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Evaluation of *Oryza Sativa* (Var. Joha Rice) for Anti-Hyperlipidemic Activity in Rats

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Abstract:
The present study evaluated the anti-hyperlipidemic activity of the ethanolic extracts of *Oryza sativa* (var. Joha rice) (EEJR) on blood cholesterol of albino rats. Ethanolic extract of joha rice was administered at doses of 200 and 400 mg/kg body weight respectively on cholesterol induced hyperlipidemic rats for 7 days. Hyperlipidemic rats had much reduced body weight than normal rats. Administration of the extracts at the dose of 200 & 400 mg/kg body wt. /day produced a significant effect on lipid profile, which had shown anti hyperlipidemic activity on cholesterol induced hyperlipidemic rats. Cholesterol induced hyperlipidemic rats treated with Ethanolic extract of joha rice (200 & 400 mg/kg) significantly reversed all these changes to near normal. These results suggest that Ethanolic extract of joha rice possess anti hyperlipidemic activity in cholesterol induced hyperlipidemic rats.

Keywords: joha rice, hyperlipidemia, cholesterol, albino rats, lipid profile.

I. INTRODUCTION:

Cardiovascular disease is the leading cause of mortality all over the world and is a major health concern of the public nowadays. Hyperlipidemia is described as the contributing risk factor for cardiovascular disease (Brown & Goldstein 1986). Hyperlipidemia is also the primary cause of atherosclerosis, ischemic cerebrovascular disease, coronary heart disease and peripheral vascular diseases (Hardman & Limbard 2001). Hyperlipidemia is characterized by cluster of abnormalities like elevated serum total cholesterol, serum triglyceride, low density lipoprotein-cholesterol levels and reduced high density lipoprotein-cholesterol levels. It is well known that various factors such as lipid abnormalities, oxidative stress (Yokoyama 2004) and inflammation (Hansson 2005) have been associated in the development of atherosclerosis and subsequent cardiovascular diseases. There exists a wide consensus that hyperlipidemia in human and animals is produced by the influence of dietary cholesterol. Diet plays a pivotal role in maintenance of ideal body weight, body fat and normal levels of blood lipids (Loo et al 1991). Numerous research reports have been demonstrated in understanding the pathophysiology of hyperlipidemia.

Growing evidence suggests that prevention or treatment of atherosclerosis and cardiovascular diseases is possible through targeting hyperlipidemia by diet or drugs (LaRosa et al 1990). Hyperlipidemia disease has afflicted humankind since antiquity. In 2002, coronay heart Epidemiological evidence strongly supported the positive correlation between blood lipids, hyperlipidemia and its complications, mainly CHD. This relationship has been shown between and within cultures. The hyperlipidemia is traditionally defined as conditions in which the concentration of *cholesterol* or *triglyceride*-carrying *lipoprotein s* in plasma exceeds an arbitrary normal limit. These lipoproteins deposit in the interstitial space of arteries arising from aorta, restricting the blood supply to the heart. This phenomenon is known as atherosclerosis. Higher deposition of lipoproteins completely blocked the blood supply to the heart, and thus myocardial infarction (MI) occurs, which is commonly known as heart attack.

Causes

The predisposing factors associated with hyperlipidemia constitute (Bethesda 1991; Marshall 1992; Lipmann et al 2000)
1. Elevated low density lipoprotein-cholesterol (LDL-C) levels and decreased high density lipoprotein- cholesterol (HDL-C) levels
2. Age (male > 45 years; female > 35 years)
3. Family history of premature death
4. Diet rich in saturated fats and cholesterol
5. Diabetes Mellitus
6. Hypertension
7. Hypothyroidism
8. Cigarette smoking and alcohol abuse
9. Physical inactivity
10. Obesity or overweight
11. Overactive adrenal gland
12. Increased levels of c-reactive proteins
13. Increased Lipoprotein (a) levels
14. Liver and kidney problems
15. Certain drugs (Birth control pills)

Types of Hyperlipidemia

Hyperlipidemia is broadly classified into two types:

Primary Hyperlipidemia and Secondary Hyperlipidemia Primary Hyperlipidemia This occurs as an outcome of high consumption

of diet rich in saturated fats and cholesterol or because of some genetic defect and heredity factors (Marshall 1992; Tripathi 2008) Fredrickson classification of hyperlipidemia is given below,

Type I: Buerger Gruetz syndrome primary hyperlipoproteinemia or Familial chylomicronemia
Type II a: Polygenic Hypercholesterolemia or Familial hypercholesterolemia
Type II b: Combined Hyperlipidemia
Type III: Familial Dysbetalipoproteinemia
Type IV: Endogenous Hyperlipidemia
TypeV: Familial Hypertriglyceridemia 1.1.2.2 Secondary Hyperlipidemia This occurs as a result of other metabolic disturbances. Several disease states are associated with secondary hyperlipidemia which includes 4 diabetes mellitus, hypothyroidism, pregnancy, alcohol abuse, chronic renal failure, myeloma and obstructive liver disease (Marshall 1992).

On the basis of causing factor

Familial (Primary) hyperlipidemia–On the basis of causing factors hyperlipidemia can be designated as either primary or secondary. According to Fredrickson familial hyperlipidemia is classified into five types (table 2) on the basis of electrophoresis or ultracentrifugation pattern of lipoproteins.

Type I –Raised cholesterol with high triglyceride levels.
Type II–High cholesterol with normal triglyceride levels.
Type III –Raised cholesterol and triglycerides.
Type IV –Raised triglycerides, atheroma and uric acid.
Type V –Raised triglycerides.

This classification was later adopted by WHO. This method does not directly account for HDL and also does not distinguish among the different genes that may be partially responsible for some of these conditions. It remains a popular system of classification but is considered dated by many.

Causes of hyperlipidemia

A diet rich in saturated fat and cholesterol increases blood cholesterol and triglyceride levels. Other disorders as obesity, diabetes mellitus and hypothyroidism increase the risk of hyperlipidemia. Smoking and not exercising may lead to hyperlipidemia [40]. Excessive use of alcohol also increases the risk of hyperlipidemia. Certain drugs as steroids and β–blockers may cause hyperlipidemia. Hereditary factor is also one of the common causes for hyper-lipidemia. In some cases hyperlipidemia occurs during pregnancy. Lipoprotein lipase mutations.

Symptoms of hyperlipidemia

Hyperlipidemia usually has no noticeable symptoms and tends to be discovered during routine examination for atherosclerotic cardiovascular disease. Symptoms may include chest pain (angina), heart attack or stroke. When levels are exceedingly high, cholesterol may be deposited in tendons or just beneath the skin under the eyes. Swelling of organs such as liver, spleen or pancreas. Blockage of blood vessels in brain and heart. Higher rate of obesity and glucose intolerance. Pimple like lesions across the body.

II. MATERIALS AND METHODS:

Evaluation Of Anti Hyperlipidemic Activity:

Animals:
Healthy eight-week old female Wister albino rats (130 to 160 g) were randomly assigned to control and treated groups (six animals per group/cage). They were maintained in standard environmental conditions (22 ± 2°C, 12:12 h dark/light cycle, humidity: 45 to 50%) frequent air change and had free access to tap water and food. All animals were obtained from the animal house. All procedures used in the present study followed the "Principles of Laboratory Animal Care" and were approved by the Animal Ethics Committee of our University.

Cholesterol supplemented diet:
Hypercholesterolemia was induced using earlier modified method of Onody et al., (2003). Briefly, cholesterol (2% w/w) powder was Olorunnisola et al. 13499 thoroughly mixed with crushed pellet diet and reconstituted with water and allowed to dry properly to prevent microbial contamination.

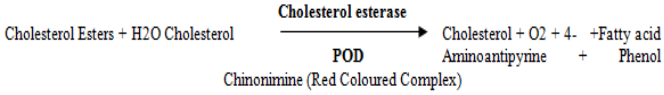
Experimental designs:
Experimental animals were divided into the following groups after two weeks of acclimatization. Each group comprised of 6 animals.
Group 1: Control rats fed with normal pellet diet for 4 weeks by orally gavage.
Group 2: Rats fed with cholesterol mixed pellet diet by orally gavage.
Group 3: Rats fed with cholesterol (2% w/w) mixed pellet diet and Joha rice extract (200mg/kg) by orally gavage.
Group 4: Rats fed with cholesterol mixed pellet and Joha rice extract (400mg/kg) by orally gavage.
Group 5: Rats fed with cholesterol (2% w/w) mixed pellet diet together with standard drug (Lovastatin) (30 mg/kg) by orally gavage.

Biochemical determinations:
Assessment of lipid profile and biochemical parameters
Blood samples were collected from overnight fasted rats using the method described by Yakubu et al. (2005). Briefly, under ether anesthesia, the neck was quickly cleared of fur and skin to expose the jugular veins. These animals were thereafter made to bleed through their cut jugular vein and their blood was collected with lithium heparinized tubes. Total cholesterol, triglycerides, low density lipoprotein (LDL), very low density lipoprotein (VLDL), high density lipoprotein-cholesterol (HDL-C) levels, aspartate amino transferase, alanine amino transferase, alkaline phosphatase, total bilirubin, lactate dehydrogenase, gamma glutamyl transferase (gGT) and glucose were determined in the blood using piccolo automated chemistry analyser.

a) Serum Cholesterol Estimation
Serum cholesterol was estimated by CHOD/POD method with the help of Clinical Chemistry Analyzer (Metro Lab, 1600 DK-R) CHOLESTROL STABLE REAGENT (Swemed Diagnostics, Bangalore).

Principle

Cholesterol reacts with hot solution of Ferric perchlorate, Ethyl acetate and sulphuric acid (Cholesterol Reagent) and gives a lavender coloured complex which is measured at 560 nm. The enzymatic reaction sequence employed in the assay of cholesterol is as follows:



Procedure

To 1000 µl of the reagent, 10 µl of standard cholesterol (100 mg/dl) was added and incubated for 10 min at 37 ºC. This incubated mixture was aspirated and concentration of standard was calibrated to show a value of 100 mg/dl. The fasting serum cholesterol was estimated by adding 10 µl of the serum sample to 1000 µl of the reagent, mixed well and incubated at 37 ºC for 5 min. This incubated mixture was aspirated and absorbance recorded against a reagent blank at 505 nm using Clinical Chemistry analyzer.

a) Serum HDL Cholesterol Estimation

Principle

The chylomicrons, VLDL (very low density lipoproteins) and LDL (low density lipoproteins) are precipitated by addition of magnesium chloride. After centrifugation the supernatant fluid contains the HDL-fraction (high density lipoproteins). This is assayed for HDL cholesterol using cholesterol reagent with the help of Clinical Chemistry Analyzer (Metro Lab 1600 DK-R) HDL REAGENT (Swemed Diagnostics, Bangalore).

Procedure

Step-1

To 500 µl of the HDL reagent was added to 250 µl of standard and mixed well, kept to stand for 10 min at 15-25 ºC and centrifuged for 15 min at approx. 4000 rpm. Determined the cholesterol concentration of the supernatant within 1 h after centrifugation.

Step-2

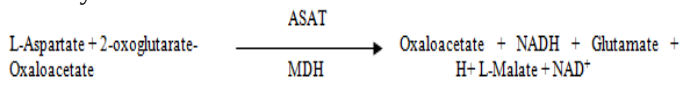
From the supernatant sample 100 µl was taken and added to cholesterol reagent. Mixed and incubated reagent blank, standard and sample for 10 min at 20 ºC or 5 min at 37 ºC, then measured the absorbance of sample against reagent blank within 1 h.

b) SGOT estimation

Serum SGOT was estimated by Modified IFCC method with help of clinical chemistry analyzer (Metro Lab, 1600 DK-R) SGOT LIQUID STABLE REAGENT (Swemed Diagnostics, Bangalore).

Principle

SGOT catalyses the transfer of amino group from L-aspartate to 2- oxoglutarate forming oxaloacetate and L-glutamate. The rate of this reaction was monitored by an indicator reaction coupled with malate- dyhydrogenase (MDH) in which the oxaloacetate formed was converted to malate in the presence of reduced nicotinamide adenine dinucleotide (NADH). The oxidation of NADH in this reaction was measured as a decrease in absorbance of NADH at 340 nm, which was proportional to SGOT activity. The enzymatic reaction sequence employed in the assay of SGOT is as follows:



Procedure

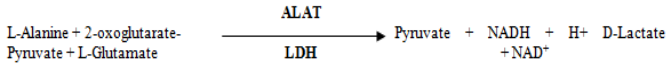
To 400 µl of R1 reagent add 100 µl of R2 reagent (total of 500 µl) add 50 µl of the serum sample and mix well take the reading after 60 sec.

c) SGPT Estimation

Serum SGPT was estimated by Modified IFCC method with help of clinical chemistry analyzer (Metro Lab, 1600 DK-R) SGPT LIQUID STABLE REAGENT (Swemed Diagnostics, Bangalore).

Principle

SGPT catalyses the transfer of amino group from L-alanine to 2-Ketoglutarate with the formation of pyruvate and L-glutamate. The pyruvate so formed is allowed to react with NADH to produce lactate. The rate of this reaction is monitored by an indicator reaction coupled with LDH in the presence of NADH (Nicotinamide adenine dinucleotide). The oxidation of NADH in this reaction was measured as a decrease in the absorbance of NADH at 340 nm, which was proportional to SGPT activity. The enzymatic reaction sequence employed in the assay of SGPT is as follows:



Procedure

To 400 µl of R1 reagent add 100 µl of R2 reagent (total of 500 µl) add 50 µl of the serum sample and mix well take the reading after 60 sec.

Statistical analysis:

Values were given as means ± standard deviation (mean ± SD). Data was statistically analyzed by using one-way analysis of variance (ANOVA).

Results of Anti hyperlipidemic activity:

The lipid profiles in control and experimental rats are depicted in Table- 1 in cholesterol induced hyperlipidemic rats. The hyperlipidemic control rats (Group II) showed significant increase in serum triglycerides, Total cholesterol, very low density lipoproteins (VLDL), low density lipoproteins (LDL), and High density lipoproteins (HDL) when compared with normal (Group I). Standard lovastatin (Group III) also reduced triglycerides, Total cholesterol, very low density lipoproteins (VLDL), low density lipoproteins (LDL), and increased High density lipoproteins (HDL) when compared with normal (Group I).The ethanolic extract showed significant decrease ($p<0.001$) in Total cholesterol, LDL, VLDL, Triglycerides and significant increase ($p<0.001$) in HDL when compared with hyperlipidemic control group (Group II). All these effects were observed on 8^{th} day. The present experimental result indicated that ethanolic extract of Joha Rice exhibited a potent blood glucose lowering properties in cholesterol induced hyperlipidemic rats. The results were shown in **Table- 1** and values were plotted in **Figure-1**

Table.1. Effect of EEJR on Lipid Profile in Cholesterol Induced Hyperlipidemic Rats

S.NO	GROUP	0N 7TH DAY						
		TCH	TG	HDL	LDL	VLDL	SC	BU
1	NORMAL CONTROL	94.17±0.477	98.0±0.632	40.67±0.666	31.5±1.232	21.5±0.763	1.11±0.047	28.0±0.730
2	HYPERLIPI-DEMIC CONTROL	134.7±1.667^{a***}	139.0±0.894^{a***}	19.8±1.352^{a***}	54.5±2.141^{a***}	60.5±1.057^{a***}	2.66±0.055^{a***}	48.33±0.802^{a***}
3	LOW DOSE	90.67±0.494^{b***}	98.33±0.333^{b***}	42.5±0.846^{b***}	33.17±1.493^{b***}	22.67±0.843^{b***}	1.35±0.042^{b***}	27.83±0.477^{b***}
4	HIGH DOSE	83.5±0.806^{b***}	93.5±1.765^{b***}	41.5±0.670^{b***}	30.67±0.494^{b***}	19.83±0.600^{b***}	1.01±0.060^{b***}	24.0±0.365^{b***}
5	STANDARD	87.83±0.749^{b***}	97.17±1.662^{b***}	40.0±0.577^{b***}	31.83±0.792^{b***}	22.33±1.085^{b***}	1.15±0.042^{b***}	27.17±0.600^{b***}

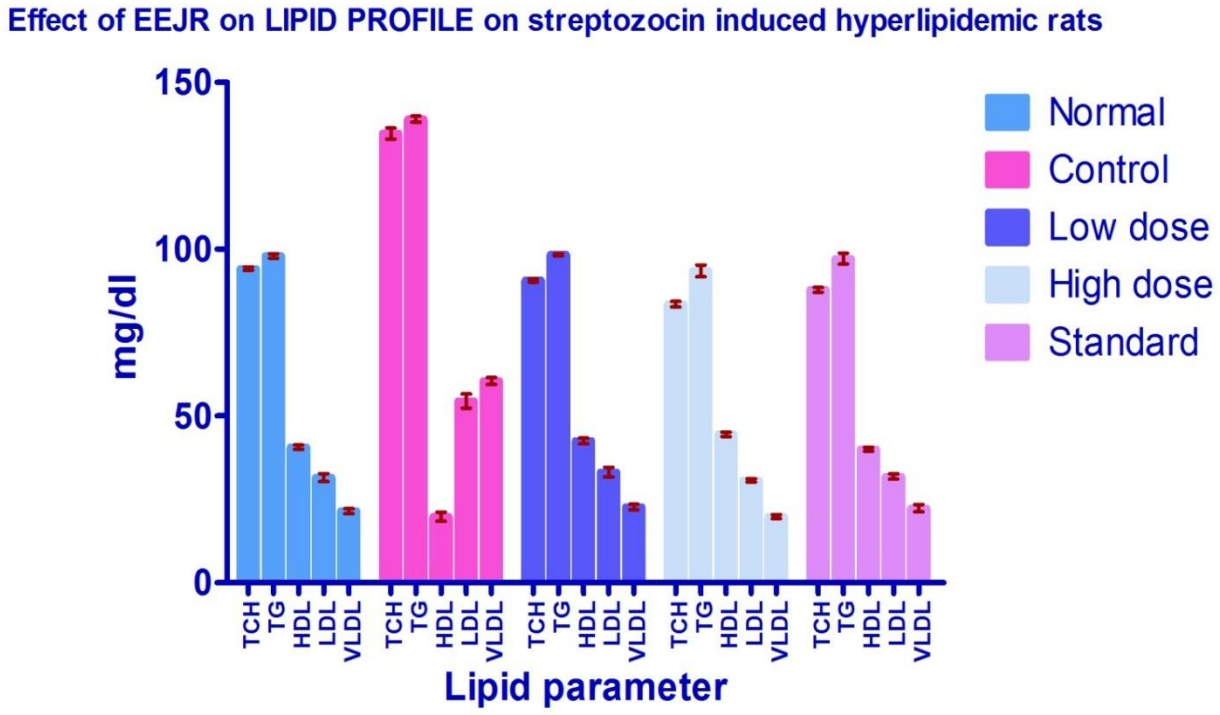


Figure.1. Effect of EEJR on Lipid profile in Cholesterol induced Hyperlipidemic rats

III. DISCUSSION:

Discussion of anti- hyperlipidemic activity:

Lipoproteins are one of the most susceptible targets of free radicals. The oxidative destruction is known as lipid peroxidation and may induce many pathological events. As the EEJR was found to possess antioxidant potential, it was evaluated for anti-hyperlipidemics activity also. The hyperlipidemia was induced by HFD containing high level of cholesterol. The treatment group received standard drug Lovastatin (20 mg/kg b.w.) and EEJR was carried out simultaneously along with HFD for a period of seven days. The blood serum collected on 8th day suggested increase in the level of TC, TG, LDL and decrease in HDL level in negative control i.e., the group that received only HFD. The estimation of these parameters in the standard group (HFD + Lovastatin) and test group (HFD + EEJR) revealed that the level of TC, TG, LDL were decreased and level of HDL was increased than the negative control group in a significant manner when evaluated statistically by using the software Graph Pad Prism 5.0 ANOVA study was done for the results of the level of parameters obtained by using Dunnet t-test. The standard drug decreased the level of TC, TG, LDL and VLDL and increased the level of HDL than the negative control. The Test drug (EEJR) decreased the level of TC, TG, LDL and VLDL and increased the level of HDL than the negative control. The differences were found to be significant. Thus it can be reported that the ethanolic extract of *Oryza sativa* (var. Joha rice) possess significant anti hyperlipidemic activity.

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