of *Crotalaria juncea* was prepared from shade dried leaf powder by successive soxhlation. Hyperlipidemia and obesity was induced in male albino Wistar rats by administering high fat diet up to 42 days. On 28th day rats with body weight more than 250 gms were considered for experiment. Ethanolic extract (200 mg/kg and 400 mg/kg) and standard simvastatin (standard) was administered to hyperlipidemic and obese rats. Body weight was recorded on the start day, 29th and 43rd day of experiment and lipid profile and blood glucose levels are estimated and recorded on 29th and 43rd day. Histopathological evaluation was performed on liver tissue after sacrificing the animals on 43rd day.

Results: Treatment with ethanolic extract showed a significant decrease in the cholesterol, triglycerides, LDL, VLDL, blood glucose and body weight levels when compared to control group. On the other hand HDL levels were increased significantly when compared to control group. Histopathological evaluation of liver tissue showed less fatty cytoplasmic vacuoles in ethanolic extract treated group when compared to control group.

Conclusion: From the experimental results it was observed that the ethanolic extract of *Crotalaria juncea* possess antihyperlipidemic and antihyperglycemic activity in a dose dependent manner.

Keywords: Antihyperlipidemic, Cholesterol, *Crotalaria juncea*, Ethanolic extract, Hypoglycemic, Simvastatin

INTRODUCTION

Hyperlipidemia is the condition of abnormally elevated levels of any or all lipids and/or lipoproteins in the blood. Hyperlipidemia comprises of a heterogeneous group of disorders whose characteristic expression is an elevation in the plasma concentration of cholesterol and/or triglyceride. These lipid disorders may occur in familial or nonfamilial form. Presumably, each variety of familial hyperlipidemia could arise from the action of a single gene (monogenic or Mlendelian inheritance) or could reflect the interaction of several genes at many different loci (polygenic inheritance) [1]. Non familial hyperlipidemias are often secondary to such factors as diet, alcohol intake and estrogen therapy or to diseases such as diabetes mellitus, hypothyroidism, or nephrosis [2]. In some cases, neither hereditary nor identifiable environmental factors can be implicated, and such cases are referred as sporadic hyperlipidemia.

Obesity is one of the common chronic disorders of cholesterol and carbohydrate metabolism is characterized by excess fat deposition in adipose tissue, liver, heart, skeletal muscle, and pancreatic islet [3, 4]. It is a major global public health issue and increasing prevalence across all sex, age-groups and ethnicity or race [5]. It is considered to be overweight [i.e. body mass index (BMI)≥25 kg/m2] and morbidly obese (BMI≥30) [6, 7].

Similar trend is continuing in developed and developing countries and it can be attributed to chronic alcoholism, excessive eating, and secondary lifestyle [4]. The prevalence of obesity-associated diseases such as dyslipidemia, cholelithiasis, ischemic heart disease and type 2 diabetes mellitus are well reported [8, 9].

Hyperglycemia is a condition in which serum glucose levels are increased and can be induced by high fat diet [10]. Several secondary complications like are associated with increased serum glucose levels.

Crotalaria juncea is an herb drug which is traditionally used for many diseases, it is very popular known as sunn hemp belongs to the family Leguminosae, subfamily Papilionaceae, which is one of the 550 species of the genus and widely distributed in the tropical and subtropical regions. Sunn hemp is an erect, stiff branched, half-woody herb, usually about one meter high, with fine hair of all the parts.

The species of crotalaria were found to contain steroids, flavonoids, saponins, phenols, glycosides and triterpenoides linoleic acid (62.36%) and other compounds which are of interest include monocrotaline, riddelline, seneciphylline, senecionine, trichodesmine, chodesmine, galactose specific lectin and cardiogenin 3-O- [OH]-d-xylopyranosid [11].

Crotalaria juncea seeds were traditionally used for the nutritional and medicinal important such as a blood purifier, psoriasis, astringent, demulcent, abortificient, emetic, purgative and in the treatment of anaemia, impetigo and menorrhagia.

Literature survey reveals that seeds of *Crotalaria juncea* were found to possess antispermatogenic, anti-ovulatory and contraceptive activities, seed oil possess antioxidant, anti-inflammatory and antibacterial activities, leaves were found to possess anti-inflammatory, anti-ulcerogenic activities and the aerial parts with antifungal activity [12-16].

In the absence of reliable antihyperlipidemic drugs in the modern medical scenario, there are numbers of medicinal plant preparations in the ayurvedic system of Indian medicine recommended for the treatment of dyslipidemic disorders.

They are in usage since centuries and are offering significant relief. But there is no scientific information available regarding the antihyperlipidemic effect of *Crotalaria juncea* leaf extract. Since, antioxidants are known to reduce the development of chemically induced hyperlipidemia, the effect of ethanolic extract of leaves of *Crotolaria juncea* (CJLE) has been evaluated and assayed for antihyperlipidemic and antihyperglycemic activities against high fat diet induced hyperlipidemic and hyperglycemic rats using simvastatin as standard drug.

MATERIALS AND METHODS

Collection of plant material

Fresh leaves of *Crotalaria juncea* will be obtained from local area of Warangal district of Andhra Pradesh and were authenticated by Dr. Md.Mustafa, Deparment of botany, Kakatiya University, Warangal.

Preparation of plant extract

Ethanolic extract of leaves of *crotalaria juncea* was prepared by successive soxhlation. The shade dried leaf powder was packed in thimble kept in the soxhlet apparatus and extraction was allowed to run successively. The extraction was carried out until the extract becomes colourless. Later, the solvent was evaporated from extract by distillation under reduced pressure and the final extract was dissolved in water

Preparation of high fat diet for 100ml of liquid diet

Dissolve 6 gms of fructose with 1.25 ml of tween 80 in 50 ml of distilled water and the solution was marked as A; Dissolve 0.4 gms of cholesterol in 50 ml of lard oil and it was marked as B. The two solutions A and B were mixed, emulsified and was administered at a dose of 10 ml/ kg [17].

Experimental Animals

Male Wistar albino rats, aged 4 months (body weight: 180-200 g) were used for the present study, procured from Sainath Agencies, Hyderabad, India. The animals were housed in acrylic poly cages (38 cm × 23 cm × 10 cm) with not more than six animals per cage, at ambient temperature of 18± 2^0C with 12-h-light/12-h-dark cycle. Rats have free access to standard chow diet and water ad libitum. The maintenance and the handling of animals were performed according to the rules and regulations of Institutional Animal Ethical Committee. (Approval No. 1105/SRRCOPSc/KNR/IAEC/2012, Dated 05.06.2012)

Acute Toxicity Studies

Acute toxicity studies were carried out following OECD guidelines and the extract was found to be safe up to 2000 mg/kg body weight in albino Wistar rats.

Experimental design

Male Albino Wistar rats weighing between 180±10gms were used for the experiment and were allowed to acclimatize for a week. Six rats were formed into one group; five such different groups of rats were formed and labeled (Group – I: Normal control (Tween 80 suspension, p.o.), Group – II: High fat diet control (10 ml/kg p.o.), Group – III: High fat diet (10 ml/kg) + leaf extract (200 mg/kg p.o), Group- IV: High fat diet (10 ml/kg) + leaf extract (400 mg/kg p.o) and Group – V: High fat diet (10 ml/kg) + Simvastatin(20 mg/kg, p.o). All the groups of animals were fed with high fat diet for 43 days (Group II-V) except normal control. Rats weighing above 250 gm and glucose levels of 150 mg/dL on 29th day were considered for experiment in group II- V. Treatment with CJLE and simvastatin was given from 29th day to 43rd day in groups III-V as prescribed. Body weight of all the rats were recorded on the start day, 28th day before treatment and 43rd day of experiment. Blood samples were collected from all the experimental animals and biochemical parameters like cholesterol, triglycerides, High density lipoproteins (HDL), Low density lipoproteins (LDL), Very low density lipoproteins (VLDL) and blood glucose levels were estimated on 29th day before treatment and on 43rd day using semi autoanalyser (Biochemical systems international) and the corresponding diagnostic kits (Crest biosystems, Tulip group).

Histopathological evaluation of liver tissue was performed after sacrificing the animals by cervical dislocation on 43rd day.

Statistical analysis of the results obtained was performed using ANNOVA followed by Dunnett's-test. All the results were expressed as mean±SEM and a probability of *p<0.05, **P<0.01 were considered as significant.

RESULTS

Ethanolic extract of *Crotalaria juncea* was found to be safe up to 2000 mg/kg body weight in albino Wistar rats and the dosage selection of 200 mg/kg and 400 mg/kg was done based on acute toxity studies as per OECD guidelines.

Administration of high fat diet in rats shows significant increase in the body weight on 29th and 43rd day when compared to control group (Table 1). Significant increase in the levels of cholesterol, triglycerides, LDL, VLDL and glucose was observed in all groups when compared to group I on day 29 prior to the treatment with ethanolic extract and standard simvastatin (Table 2). There was a significant decrease in HDL levels when compared to normal (group I) on 29th day prior to the treatment with ethanolic extract and standard simvastatin (Table 2).

Treatment with ethanolic extract (200 mg/kg p.o and 400 mg/kg p.o) and simvastatin (20mg/kg, p.o) significantly reduced the cholesterol, triglycerides, LDL, VLDL and glucose levels in the serum on day 43 when compared to the obesity control group (group II) (Table 3). There was a significant increase in HDL levels when compared to the obesity control group on 43rd day after treatment with ethanolic extract and standard (Table 3).

Histopathology study on liver tissue revealed that high fat diet fed rats showed more cytoplasmic vacuolated cells as compared to normal control. Treatment with ethanolic extract of Crotalaria juncea showed less fatty cytoplasmic vacuoles when compared to high fat diet fed rats (group II) (Figure 1).

Table 1: Body weight of different groups of rats

S. No.	Group	0 Day	29th Day	43rd Day
1	Group 1	186.17±5.15	243.17±4.87	254.32±4.1
2	Group II	183.33±4.27	275±4.85	317.67±7.08
3	Group III	187.17±4.26	289.68±5.44	285.5±6.15 *
4	Group IV	186±6	286.5±4.59	271.4±2.82 **
5	Group V	186.33±4.22	287.33±3.2	267.17±2.4 **

Data is expressed as mean±SD (n=6). *p<0.05 and **p<0.01 when obesity control (Group II) is compared with different treatment groups. **Group-I**: Normal control, **Group-II**: Obesity control (High fat diet (10 ml/kg)), **Group-III**: High fat diet (10 ml/kg) + leaf extract (200mg/kg p.o), **Group-IV**: High fat diet (10 ml/kg) + leaf extract (400mg/kg p.o) and **Group-V**: High fat diet (10 ml/kg) + Simvastatin (20mg/kg, p.o)

Table 2: Various biochemical parameters in different animal groups on 29th day (Before treatment)

Parameters	Animal Groups				
	Group- I	Group- II	Group- III	Group- IV	Group- V
Cholesterol (mg/dL)	80.21±2.01	293.18±3.29	292.83±2.88	297.48±4.16	298.15±4.5
Triglycerides (mg/dL)	77.57±3.02	177.01±4.37	177.82±4.83	180.14±2.67	178.41±2.18
HDL (mg/dL)	38.88±1.13	21.07±1.65	19.97±1.18	20.31±1.34	20.05±1.24
LDL (mg/dL)	25.82±1.88	236.7±2.89	237.29±2.4	241.15±4.87	242.42±5.01
VLDL (mg/dL)	15.51±0.6	35.47±0.87	35.56±0.96	36.03±0.53	35.68±0.43
Blood Glucose (mg/dL)	89.25±2.12	155.75±3.6	156.1±2.38	155.28±4.54	157.35±3.27

Data is expressed as mean±SD (n=6).

Group-I: Normal control, **Group-II**: Obesity control (High fat diet (10 ml/kg)), **Group-III**: High fat diet (10 ml/kg) + leaf extract (200mg/kg p.o), **Group-IV**: High fat diet (10 ml/kg) + leaf extract (400mg/kg p.o) and **Group-V**: High fat diet (10 ml/kg) + Simvastatin (20mg/kg, p.o)

Table 3: Various biochemical parameters in different animal groups on 43rd day (After treatment)

Parameters	Animal Groups				
	Group- I	Group- II	Group- III	Group- IV	Group- V
Cholesterol (mg/dL)	81.2±1.54	304.42±6.19	260.06±3.43 *	236.8±4.24 **	214.48±2.39 **
Triglycerides (mg/dL)	77.76±2.53	188.19±5.35	162.86±4.47 *	122.58±3.67 **	115.96±3.5 **
HDL (mg/dL)	39.9±1.64	18.53±0.84	28.36±1.77 *	31.43±2.58 **	37.3±2.07 **
LDL (mg/dL)	25.75±1.88	248.25±6.01	199.13±4.68 *	180.16±4.15 **	153.98±2.87 **
VLDL (mg/dL)	15.55±0.5	37.64±1.07	32.57±0.89 *	24.52±0.73 **	23.19±0.7 **
Blood Glucose (mg/dL)	89.67±2.1	177.53±3.84	142.54±2.59 *	130.82±2.35 **	122.89±3.68 **

Data is expressed as mean±SD (n=6). *p<0.05 and **p<0.01 when obesity control (Group II) is compared with different treatment groups. **Group-I**: Normal control, **Group-II**: Obesity control (High fat diet (10 ml/kg)), **Group-III**: High fat diet (10 ml/kg) + leaf extract (200mg/kg p.o), **Group-IV**: High fat diet (10 ml/kg) + leaf extract (400mg/kg p.o) and **Group-V**: High fat diet (10 ml/kg) + Simvastatin (20mg/kg, p.o)

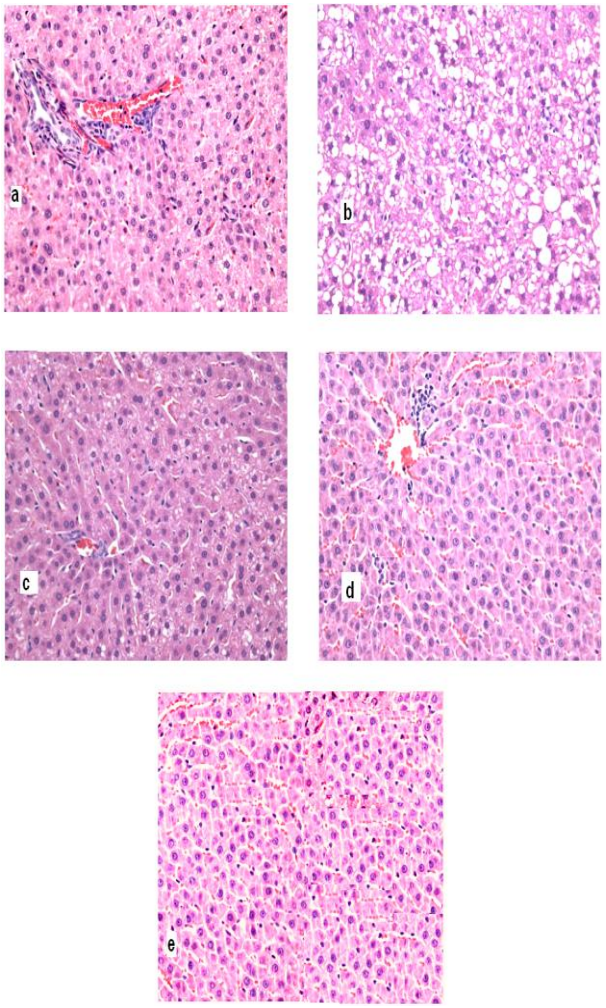

Fig. 1: Histopathological evaluation of liver tissue in different groups of animals

a: Normal control, **b**: Obesity control (High fat diet (10 ml/kg)), **c**: High fat diet (10 ml/kg) + leaf extract (200mg/kg p.o), **d**: High fat diet (10 ml/kg) + leaf extract (400mg/kg p.o) and **e**: High fat diet (10 ml/kg) + Simvastatin (20mg/kg, p.o).

DISCUSSION

The results of the present study reveals that treatment with ethanolic leaf extract of *Crotalaria juncea* (200 mg/kg and 400 mg/kg p.o) significantly reduced cholesterol, triglycerides, LDL, VLDL and glucose levels and increased HDL levels of serum in high fat diet induced hyperlipidemic and hyperglycemic albino Wistar rats in a dose dependant manner. High fat diet acts as a source of saturated fat resulting in increase in body weight, increase in blood lipid concentration and increase in blood glucose levels [10, 18, 19]. Dietary cholesterol is used in composition of high fat diet, to induce obesity. Dietary cholesterol causes increase in serum total cholesterol, VLDL, and decrease in HDL [20]. CJLE decreased serum cholesterol, VLDL and increased HDL levels, this may be attributed to the fact that Crotalaria sps. were found to contain saponins and flavonoids [11, 21]. Plant containing flavonoids have the antioxidant, antidyslipidemic and antidiabetic activity and possess appetite-suppressive properties. Reports suggest that several active constituents like glycosides, saponins and flavonoids possess appetite-suppressive capabilities [22]. The activity of CJLE over hyperlipidemia may due to presence of saponins because they acts as bile acid sequestrants so, administration of *crotolaria juncea* leaf extracts significantly reduce both fat deposition and serum cholesterol, VLDL and increased serum HDL levels when compare to obesity induced normal group. Obesity results in increased level of oxidized LDL. LDL is found to cause endothelial damage, oxidative stress and inflammation, which further aggravates obesity. CJLE significantly decreased LDL levels because of the presence of flavanoids. Mechanistic studies have shown that flavonoids lower LDL levels by inhibiting the synthesis of both apolipoprotein B and triglycerides [23]. Release of free fatty acids by lipoprotein lipase from increased serum triglycerides cause lipotoxicity which results in insulin-receptor dysfunction. Free fatty acids also produce oxidative stress. The release of excessive free fatty acids provokes lipotoxicity, as lipids and their metabolites create oxidative stress. This affects adipose as well as nonadipose tissue, accounting for its pathophysiology in many organs, such as the liver and pancreas, and resulting in the metabolic syndrome. CJLE significantly decreased the triglyceride level by inhibhiting the synthesis of apolipoprotein B and triglycerides because of the presence of flavanoids thereby, reduces the free fatty acids production [23]. Histopathological study confirmed the biological changes and found that liver tissue showed less fatty cytoplasmic vacoules in ethanolic extract and simvastatin treated groups when compared to control group. The present findings also revealed that CJLE prevents body weight gain in high fat diet fed rats by preventing the absorption of high fat diet due to the presence of saponins.

CONCLUSION

By the above findings it can be concluded that CJLE can be useful in the treatment of hyperlipidemia and hyperglycemia because of the presence of flavanoids and saponins.

Conflict of interest

Authors have no conflict of interest

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