

# Antihyperlipidemic and anti-hyperglycemic effects of *Cymbopogon jwarancusa* in high-fat high-sugar diet model

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**Abstract:** Hyperlipidemia is the root cause for development of atherosclerosis, coronary heart disease, diabetes mellitus, obesity, hypertension and cerebral palsy. *Cymbopogon jwarancusa* is an aromatic grass (Rush grass, khavi grass) belonging to family Poaceae. *C. jwarancusa* essential oil is famous for its use in perfumery, soaps cosmetics, detergents, medicine and pharmaceuticals. The anti-pyretic, anti-fungal, antibacterial, anti-oxidant and cytotoxic activities of *C. jwarancusa* have been reported in literature. In the present study different doses of *C. jwarancusa* extract have been investigated for anti-hyperlipidemic and anti-hyperglycemic activities in high-fat high sugar diet model in rats. Hyperlipidemia and hyperglycemia were assessed by measuring body weight, serum lipid profile and fasting blood glucose levels. Administration of ethanol leaves extract of *C. jwarancusa* exhibited significant dose-dependent reduction in body weight, lipid parameters and blood sugar levels. Hence it may be concluded that *C. jwarancusa* aids in ameliorating hyperlipidemic, hyperglycemic conditions and has potential to reduce the risk of cardiovascular problems.

**Keywords:** *Cymbopogon jwarancusa*, high-fat high-sugar diet, anti-hyperlipidemic, anti-hyperglycemic, atherogenic index.

## INTRODUCTION

Dietary changes, genetic factors and life style modification play crucial role in the development of hyperlipidemic state, which ultimately is the root cause of cardiovascular problems. Researches showed the direct relationship between the increased lipid blood profile level and CAD, stroke, angina, atherosclerosis, cerebral palsy, hypertension, diabetes mellitus, liver fibrosis (Panchal and Brown, 2010). According to WHO, CHD is the 1<sup>st</sup> and stroke is 4<sup>th</sup> major cause of death in people all over the world. It is expected that the mortality rate from coronary heart disease (CHD) double from 13.1 million in 1990 to 24.8 million in 2020 (Poulter, 2003).

Coronary artery diseases are associated with increased mortality rate all over the world. Factors attributed in the rise of CAD are obesity, diabetes mellitus, arteriosclerosis, hypertension, physical inactivity and elevated prothrombotic factors (Yusuf *et al.*, 2002).

Hyperlipidaemia and insulin resistance are linked to each other. Persons with type 2 diabetes are at increased risk for developing atherosclerosis and cardiovascular disease. Low HDL levels are not only associated with progression of CVD but also an independent factor for development of diabetes. The mortality rate due to stroke and heart problems is 2 to 4 times more in diabetic persons than in non-diabetic persons (Howard, 1995).

Adaptation of healthy life style (control of fat rich diet, weight loss, no smoking and proper aerobic exercise) is

first step to control the cholesterol level. If remain untreated by life style modification then switch towards medical approach (Nelson, 2013). HMG-CoA reductase inhibitors or statins are drug of choice for the management of hypercholesterolemia. They blocked the enzyme HMG-CoA reductase, which is the rate determining step in cholesterol synthesis. Beside statins fibrates, bile acid binding resins and Ezetimibe are also used. Statins commonly cause rhabdomyolysis while, other adverse effects are myalgia and increased hepatic transaminases levels which limits its use (Pascual *et al.*, 2008). Most commonly used anti-hyperglycemic medications are sulfonylureas (e.g. glimepiride), beside that biguanides, alpha-glycosidase inhibitors and thiazolidinedione's are also used. Hypoglycemia is common side effect of sulfonylureas, while biguanides lead to lactic acidosis (Berger, 1985).

In addition to medications, nutraceuticals and herbal preparations also used to control cholesterol and glucose levels, as these substances are natural, cost effective and do not require prescription. *C. jwarancusa* is an aromatic grass (Rush grass, khavi grass) belonging to family Poaceae. The volatile oil extracted from *C. jwarancusa* provides medicinal benefits economically. The *jwarancusa* is a fusion of two Sanskrit words *jwar* and *khusha* means fever breaker (Jones *et al.*, 1796). Phytochemical investigation showed that essential oil of plant is rich in monoterpenes and sesquiterpenes. Among monoterpenes, Piperitone (64.71%) constitute the highest concentration in plant essential oil (Saeed *et al.*, 1978). Beside the phytochemical constituents some minerals, trace and biochemical elements are also present in essential oil (Mahmud *et al.*, 2002).

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The anti-pyretic (Alam *et al.*, 2016), anti-fungal (Bhuyan *et al.*, 2010), antibacterial (Bose *et al.*, 2013), anti-oxidant and cytotoxic activities (Dar *et al.*, 2011) of *C. jwarancusa* have been reported in literature, while there is lack of documented evidence regarding its anti-hyperlipidemic and anti-hyperglycemic activity, hence present study is designed to explore the anti-hyperlipidemic and anti-hyperglycemic potential of *C. jwarancusa* leaves extract by developing *in vitro* high fat and high sugar model in rats.

## MATERIALS AND METHODS

### *Preparation of extract*

Aerial parts of *C. jwarancusa* were collected from University of Karachi and identified by herbarium, Botany Department (Voucher specimen no 93325). Plants were washed to remove impurity, dried under shade for 3 weeks, chopped and soaked in ethanol for 20 days, then filtered and extract was evaporated using Rota evaporator. Final crude extract was obtained by freeze drying and kept in refrigerator for further examination.

### *Experimental animals*

Forty two Sprague-Dawley male rats, bred at animal house of Department of Pharmacology University of Karachi were used in the study. Animals were kept in plastic cages under controlled condition of temperature (25±2°C) and humidity (50-60%). Animals were provided water and standard laboratory diet ad libitum.

### *Test and standard drug*

Test drugs (150, 300 and 500 mg/kg of *C. jwarancusa*) and standard drugs (atorvastatin 2.1mg/kg/day and glimepiride 2.7 mg/kg/day) were prepared in 10% DMSO and administered by oral intubation tube.

### *High-fat high-sugar diet*

Hyperlipidemia in rats was induced by giving coconut oil and Vanaspati ghee in ratio of 1:3 v/v in the dose of 3 ml/kg/day in addition to normal rat diet. While hyperglycemia was induced by adding 25% fructose solution to drinking water. high-fat high-sugar (HFHS) diet was given continuously for 14 weeks (Munshi *et al.*, 2014).

### *Animal grouping & experimental design*

The study was carried out on 42 Sprague-Dawley male rats weighing about 180-200g. The animals were randomly divided into seven groups each containing 6 animals. Normal control group received standard laboratory diet and water ad libitum for 14 weeks. The remaining six groups received HFHS diet for 14 weeks. Among these six groups, one served as diet control and did not receive any drug, three groups served as treated and received ethanol extract of *C. jwarancusa* in the dose of 150, 300 and 500mg/kg from 10<sup>th</sup> to 14<sup>th</sup> weeks while

two other groups received standard drugs atorvastatin and glimepiride for 4 weeks i.e. from 10<sup>th</sup> to 14<sup>th</sup> week in the doses of 2.1mg/kg and 2.7mg/kg respectively (Munshi *et al.*, 2014).

Body weight were recorded at the start of experiment and then noted weekly till the end of experiment. The blood sugar levels were measured initially and then evaluated after induction of hyperglycemia and treatment with standard and test drugs.

### *Blood biochemical analysis*

At the end of 14 week all animals were fastened for 12 h and blood glucose level was determined by accu-check glucometer. Animals were then sacrificed and blood samples collected by cardio puncture technique. Serum was isolated by centrifuging at 4000 rpm for 10 min. Serum lipids parameters were measured by diagnostic kits. Atherogenic index and % protection were calculated by following formula (Munshi *et al.*, 2014).

$$\text{Atherogenic index (AI)} = \frac{\text{Total cholesterol} - \text{HDL cholesterol}}{\text{HDL cholesterol}}$$

$$\text{Protection \%} = \frac{\text{AI of treated group}}{\text{AI of control}} \times 100$$

VLDL was calculated by (Friedewald *et al.*, 1972) equation:

$$\text{VLDL} = \frac{\text{Triglycerides}}{5}$$

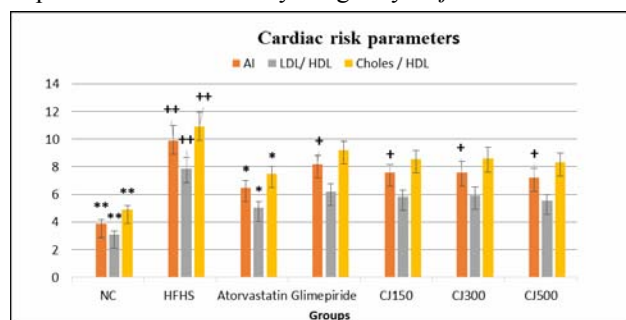
## STATISTICAL ANALYSIS

All statistical calculations were done using SPSS 20. Comparison studies were carried by one way ANOVA, followed by (post hoc in which Tukey's test was used for comparing control with other groups). All values expressed as mean ±S.E.M. Results were considered significant when p values were <0.05 and highly significant when p<0.001. All graphical results were manipulated by Microsoft excel sheet.

## RESULTS

Table 1 shows the comparison of body weight of rats received HFHS and regular diet along with *C. jwarancusa* extract in different doses and standard drugs. Animals on regular diet initially showed increase in body weight at regular pace, however there was highly significant decrease in body weights on 10th and 14th week in comparison to animals on HFHS diet. Animal groups on HFHS diet and treated with atorvastatin and *C. jwarancusa* extract at 500 mg/kg showed a highly significant decrease in body weight as compared to the animals who received only HFHS diet. The decrease in body weight by *C. jwarancusa* extract at 500 mg/kg was

almost comparable to standard drug atorvastatin. While animals received glimepiride did not reveal significant reduction in body weight. However animals who received *C. jwarancusa* extract in the doses of 150 and 300mg/kg showed a significant decrease in body weight as compared to the animals on HFHS thus there was dose-dependent decline in body weight by *C. jwarancusa*.



n=6

Data is represented as mean ± S.E.M

Values are considered as significant \* p<0.05 as compare to HFHS control

Highly significant \*\* p<0.001 as compare to HFHS control

Significant + p<0.05 and highly significant ++ p<0.001 when compare to normal control

CJ= *Cymbopogon. jwarancusa*

**Fig. 1:** Effect of *C. jwarancusa*, atorvastatin and glimepiride on LDL/HDL, Cholesterol/HDL ratio and AI value

Table 2 shows the effect of normal diet, HFHS diet, *C. jwarancusa* extract, atorvastatin and glimepiride on lipid profile and blood glucose concentration. Animals who received normal diet showed a significant decrease in lipid profile and glucose levels as compared to the animals on HFHS diet. Animals who received atorvastatin and *C. jwarancusa* extract at 500mg/kg showed a highly significant decrease in total cholesterol, while triglycerides, LDL and blood glucose were reduced significantly as compared to the animals on HFHS diet. Whereas animals received *C. jwarancusa* at 150 and 300mg/kg showed significant decrease in total cholesterol, LDL and blood glucose level. Hence results indicate a dose dependent decline in lipid profile and blood glucose level by *C. jwarancusa* extract.

Table 3 and figure 1 shows cardiovascular protection trend of normal diet, HFHS diet and different drugs. Highest AI value and cardiovascular risk was observed in animals who received HFHS diet, while lowest AI was displayed by 500mg/kg *C. jwarancusa* extract and atorvastatin. AI value has a direct relation with dose of *C. jwarancusa* extract as risk decreases for CVD when dose increases.

## DISCUSSION

Asian countries have undergone a rapid change in their eating habits in the past few years (Shridhar et al., 2015). People instead of consuming low fat and high fiber diet

like vegetables and fruits are processed food like potato chips, bakery items, burgers and chocolates. Another reason for raised blood cholesterol is use of cooking oil obtained from processed saturated fat. While increased consumption of soft drinks, sauces, topping and synthetic fruit juices is the cause of hyperglycemia.

In present study high fat high sugar diet model was used to evaluate anti-hyperlipidemic and anti-hyperglycemic effect of *C. jwarancusa*. The high sugar diet also increases triglycerides level and ultimately risk of CVD (Bray, 2007).

Present study supports the claim of unani physicians that herbs reduce total cholesterol and hence decrease body weight. This is evident by significant reduction in body weight as well as TG, TC, LDL and VLDL of all animal's except those treated with glimepiride. Anti-hyperlipidemic effect of *C. jwarancusa* extract at 500mg/kg was almost comparable to the effect of standard drug atorvastatin. This effect may be attributed due to presence of active phytochemicals e.g. isoprenoids and terpenoids. Isoprenoids e.g. geraniol, limonene and linalool in *C. jwarancusa* extract have been reported to inactivate HMG-CoA reductase (Middleton and Kok-Pheng 1982) and hence may be responsible for anti-hyperlipidemic. Terpenoid possesses cardio protective effect since inhibit the production of free radical (Pietri et al., 1997).

Blood sugar levels declined in all groups of animals received standard and test drug, in comparison to HFHS diet group. Normal control group also showed significant reduction in blood glucose. Anti-hyperglycemic activity of *C. jwarancusa* reflects the presence of active chemical constituents. Essential oil of *C. jwarancusa* contains higher percentages of sesquiterpenes and monoterpenes (Dar et al., 2011; Bose et al., 2013), which are reported to possess anti-diabetic and anti-hyperlipidemic activity (Boukhris et al., 2012). Terpenes play a significant role in the management of type 2 diabetes by modulating PPAR- $\gamma$  (Goto et al., 2010).

*C. jwarancusa* essential oil also contains manganese (Mahmud et al., 2002), which is reported to contribute in decreasing blood glucose and cholesterol levels, since it enhances the insulin secretion and prevent oxidation of lipids (Lee et al., 2013).

AI value is predictor of coronary artery diseases and atherosclerosis in patient. However, animals treated with *C. jwarancusa* reduce AI index and showed dose-dependent cardio protection by 27.57%, 23% and 20.94% in the doses of 500, 300 and 150mg/kg respectively, which was almost comparable to atorvastatin (31.31%) in HFHS diet group. Thus atorvastatin and *C. jwarancusa* reduced AI value and risk of atherogenesis.

**Table 1:** Effect of Normal diet, HFHS diet, *C. jwarancusa*, atorvastatin and glimepiride on body weight in rats

Groups	Body weight (gm.)			
	Dose mg/kg	0 day initial weight	10 <sup>th</sup> week	14 <sup>th</sup> week weight
Normal Control	-	146.8±4.84	175.2**±6.17	181.7**±5.43
HFHS Control	-	172.3±8.67	220.3 <sup>++</sup> ±10.04	251.2 <sup>++</sup> ±9.91
Atorvastatin	2.1	193.7±10.64	238 <sup>++</sup> ±8.87	200.5**±9.21
Glimepiride	2.7	180.3±4.11	227.3 <sup>++</sup> ±1.80	239 <sup>++</sup> ±1.67
<i>C. jwarancusa</i>	150	182.5±3.42	234 <sup>++</sup> ±2.90	224.2 <sup>++</sup> ±2.30
<i>C. jwarancusa</i>	300	185.2±2.25	231.16 <sup>++</sup>	215.3 <sup>++</sup> ±3.48
<i>C. jwarancusa</i>	500	182.3±2.89	230 <sup>++</sup> ±3.56	209.8 <sup>++</sup> ±3.19

**Table 2:** Effect of *C. jwarancusa* extract, atorvastatin and glimepiride on plasma lipid profile and blood glucose levels

Parameters	Normal control	HFHS control group	Atorvastatin	Glimepiride	<i>C. jwarancusa</i> 150mg/kg	<i>C. jwarancusa</i> 300mg/kg	<i>C. jwarancusa</i> 500 mg/kg
Cholesterol mg/dl	120.3±3.1**	243.3±6.1 <sup>++</sup>	178.7±10.6 <sup>++</sup>	210.6±3.1 <sup>++</sup>	201.5±10.4 <sup>+++</sup>	199.5±7.6 <sup>+++</sup>	191.9±10.6 <sup>+++</sup>
Triglycerides mg/dl	97.5±3.3**	232±4.3 <sup>++</sup>	171.8±5.8 <sup>+++</sup>	232.7±10.7 <sup>++</sup>	203.3±9.9 <sup>++</sup>	198.8±4.8 <sup>+++</sup>	194.7±8.2 <sup>++</sup>
HDL mg/dl	25±1.4	23.1±1.9	24±1.1	23.5±1.7	23.7±1.1	24±2	24.3±2.9
LDL mg/dl	75.8±2.9**	173.8±7.4 <sup>++</sup>	120.2±9.7 <sup>+++</sup>	140.6±6 <sup>++</sup>	137±9 <sup>+++</sup>	135.7±7.5 <sup>+++</sup>	128.8±8.7 <sup>+++</sup>
VLDL mg/dl	19.5±0.67**	46.4±0.87 <sup>++</sup>	34.4±1.1 <sup>+++</sup>	46.5±2.1 <sup>++</sup>	40.7±2 <sup>++</sup>	40±1 <sup>+++</sup>	38.9±1.6 <sup>+++</sup>
LDL/ HDL	3.1±0.27**	7.8±0.9 <sup>++</sup>	5±0.4*	6.2±0.6 <sup>+</sup>	5.9±0.5 <sup>+</sup>	5.9±0.7 <sup>+</sup>	5.6±0.4
Cholesterol/ HDL	5±0.3**	10.9±1 <sup>++</sup>	7.5±0.5*	9.2±0.7 <sup>+</sup>	8.6±0.6 <sup>+</sup>	8.6±0.8 <sup>+</sup>	8.3±0.7 <sup>+</sup>
Fasting blood glucose mg/dl	71±1.8*	155±4 <sup>+</sup>	103.7±2 <sup>+</sup> *	84±4*	133±3.2 <sup>+</sup> *	128±2.8 <sup>+</sup> *	122.5±4.5 <sup>+</sup> *

n=6

Values are mean ±S.E.M

\*p < 0.5 significant as compare to HFHS control

\*\*p < 0.001 highly significant as compare to HFHS control

<sup>++</sup>p < 0.001 highly significant as compare to normal control

<sup>+</sup>p < 0.05 significant as compare to normal control

**Table 3:** Effect of various drugs on AI and % protection

Parameters	Normal control	HFHS control group	Atorvastatin	Glimepiride	<i>C. jwarancusa</i> 150mg/kg	<i>C. jwarancusa</i> 300mg/kg	<i>C. jwarancusa</i> 500 mg/kg
AI	3.8±0.31**	9.5±1.05 <sup>++</sup>	6.4±0.51*	7.95 <sup>+</sup> ±0.67 <sup>+</sup>	7.5±0.57 <sup>+</sup>	7.3±0.81 <sup>+</sup>	6.9±0.65 <sup>+</sup>
% protection	-	-	31.31	23.68	20.94	23	27.57

n=6

Data is represented as mean ± S.E.M

Values are considered as significant \* p < 0.05

Highly significant \*\* p < 0.001 when compare to HFHS control

Significant <sup>+</sup> p < 0.05 and highly significant <sup>++</sup> p < 0.001 when compare to normal control

AI= Atherogenic index

LDL/HDL and cholesterol/HDL ratio also helps in predicting the risk for cardiovascular problems, higher values are indicator of increased tendency cardiovascular disease. Animals on HFHS diet has shown highest ratio for LDL/HDL and cholesterol/HDL. However, animals received extract of *C. jwarancusa* in the dose of 500mg/kg showed LDL/HDL and cholesterol/HDL ratios almost equivalent to atorvastatin.

Thus, present study reveals hypoglycemic and hypocholesterolemic effect of *C. jwarancusa* extract in all doses but more significant effects were observed at 500mg/kg which were almost comparable to atorvastatin and glimepiride. These two activities attributed the use of plant in ameliorating cardiovascular diseases, atherogenesis and non-insulin independent diabetes mellitus.

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