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PHARMACOGNOSTICAL AND PHARMACOLOGICAL EVALUATION OF THE POLYHERBAL EXTRACT ON RODENTS

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ABSTRACT

Hyperlipedemic is the greatest risk factor for coronary heart diseases. It is characterized by elevated serum total cholesterol, low density lipoprotein, very low density lipoprotein and decreased high density lipoprotein levels. Methanolic extract of *Terminalia arjuna* (bark), *Phyllanthus emblica* (fruits), *Withania somnifera* (leaves), *Convolvulus pluricaulis* (whole plant), *Piper betle* (leaves), *Allium sativum* (bulb), *Piper longum* (dry fruits), *Zingiber officinale* (rhizomes), *Tribulus terrestris* (whole plant) and *Cardamom* (dry fruits) these herbs were tested against high cholesterol diet induced hyperlipedemic in adult albino rats. The therapeutic dose is calculated as 200mg/kg as per the toxicity guidelines OECD 423. Fenofibrate 65mg/kg is used as a standard drug. The methanolic extracts shows a significant decrease in the levels of serum cholesterol, Triglycerides, LDL, VLDL and significant increase in the level of serum HDL against high cholesterol diet induced hyperlipedemic rats. The results shows that the polyherbal extract possess significant ($p<0.001$) antihyperlipedemic activity suggesting the potential role in coronary artery disease and in hyperlipidemia.

INTRODUCTION

Herbal medicine is the oldest form of healthcare known to mankind. Herbs had been used by all cultures throughout history. Many drugs commonly used today are of herbal origin. Herbal medicinal products are defined as any medicinal product exclusively containing one or more active substances[1]. Many such practices were experimentally proved depicting the scientific insight behind their traditional adoption. *Terminalia arjuna*[2] (bark), *Phyllanthus emblica*[3] (fruits), *Withania somnifera*[4] (leaves), *Convolvulus pluricaulis*[5] (whole plant), *Piper betel*[6] (leaves), *Allium sativum*[7] (bulb), *Piper longum*[8] (dry fruits), *Zingiber officinale*[9] (rhizomes), *Tribulus terrestris*[10] (whole plant) and *Cardamom*[11] (dry fruits) were traditionally used medicines from time immemorial. It was found that all plants possess medicinal property and exclusively antioxidant action majorly. Besides, in the last few years, interest in the antioxidant activity of the plant extracts has increased tremendously which is very important due to the fact that free radicals can be responsible for various diseases like; heart diseases, strokes, arteriosclerosis, cancer as well as for ageing processes. The main objective of the present study was to standardise pharmacognostically the selected polyherbs and to screen anti-hyperlipedemic activity. World Health Organization reports that high plasma cholesterol contributes to approximately 56% of cardiovascular diseases worldwide and causes about 4.4 million deaths each year[12]. Lipid-lowering strategy may have a beneficial role in normalizing vascular function and greatly decreasing the frequency of clinical events associated with atherosclerosis, combined with the ability of antioxidants to alleviate vasomotor disturbances in hypercholesterolemia and to slow the progression of atherosclerosis. Thus the desirable medicament must not be confined just to reduce the lipid levels in plasma but also should be efficient to protect from free radical damage[13].

MATERIALS AND METHODS

The plant materials were collected locally and they identified and authentified by Dr.P.Jayaraman, Director, Plant Anatomy and Research Centre, Chennai. Fenofibrate was purchased from Moral Labs, Chennai. Cholesterol and HDL-Cholesterol enzyme kit purchased from Span Diagnostics Ltd., Triglycerides and LDL-Cholesterol kits from Euro Diagnostic System. All other chemicals were of analytical grade and obtained locally.

EXTRACTION

All drugs were shade dried at room temperature and were powdered in a Wiley mill[14]. One kilogram of powdered drug was packed in a Soxhlet apparatus, extracted with petroleum ether and methanol. The percentage yield of both the extract was calculated[15]. The pet ether

and methanolic extract was concentrated in a rotary evaporator. They were used for the phytochemical, instrumental, toxicity study and pharmacological validation.

PHYTOCHEMICAL ANALYSIS

The pet ether and methanolic extracts of the polyherbal subjected to systematic qualitative phytochemical screening to identify the phytoconstituents.

PHARMACOGNOSTICAL STANDARDISATION

Determination of Ash values

Determination of Total ash

Accurately about 3 gm of the powdered drug in a tared crucible was taken. Incinerated the powdered drug by gradually increasing the heat until free from carbon and cool. Keep it dessicator. The ash weight was noted and the percentage yield was calculated.

Determination of Acid-Insoluble ash

About 3 gm of the powder drug was taken in a tarred crucible and incinerated by gradually increasing the neat until free from carbon and made it cool. The ash obtained was boiled with 25 ml of dilute hydrochloric acid for 5 minutes and filtered. The insoluble matter was collected on the ash less filter paper, washed with hot water and ignited in tarred crucible, cooled, kept in dessicator. The residue was weighed, acid insoluble ash value was calculated.

Determination of Sulphated ash

3 gm of powdered drug accurately weighed, moistened with sulphuric acid, ignited gently and again moistened with sulphuric acid, reignited again, cooled and weighed. The percentage yield of the sulphated ash was calculated.

Determination of Water soluble ash

Accurately 3 gm of the powdered drug was weighed in a tared platinum or silica dish previously ignited and weighed. The ground drug was scattered in a fine even layer at the bottom of the dish. It was incinerated by gradually increasing the heat not exceeding dull red heat until free from carbon, cooled and weighed. Boil the ash for five minutes with 25ml of water, the insoluble matter was collected in a Gooch crucible or on an ash less filter paper washed with hot water and ignited to constant temperature. The difference between the insoluble matters from the weight of the ash to the water soluble ash was noted down. The percentage yield of water soluble ash was calculated.

Extractive values

Determination of Alcohol – soluble extractive value

About 5 gm of air dried coarse powder mixed with 100ml of alcohol (90%) in a stoppered flask for 24 hours, shaked frequently during first 6 hours. Filtered rapidly through filter paper with taking precaution against excessive loss of alcohol. Evaporated 25 ml of alcoholic extract to dryness in a tarred flat-bottomed shallow dish. Dried at 105° C and weighed. Kept it in a dessicator. Percentage W/V of alcohol (90%) soluble extractive with reference to the air dried drug.

Determination of Water soluble extractive value

About 5 gm of coarsely powdered drug was macerated with 100 ml of water in a stoppered flask for 24 hours, shaked frequently during first six hours. Filtered rapidly through filter paper taking precaution against excessive loss of water. Evaporated 25 ml of water extract to dryness in a tarred flat bottomed shallow dish. Dried at 110° C and weighed. Kept in a dessicator. Percentage yield W/W of water soluble extractive was calculated with reference to the air dried drug.

Determination of moisture content (Loss on drying)

About 2 gm of powdered drug was accurately weighed in a tarred dish and dried in an oven at 105°C for one hour. It was cooled, weighed and kept in a dessicator. The loss on drying was calculated with air dried reference drug and the values are recorded.

Animals

Normal healthy adult albino rats of either sex (180 – 250 gm) were housed under standard environmental conditions at temperature (25±2° C) and light and dark (12:12h). Rats were fed with standard pellet diet (National Institute of Nutrition, Hyderabad) and water *ad libitum*. The experiment was carried out according to the guidelines of the CPCSEA and IAEC, Department of Pharmacology, Teegala Ram Reddy College of Pharmacy, Meerpet, Hyderabad.

Acute toxicity study [16]

Acute oral toxicity study was performed as per OECD – 423 guideline (Acute Toxic Oral Class method), albino rats (n=6) of either sex selected by random sampling were used for acute toxicity study. The animals were kept fasting for over night and provided only with water, after which the extract was administered orally at 5, 50, 300, 2000mg/kg/b.w. by gastric intubations and observed for 14 days. If mortality was observed then the dose

administered was assigned as toxic dose. From that $1/10^{th}$ of the dose will be taken as the therapeutic dose *i.e*) $1/10^{th}$ of $LD_{50} = ED_{50}$ [17]

PHARMACOLOGICAL SCREENING

High cholesterol diet induced hyperlipidaemia [18]

The animals were given high cholesterol rich diet like chocolates, wool fat nuts, coconut biscuit, chips, ghee sweets etc for period of 4 (28days) weeks. The weight of the animals was noted every week. On 29^{th} day animals were kept overnight fasting with only water for free access. From 30^{th} day onwards every day morning the ethanolic extract 200mg/kg was given orally for upto 7 days.

Group 1 - Control received distilled water (1 ml/kg)

Group 2 - High cholesterol diet

Group 3 - Methanolic extract (200 mg/kg)

Group 4 - Fenofibrate (65 mg/kg)

8^{th} day blood collection and analysis of Total cholesterol, Triglycerides, High density lipoproteins, Low density lipoproteins, Very low density lipoproteins and Total protein.

BIOCHEMICAL ESTIMATION[19]

The rats were sacrificed after 48 hours of last dose by cervical decapitation. The blood samples were collected separately by cardiac puncture and allowed to clot, for 30 min at room temperature. The clear serum was separated by centrifugation at 2500 rpm for 10min. The serum triglycerides (STG) and serum cholesterol (SC) levels, were determined by standard kits by using Auto analyzer and Very low density lipoprotein cholesterol (VLDL-c) was calculated by using Friedwald formula VLDL-c = TG/5.

STATISTICAL ANALYSIS

Statistical evaluation of the data was done by one-way ANOVA followed by Dunnet's multiple comparison tests using Graph pad prism software version 5.0 and the values were expressed as Mean±SEM.

RESULTS AND DISCUSSION

The percentage yield for petroleum ether extract 9.11% and methanolic extract 51.3% shows the number and quantity of active phytocontituents soluble in the particular solvent used based upon their polarity nature. The phytochemical analysis showed the presence of a few and traces of phytoconstituents in the pet ether extract and alkaloids, tannins, saponins, flavonoids, phenol, sugar were present in the methanolic extract. The pharmacognostical parameters like ash values, extractive values, crude fibre content, loss on drying reveals the

standardization of the herbal drug. All the parameters are within the limit of official pharmacopoeial standards. Hence, the selected poly herbal obeys and comes under the limit of the specified standards. Table - 1

Oral administration of methanolic extract significantly reduced the cholesterol, triglycerides, low density lipoproteins, very low density lipoproteins and significantly increased the HDL – cholesterol level as compared with high cholesterol diet induced hyperlipedemic animals. The results were significant with the p value ($p<0.001$).

There is an inverse relationship between plasma HDL-cholesterol level and coronary heart disease. The levels of serum lipid profile, total cholesterol, triglycerides, LDL, VLDL and HDL in control, test and standard drug treated were presented in the table. Lowering of serum lipid profiles through dietary or drug therapy seems to be associated with a decrease in the risk of vascular disease. The increased fatty acid concentration also increases the beta-oxidation of fatty acids, producing more acetyl Co-A and cholesterol[20]. The increased concentration of free fatty acid may be due to lipid break down and this may cause increased generation of NADPH dependent microsomal lipid peroxidation. Phospholipids were increased in animals treated with high cholesterol diet. Phospholipids present in the cell membrane and make up vast majority of the surface lipoprotein forming a lipid layer that acts as an interface with both polar plasma environment and non-polar lipoprotein of lipoprotein core. Administration of the extracts showed decreased the levels of phospholipids[21]. As a conclusion the methanolic polyherbal extract shows significant anti hyperlipedemic action when compared with the standard drug Fenofibrate. Table – 2 hence the methanolic polyherbal extract can be safely used as an antihyperlipedemic medicine used against hyperlipidemia.

CONCLUSION

Lipid lowering strategy may have a beneficial role in normalizing vascular function and greatly decreases the frequency of clinical events associated with atherosclerosis, combined with the ability of antioxidants to alleviate vasomotor disturbances in the hypercholesterolemia and to slow the progression of atherosclerosis. The herbal chosen were based upon the traditional knowledge and it shows significant anti hyperlipedemic activity against high cholesterol diet induced hyper lipedemia compound with that of the standard drug Fenofibrate. The phytochemical studies, pharmacognostical parameters and the pharmacological evaluation reveal the official standardisation and anti-hyperlipedemic effects of the polyherbs chosen.

Table – 1 Pharmacognostical standardization

S.No	Parameters	Percentage yield %W/W
1	Total ash	1.0
2	Acid insoluble ash	0.9
3	Sulphated ash	0.9
4	Water soluble ash	0.8
5	Alcohol soluble extractive	2.0
6	Aqueous soluble extractive	1.8
7	Loss on drying	1.0
8	Crude fibre content	1.2

Table – 2 Effect of polyherbal extract on High cholesterol diet induced hyperlipidemia

Treatment	Total Cholesterol mg/dl	Triglycerides mg/dl	HDL mg/dl	LDL mg/dl	VLDL mg/dl
Control	103.41±3.46	88.41±2.81	51±2.11	162.4±2.33	30.5±1.17
High cholesterol diet	313.1±1.46	195.1±2.20	38.01±3.2	199.21±3.21	48.5±2.31
Methanolic extract 200mg/kg	260.3±3.21*	158.2±1.30*	46.2±1.20*	170.32±5.41*	34.3±2.41*
Fenofibrate 65mg/kg	254.2±2.21*	152.2±2.13*	49.1±2.1*	167.2±4.31*	32.5±1.51*

n=6, Values are mean±SEM, One way ANOVA followed by Dunnett's multiple comparison test. *P<0.001

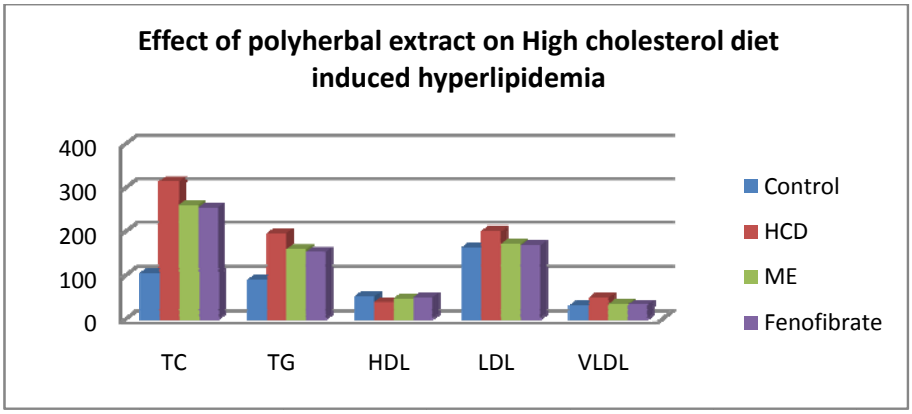

Figure – 1 Diagramatic representation of effect of polyherbal extract on high cholesterol diet induced hyperlipidemia

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