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Evaluation of anti-diabetic, anti-hyperlipidemic and anti-oxidant properties of hydro-alcoholic extract of *Euphorbia cotinifolia*

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Abstract
Indian people have 77 million belong to India. Diabetes describes a group of metabolic diseases characterized by high blood sugar levels and has hyperlipidemia condition. The present study was carried out to investigate the antidiabetic, antihyperlipidemic and antioxidant activity properties of hydroalcoholic extract of *Euphorbia cotinifolia* stem. Streptozotocin induced diabetic Wister rats were used in this study consisting of five groups of five animals each. Groups (1) normal control, (2) diabetic control, (3) HEEC 300mg/kg b.w, (4) HEEC 600mg/kg b.w (5) Glibenclamide respectively. Fasting blood sugar was recorded on 1st, 7th, 14th, 21st and 28th day. At the end of the experiment lipid profile and levels of antioxidants were determined. Safety profile of both extracts was evaluated using acute and chronic toxicity studies in streptozotocin-induced diabetic male rats. Higher dose of HEEC and glibenclamide significantly lowered blood glucose level from 7th day onwards however glibenclamide was found to be more effective but lower dose not show significant decrease blood glucose on same day. HEEC both dose and standard show significant decrease glucose remain days. Glibenclamide and both dose of stem HEEC extract significantly reduced the total cholesterol (Including HDL and LDL), triglyceride levels and significantly increased the high-density lipoprotein cholesterol level. HEEC possesses significant anti-diabetic, hypolipedimic and antioxidant properties. This study supports the traditional use of *Euphorbia cotinifolia* stem in diabetes.

Keywords: Antidiabetic, antihyperlipidemic, *Euphorbia cotinifolia,* glibenclamide, antioxidant

Introduction
Diabetes is a chronic disease that occurs when the pancreas does not produce enough insulin (a hormone that regulates blood sugar) or alternatively, when the body cannot effectively use the insulin it produces. The overall risk of dying among people with diabetes is at least double the risk of their peers without diabetes. Diabetes mellitus is a group of metabolic diseases characterized by hyperglycaemia resulting from defects in insulin secretion, insulin action, or both. The chronic hyperglycaemia of diabetes is associated with long-term damage, dysfunction, and failure of various organs, especially the eyes, kidneys, nerves, heart, and blood vessels. Several pathogenic processes are involved in the development of diabetes. These range from autoimmune destruction of the cells of the pancreas with consequent insulin deficiency to abnormalities that result in resistance to insulin action. The basis of the abnormalities in carbohydrate, fat, and protein metabolism in diabetes is deficient action of insulin on target tissues. Deficient insulin action results from inadequate insulin secretion and/or diminished tissue responses to insulin at one or more points in the complex pathways of hormone action. Impairment of insulin secretion and defects in insulin action frequently coexisting the same patient, and it is often unclear which abnormality, if either alone, is the primary cause of the hyperglycaemia [1]. Hyperlipidemia is common in diabetic patients. While our understanding of lipid and lipoprotein metabolism in diabetes is incomplete, a pathophysiologic approach to this problem is presented. It is based on the recognition that diabetes is metabolically heterogeneous. Thus, the roles of insulin deficiency, insulin resistance, obesity, and genetic factors are discussed in relation to their effects on lipoprotein production and catabolism. The most important defect in insulin-deficient subjects appears to be a deficiency of lipoprotein lipase, which is responsible for the removal of the triglyceride-rich lipoproteins. In non-insulin-dependent subjects there is evidence for a removal defect as well as, in some patients, for overproduction of VLDL-triglyceride. Cholesterol levels may be elevated and it is important to distinguish between VLDL, LDL, and HDL as the causes for these increases. HDL-cholesterol levels may be increased in insulin-dependent subjects,

whereas they may be decreased in obese non-insulin-dependent patients. Mild elevations of LDL-cholesterol may occur in inadequately controlled type I and II diabetic patients, while elevated VLDL may raise the serum cholesterol in addition to the triglyceride levels. The rationale for therapy is based on the complications of severe hypertriglyceridemia and the risk of occlusive atherosclerosis. Management is directed at improving glycemic control, altering dietary composition, and reducing calories in obese patients. Improved glycemic control is effective in reducing triglyceride and cholesterol levels in insulin-deficient subjects. The response of the non-insulin-dependent diabetic patient to improved control may be complicated by associated obesity or familial hyperlipidaemia. The advantages and disadvantages of fat versus carbohydrate restriction in the diet are discussed. Finally, resistant hyperlipidaemia may require drug therapy. Diabetic hyperlipidaemia should be viewed as resulting from an interaction between the diabetic syndrome, the genetic background of the patient, and the environment [2]. High level of glucose in blood is known to produce free radicals or reactive oxygen species (ROS) which react with lipids, initiating their peroxidation leading to abnormalities in lipid metabolism and dyslipidaemia in diabetes mellitus which followed by atherosclerosis. Thus, oxidative load lip toxicity as well as hyperglycaemia-mediated glucotoxicity in diabetic dyslipidaemia may causes complications in diabetes [3]. Medicinal plants are a rich source of bioactive phytochemicals which have the potential for preventing chronic disease such as cancer, Alzheimer, diabetes, cardiovascular disease etc. Several phytochemicals including alkaloids, flavonoids, glycosides, glycolipid, polysaccharides, peptidoglycans, carbohydrates, amino acids and saponin obtained from plant sources have been reported to possess antioxidant, anti-inflammatory, hepatoprotective, antidiabetic, antidyslipidemic activities [4]. *Euphorbia cotinifolia* is Caribbean copper plant which has antibacterial and dipeptidyl peptidase-IV inhibitory is reported previously. *Euphorbia cotinifolia* compounds such asjatrophane, ingol, myrsinane diterpenoids, leucocyanidol, quercitol, camphol, quercetrin, dihydroellagitannins and dimeric hydrolysable tannins (euphorbins).

Materials and methods

Plant material

The plant *Euphorbia cotinifolia* stem was collected from garden of Dr A.P.J. Abdul Kalam University, Indore and was authenticated by Dr. Karunakar Shukla, Professor & Head, Department of Pharmacognosy, College of Pharmacy, Dr. APJ Abdul Kalam University Indore M.P. and Voucher specimen No. APJAKU/COP/2019/041 was obtained.

Chemicals and reagents

2,2-diphenyl-1-picrylhydrazyl (DPPH), ethylene diamine tetra acetic acid (EDTA) and 2, 4-dinitrophenyhydrazine (DNPH), streptozotocin (STZ), citral were purchased from Sigma-Aldrich, Chemicals Pvt. Ltd., India. All other chemicals used in this study were obtained from SRL Pvt. Ltd. (Mumbai, India), Hi Media Laboratories Pvt. Ltd. (Mumbai, India), SD Fine-Chem. Ltd. (Mumbai, India). All other chemicals and solvents used were of analytical grade available commercially.

Animals

Male albino Wistar rats weighing 200-250 gm were used in this study. All rats were kept at room temperature of 20^0C in the central Animal house of the Pinnacle Biomedical research Institute (PBRI), Bhopal. They were maintained on Standard diet-pellets (Ashirvad Industries, Chandigarh) and water *ad libitum*. All experiments were performed as per the directives of the institutional Animal Ethics Committee (PBRI/IAEC/PN-19025b).

Extraction process

The powder form of stem of *Euphorbia cotinifolia* defatted by petroleum ether to remove fatty or fatty acid content at room temperature. When the solvent become concentrated, the petroleum ether content were filtered through muslin cloths after the defatting process residue again macerate with hydro-alcoholic (ethanol) solvent in equal ratio (1:1) or 50% both at room temperature. The bottle was kept at room temperature and allowed to stand for several 8-11 days with occasional shaking and stirring. When the solvent become concentrated, the liquid hydroalcoholic contents were filtered through cotton and then through filter paper (Whatman Fitter Paper No. 1). Finally, a highly concentrated hydroalcoholic extract was of *Euphorbia cotinifolia* (HEEC) obtained [5].

Phytochemical investigation

Preliminary photochemical screening was carried out for qualitative identification of phytochemical constituents employing standard methods. Chemicals and reagents used were of analytical grade. All extracts were subjected to preliminary phytochemical screening to identify the various phyto-constituents present in them i.e. steroids, triterpenoids, flavonoids, carbohydrates, alkaloids, terpenoids, glycosides, saponins and tannins [6].

Acute toxicity study and dose selection

The acute toxic class method set out in this Guideline is a stepwise procedure with the use of 3 animals of a single sex per step. Depending on the mortality and/or the moribund status of the animals, on average 2-4 steps may be necessary to allow judgement on the acute toxicity of the test substance. The substance is administered orally to a group of experimental animals at one of the defined doses. The substance is tested using a stepwise procedure, each step using three animals of a single sex (normally females). Absence or presence of compound-related mortality of the animals dosed at one step will determine the next step, i.e.; no further testing is needed, dosing of three additional animals, with the same dose and, dosing of three additional animals at the next higher or the next lower dose level. Three animals are used for each step. The dose level to be used as the starting dose is selected from one of four fixed levels, 5, 50, 300 and 2000 mg/kg body weight [7].

Streptozotocin induced antidiabetic activity

Before the induction the all animal or rats were fasted overnight before with streptozotocin (STZ). A freshly prepared solution of STZ (45 mg/kg) in 0.1-M cold citrate buffer (pH 4.5) was injected intraperitoneally in a volume of 1 ml/kg, and the control rats were injected with citrate buffer alone. Hyperglycaemia was confirmed by the elevated fasting glucose levels in blood determined at 48 h and then on day 6 after injection. Rats with moderate diabetes exhibiting fasting blood glucose levels in the range of 260-325 mg/100 ml were selected for the studies. All extracts/drugs dissolved in distilled water were administered once orally/day in the morning between 9 and 10 am for 28 days. In all groups

fasting blood glucose (FBG) levels were recorded on 1st (First time recording FBS levels), 7th, 14th, 21st and 28th day. FBS levels were taken after overnight fasting. Body weights of animals were also recorded on 1st, 14 th and 28th day in all groups. Overnight fasted rats were divided into five groups of six each. The animals were treated (HEEC) orally once daily for 28 consecutive days as follows [8, 9].

Experimental designs

Groups 1; Normal or Untreated animal
Groups 2; Diabetic control rats
Groups 3; Hydroalcoholic extract of *Euphorbia cotinifolia* (HEEC) 300mg/kg
Groups 4; Hydroalcoholic extract of *Euphorbia cotinifolia* (HEEC) 600mg/kg
Groups 5; Standard Glibenclamide600 mg/kg of body weight (IP)

Serum lipid profile analysis

In order to detect the serum biochemical parameters like total cholesterol, low density lipoprotein (LDL), high density lipoprotein (HDL),triglycerides, etc. the blood of the overnight fasted rats were collectedon28th day [10].

DPPH radical scavenging activity

The DPPH radical (Hi-media) is stable due to the delocalization of a spare electron over the molecule, thus preventing dimer formation. Prepared 0.1mM DPPH solution (4mg/100ml) in methanol. This radical is used in the DPPH radical scavenging capacity assay to quantify the ability of antioxidants to quench the DPPH radical. The dark purple colour of DPPH will be lost when it is reduced to its nonradical form stable organic nitrogen cantered free radical with a dark purple colour which when reduced to its nonradical form by antioxidants becomes colourless. DPPH radicals are widely used in the model system to investigate the scavenging activities of several natural compounds. When the DPPH radical is scavenged, the colour of the reaction mixture changes from purple to yellow with decreasing of absorbance at wavelength 517 nm. 200 mg of sample was taken in centrifuge tube (in triplicate). Two hundred microliter distilled water was taken in blank instead of the sample. Then 1 ml of DPPH (8 mg/100 ml of ethanol) solution was added to the sample and the blank. This setup was left at room temperature for 30 minutes (vertexed in between). Tubes were then centrifuged at 4000 rpm for 10 min. After that, 0.5 ml supernatant was poured in fresh tubes containing 1 ml of ethanol and the absorbance was taken at 517 nm against the ethanol by using UV-1800 spectrophotometer (Shimadzu, Japan). Each crude extract was analysed in triplicate. The percentage of inhibition was calculated against blank:

$$I\% = \frac{A_{blank} - A_{sample}}{A_{blank}} \times 100$$

Where I is the absorbance of the control reaction (containing all reagents except the test compound) and is the absorbance of the test compound [11, 12].

Statistical analysis

Data was analysed using Sigma stat 3.5 version statistical software. Results are expressed as mean ± standard deviation statistical analysis was performed using analysis of variance followed by post-hoc test (Bonferroni). $P < 0.05$ was considered as statistically significant.

Results

The result of qualitative phytochemical analysis of the crude powder flower of *P. indica* was shown in Table 1. Hydroalcoholic extract of stem of *Euphorbia cotinifolia* sample showed the presence of flavonoids, terpenoids, tannin, phenol, and steroid. Acute toxicity was performed on albino rats using OECD 423 guidelines. Oral administration of test extract of hydro-alcoholic extract of *Euphorbia cotinifolia* in rats did not produce any signs of toxicity and mortality up to 2000 mg/kg. These findings indicated that extract is safe and can be used even for prolong duration, based on toxicity data the selected dose 300 and 600 mg for the further studies. The effect of intraperitoneal injection of 600mg/kg b.w of Glibenclamide on blood glucose levels in normal and STZ-induced diabetic rats are shown in Table 2 on 0^{th} to 7^{th} days day there is no statistically significant response between group 2 and HEEC treated group where as standard Glibenclamide show significant response's(P = <0.001) and this is due to short time dosing response but after 14^{th} to 28^{th} day HEEC 300 and 600 mg show significant decrease blood glucose level in Streptozotocin induce blood glucose level (P = <0.001) whole study standard group is marked decreases glucose level [13]. This study showed an important rise in lipid profile parameters (TCH, TG, and LDL) induced by high leave of blood glucose insignificantly (p <0.001) decrease by HEEC treatment group (300 and 600) and standard (Glibenclamide600 mg/kg) group, moreover HDL also significantly (p <0.001) increase with compare to control group table 3. The antioxidant activity of HEEC was assessed by in vitro DPPH free radical scavenging assay. The capability of HEEC on reducing production of DPPH radicals at all concentrations are shown in table 4. Ascorbic acid, used as standard was highly effective in inhibiting the DPPH free radicals, showing an IC50, 11.79µg/ml where as HEEC exhibited IC50, 79.44µg/ml**.** The antioxidant activity of GAE was assessed by in vitro DPPH free radical scavenging assay. The capability of GAE on reducing production of DPPH radicals at all concentrations are shown in Fig. 1. Ascorbic acid used as standard was highly effective in inhibiting the DPPH free radicals, showing an IC50, 12.50µg/ml where as GAE exhibited IC 50, 76.45µg/ml.

Table 1: Phytochemical characterization of *Euphorbia cotinifolia* extract

S. No.	Phytoconstituents	HEEC Extract
1	Alkaloids	-
2	Flavonoids	+
3	Terpenoids	+
4	Tannins	+
5	Steroids	+
6	Carbohydrates	-
7	Proteins	-
8	Saponins	-
9	Phenols	+

+: Present, −: Absent

Table 2: Streptozotocin induced antidiabetic activity

Group	Name	0 day (mg/ml)	After 7days (mg/ml)	After 14days (mg/ml)	After 21days (mg/ml)	After 28days (mg/ml)
I	Normal or Untreated animal	70.64± 4.03	79.52± 1.75	85.19± 3.29	90.42± 3.12	84.91± 2.31
II	Diabetic control rats	240.20±3.65	221.77± 4.21	201.01± 2.95	196.93± 6.69	189.01± 2.39
III	(HEEC) 300 mg/kg	238.78±5.62	198.56± 2.87	187.03± 3.32	150.28± 3.22*a	121.04± 2.55*a
IV	(HEEC) 600 mg/kg	228.18±4.72	180.22± 3.88	152.96± 2.98*a	100.42± 2.60	95.24± 2.99
V	Standard (Glibenclamide 600 mg/kg)	180.74±3.78*a	90.88± 1.78*a	84.74± 3.12*a	82.20± 2.87*a	75.08± 3.26*a

Values are mean ± SD, n=5 One way ANOVA followed by Bonferroni multiple comparison tests. a is compared to group 2 which is a diabetic control group, *p <0.001; there is a statistically significant difference (P = <0.001).

Table 3: Serum lipid profile analysis

Group	Name	Total cholesterol	LDL	Triglycerides	HDL
I	Normal or Untreated animal	121.46±3.10	41.27±2.26	77.10±3.46	60.11±2.05
II	Diabetic control rats	190.91±3.06	90.15±3.06	120.13±2.93	30.45±2.86
III	(HEEC) 300 mg/kg	149.95±2.95*a	65.45±3.44*a	90.77±2.94*a	45.23±2.70*a
IV	(HEEC) 600 mg/kg	137.29±2.63*a	56.27±2.32*a	87.67±1.28*a	55.21±2.67*a
V	Standard (Glibenclamide600 mg/kg))	125.82±3.66*a	45.02±2.24*a	82.12±2.92*a	69.09±2.69*a

Values are mean ± SD, n=5 One way ANOVA followed by Bonferroni multiple comparison tests. a is compared to group 2 which is a diabetic control group, *p <0.001; there is a statistically significant difference (P = <0.001).

Table 4: DPPH radical scavenging activity

Conc.	% inhibition (HEEC)	% inhibition (AA)
20	25.49±1.3	52.28±1.4
40	37.76±1.7	58.54±4.6
60	51.53±1.4	69.95±1.3
80	59.71±1.2	71.85±1.4
100	69.59±1.5	78.24±1.2

Values are expressed as the means ± SD of three independent assays (n=5)

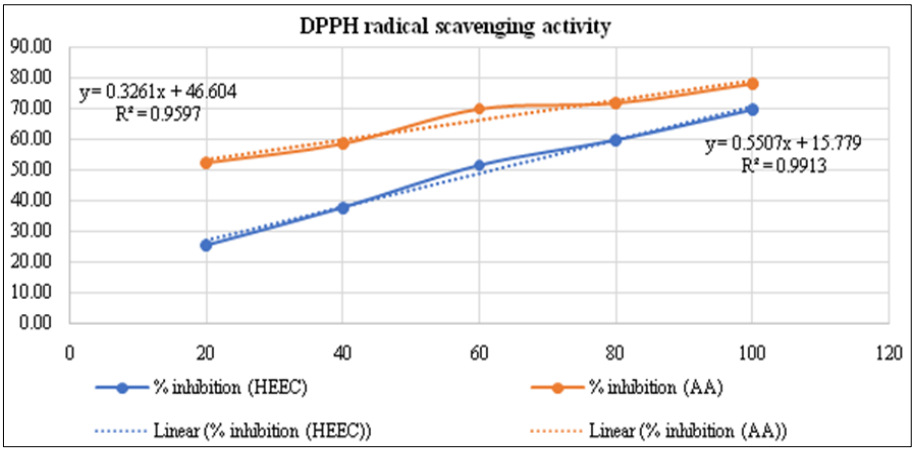

Fig 1: DPPH radical scavenging activity

Discussion

In the recent days, many researchers and investigators tested various traditional medicinal plants for their potential anti-diabetic effect in experimental animals diabetes, a disorder of glucose metabolism, is often associated with hyperlipidemia. The conventional hypoglycaemic drugs like glipizide, metformin and other cannot benefit high cholesterols and triglyceride level. This warrants co-administration of lipid lowering agents, for example, statins which have many limiting adverse effects. Preliminary phytochemical investigations of the hydroalcoholic extracts of Stem of HEEC revealed the presence of flavonoids, phenolic compounds, steroids, tannin. Products of herbal origin like HEEC extract have been shown to possess both anti-diabetic as well as anti-hyperlipidemic properties in earlier studies. HEEC also have antioxidant activity at higher concentration. Antioxidant activity is important for scavenge of free radical which is responsible born various kind of disease like diabetes, atherosclerosis. The result indicates that HEEC extract act as electron donors and could react with free radicals to convert them into more stable products and then terminate the free radical chain reactions. During the study, it was found that antioxidant activity was produced due to the presence of phenolic and flavonols [14]. Compounds in the present study, we investigated HEEC hydro-alcoholic extract further for its potential antidiabetic and antihyperlipidemic effects in experimental models. Besides, attempts were made to assess, if HEEC extract treatment could have synergistic effect with standard oral hypoglycaemic and lipid lowering agents like glipizide and atorvastatin, respectively. The HEEC extract and alone Glibenclamide showed a greater glucose-lowering potential. And, this trend was gradually more pronounced with longer duration of treatment namely 2, 3, or 4 weeks. However, when comparison was done between the findings of Groups 3 and 4 it was evident that group is comparable slightly less remove glucose effect from blood.

Glibenclamide is potent blood glucose lowering activity at a higher dose level (600 mg/kg) and have significantly show both anti diabetics and antihyperlipidemic activity. Group 4, 600 mg of HECC extract also have both antidiabetic and lipid lowering activity long term dosing when compare to control group 2, Due to antioxidant property of HEEC extract improving high density cholesterol level. In general, it has been observed that hyperlipidemia is a complication associated with hyperglycemias. During the study, it was observed an increase in total cholesterol, TGL, LDL, and decrease in HDL in STZ induced diabetic rats as compared to normal animals. The HEEC 300 and 600mg/kg showed a significant reduction in total cholesterol, LDL, and TGL and a significant rise in HDL when compared with diabetic control group. The potent antidiabetic effect of the plant extract suggests the presence of potent antidiabetic active principles, which produced antihyperglycemic effect indiabetic rats. The outcomes of lipid profile confirmed the potent antidiabetic activity and lipid lowering property of the drugs.

Conclusion

Based on the evaluation of antidiabetic, antihyperlipidemic and antioxidant properties of hydroalcoholic extract of *Euphorbia cotinifolia* stem show have all three properties as antioxidant, antidiabetic and antihyperlipidemic drugs. The main rational cause of all three activities is that extract have found phenol and flavonols as biochemical agents. further study is need to separate out and isolate the HEEC extract phyto-compound and determine that which contain is mainly have potent antidiabetic, antihyperlipidemic and antioxidant properties

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