



COMPARISON OF METHANOLIC AND AQUEOUS EXTRACTS OF *QUISQUALIS INDICA* (Linn) ON PASSIVE SMOKING INDUCED HYPERLIPIDEMIA IN WISTAR RATS

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ABSTRACT

Quisqualis indica (Combretaceae) is commonly known as Rangoon creeper and Madhumalti in Hindi, an evergreen plant growing as an ornamental plant all over the country and also used as traditional medicines. In the present study, the effects of two different doses (100 mg/kg and 200 mg/kg) of methanolic and aqueous extract of aerial part of *Quisqualis indica* on passive smoking induced hyperlipidemia had been investigated in rats. Hyperlipidemia is an excess of fatty substances called lipids, largely cholesterol and triglycerides, in the blood. The present results showed that there is increase in concentration of total cholesterol, triglycerides, low density lipoprotein cholesterol and very low density lipoprotein cholesterol when compared to control group due to passive smoking. Co-administration of both extracts and passive smoking simultaneously there is reduced level of harmful lipid had been found when compared to only passive smoking taken group with normal food and water ad libitum. The main principle behind this reduction is may be due to decrease in lipid peroxidation as the plants contain flavonoid (rutin) and other compound acting as a free radical scavengers obstructing the formation of free oxygen in the endothelial space. The effect of methanolic and aqueous extract of aerial part of *Quisqualis indica* had been compared at two different doses level i.e. 100 and 200 mg/kg with atorvastatin drug (10 mg) and it had been concluded that methanolic extract was more effective than aqueous extract but at the dose of 200 mg/kg of methanolic extract had been markedly showed effect comparable anti hyperlipidemic as that of standard Atorvastatin (10 mg/kg).

Keywords: Hyperlipidemia, passive smoking, *Quisqualis indica*, flavonoids, atorvastatin.

INTRODUCTION

Hyperlipidemia is mainly associated with endothelial dysfunction and cause due to disorderliness of lipid metabolism, also called hyperlipoproteinemia, which results in abnormally high levels of cholesterol, triglycerides, and lipoproteins in the blood circulation and a key contributor to atherosclerosis, coronary artery disease (CAD), and peripheral vascular disease. Some forms of hyperlipidemia are familial or hereditary and may manifest regardless of lifestyle¹.

The fat-protein complexes in the blood are called lipoproteins. The best-known lipoproteins are LDL (low density lipoprotein) and HDL (high density lipoprotein). Excess LDL cholesterol contributes to the blockage of arteries as they stick on blood vessel and form plaque which eventually leads to heart attack. Population studies have clearly shown that the higher the level of LDL cholesterol, the greater the risk of heart disease. Hence, LDL cholesterol has been labeled the "bad" cholesterol. In contrast, lower the level of HDL cholesterol, the greater the risk of coronary heart disease. As a result, HDL cholesterol is commonly referred to as the "good" cholesterol. Low HDL cholesterol levels are typically accompanied by an increase in blood triglyceride levels. Studies have shown that high triglyceride levels are associated with an increased risk of coronary heart disease².

Passive smoking causes approximately 50,000 deaths annually in the United States, with the vast majority of

these deaths due to heart disease. The effects of secondhand smoke on many pathophysiological mediators of coronary artery disease are nearly as large as those of active smoking, including impaired platelet function, damage to vascular endothelium and its associated repair mechanisms, a rise in inflammatory molecules, and dysfunctional lipid metabolism by forming free radicals³. Nicotine increases the amount of bad fats (LDL, triglycerides, cholesterol) circulating in the blood vessels and decreases the amount of good fat (HDL)⁴ and are absorbed through the lungs into the blood stream and are circulated throughout the body. These substances damage the blood vessel walls, which allow plaques to form at a faster rate than they would in a non-smoker. In this way, smoking increases the risk of heart disease by hastening atherosclerosis⁵.

Oxidative damage can directly lead to a breakdown or even hardening of lipids, which composition of all cell walls made up of unsaturated lipid molecules susceptible to free radicals. Epidemiological studies suggest that increased dietary intake of antioxidants reduces the risk of coronary artery disease⁶ and there are so many natural things found in world which could have been better than the synthetic products because of their side effects or toxicity. *Quisqualis indica* Linn showing various pharmacological activities such as anti-inflammatory activity, antipyretic activity, immunomodulatory activity, anti-staphylococcal activity, anthelmintic activity, antiseptic activity, antioxidants etc due to its presence of various active constituents all over the parts of plants



which had been proved⁷⁻¹². On the contrary the presence of mainly flavonoids and other constituent together act as antihyperlipidemic as it gives an antioxidant activity¹³.

MATERIALS AND METHODS

Plant Material

The mature aerial parts of *Quisqualis indica* were collected in the morning from Bhopal, Madhya Pradesh, India, in the month of January 2012. Identification and authentication of herb by Dr. Zia ul Hassan, Professor of Botany, Safia College of Science, Bhopal, Madhya Pradesh, India (Voucher. No 323/Bot/Safia/2010). The collected parts were washed with a normal tap water so that the stucked dirt particle had been washed and then dried in a shed area, after dried it had been crushed into small pieces for extraction process. About 80 gm of dry powder was taken in a soxhlet apparatus and extracted with 400 ml methanol for about 8 days at 10-15 degree centigrade and further it was extracted with 400 ml distilled water after the collection of marc. The marc left after successive extraction was taken out and dried it separately under room temperature to get a dry mass i.e. free of solvent. The both final obtained extract was weighed, packed in a paper bags & stored in air tight container at cool place until use.

Phytochemical Analysis

Preliminary Phytochemical studies of methanolic and aqueous extract of *Quisqualis indica* was performed for major classes of constituents like alkaloids, carbohydrates, protein and amino acid, Saponins, glycosides, steroids, tannins, flavonoid and phenolic compounds according to published standard methods¹⁴. The dose limits were selected on the basis of previously performed oral acute toxicity studies in albino mice, in accordance with the OECD (423) guidelines.

Preparation of dosage forms

For in vivo studies, the standard drug Atorvastatin (10 mg/kg) was administered orally after suspending in 0.05% CMC and the concentrated methanolic and aqueous extract of *Quisqualis indica* was administered orally after suspending in Distilled water. The freshly prepared solution of both standard drug Atorvastatin & *Quisqualis indica* extract was used in each experiment. Test doses i.e. 100 and 200 mg/kg which were selected on the basis oral acute toxicity study in rat.

Animals and Animals Diet

Albino wistar rats (100-200 gm) of either sex had been taken which were obtained from Sapience Bio Analytical Research laboratory, Bhopal (M.P.) animal house (Reg. No. 1413/A/11 CPCSEA) and housed 6 animals per cage made up of polypropylene, habituated at laboratory condition for 2 days prior to experiment procedure which were maintained at environment [(25°C ± 2) temperature, 30-50 % humidity and 12 hr light and dark condition alternately)]. The animals were fed with standard pellet diet and water ad libitum.

Passive Smoking Induced Hyperlipidemia

Animals was provided with cigarette smoke twice a daily along with normal diet to induce hyperlipidemia i.e. 1 cigarettes provided to a group of 6 animals at morning and evening by the use of smoking chambers having 1 ventilacle hole at both sides throughout the experiments except control group. The animals were divided into 3 groups containing 6 animals in each group i.e. Control Group, Passive smoking induced group and Passive smoking + Test Methanolic extract (200 mg/kg p.o.) group. After 28 days rats were fasted for 10-12 hours and then they were anaesthetized with mild chloroform, blood sample was collected by retro orbital sinus puncture. Collected blood was poured slightly into tubes marked and immediately centrifuged for 2000 rpm for 15 minutes to obtain clear serum. The amount of blood parameters was calculated in mg/dl.

Biochemical Analysis

The blood serum were assayed for total cholesterol (TC), triglycerides (TG), high-density lipoprotein (HDL), low-density lipoprotein (LDL), very low-density lipoprotein (VLDL) using Span diagnostic kit having standard kit formula. The amount of LDL-Cholesterol and VLDL-Cholesterol were calculated using friedewald's equation. $LDL\text{-Cholesterol} = Total\ Cholesterol - Triglycerides/5$ - $HDL\text{-Cholesterol}$ and $VLDL\text{-Cholesterol} = Triglycerides/5$. (Kaplan and Szabo, 1983)¹⁵⁻¹⁷.

Statistical Analysis

The results were expressed as mean ± S.E (Standard Error). Statistical analysis was carried out by using ANOVA followed by Tukey's multiple comparison tests using Graph pad PRISM software version 5.04 (2010). P values < 0.05 were considered as statistically significant.

RESULTS

By considering the acute toxicity test doses of both the extract were taken as 100 and 200 mg/kg. The present investigation showed that the passive smoking induced hyperlipidemia in rats by raising the harmful lipid level i.e. LDL, VLDL, TC and TG and lowering of HDL shown in table 1. The results were discussed mainly under lipid layer. Both the extracts had maintained the total cholesterol (TC), triglycerides (TG), low-density lipoprotein (LDL), and very low-density lipoprotein (VLDL) and increase the high-density lipoprotein (HDL) level compared to induced group which was may be due to inhibition of lipid peroxidation due to presence of flavonoids and phenolic compounds in plants which act as antioxidants. By the results it had been concluded that the extracts is acting as antihyperlipidemic drugs at the dose level of 100 and 200 mg/kg by lowering the harmful effects of lipid level and increased HDL in a dose dependent manner but the methanolic extracts at dose of 200 mg/kg was found to be more effective than others extracts comparable to standard drug Atorvastatin clearly shown in graph figure III.





Figure 1: Smoking Chamber used for providing passive smoking to rats

Table 1: Effect of Methanolic and aqueous extract of *Quisqualis indica* Linn on TC, TG, HDL

Treatment	TC	TG	HDL	LDL	VLDL
Control	52.15±0.39	41.37±1.37	21.65±0.44	24.66±1.03	8.275±0.27
PS	64.05±0.89***	55.49±0.52***	12.45±0.40***	40.50±0.49***	11.10±0.10***
Atorvastatin	56.71±0.73***	45.69±0.52***	17.35±0.39***	30.23±0.37***	9.14±0.10***
PS + ME (200 mg/kg)	58.57±0.37**	47.94±0.78***	16.49±0.61***	32.88±0.27***	9.588±0.16***
PS + AE (200 mg/kg)	60.30±0.61*	48.14±0.60***	16.10±0.08***	34.17±0.40***	9.63±0.12***
PS + ME (100 mg/kg)	60.17±0.89*	48.92±0.97**	15.85±0.19**	34.54±0.99***	9.784±0.19**
PS + AE (100 mg/kg)	62.57±0.54	50.59±0.68*	15.49±0.18**	36.97±0.40*	10.12±0.14*

Values are in mean ± SE; n- number of animals in each group = 6; ***p < 0.001, **p < 0.01, *p < 0.05 compared with induced groups using ANOVA software followed by Tukey's multiple comparison tests; Where, PS- Passive Smoking, ME- Methanolic Extract, AE- Aqueous Extract, TC- Total cholesterol, TG- Triglyceride, HDL- High density lipoprotein, LDL- Low density lipoprotein and VLDL- Very low density lipoprotein.

DISCUSSION

Cell membranes are made of unsaturated lipids and these unsaturated lipid molecules of cell membranes are particularly susceptible to free radicals. Oxidative damage can direct to a breakdown or even hardening of lipids, which is a composition of all cell walls. Breakdown or hardening is due to lipid peroxidation leads to death of cell or it becomes unfeasible for the cell to properly get its nutrients or get signals to achieve another. Antioxidants cause protective effect by neutralizing free radicals i.e. inhibition of lipid peroxidation or lipolysis, which are toxic byproducts of natural cell metabolism¹⁸.

Active smoking is associated with reduced HDL cholesterol levels in adults and young persons. In adults, passive smoking had been reported to have a similar effect. Passive smoking causes approximately 50,000 deaths annually in the United States, with the vast majority of these deaths due to heart disease³. The present results shows that the extracts of *Quisqualis indica* linn produce a significant reduction in harmful lipids and raised the HDL level which is good cholesterol and thus it act as antihyperlipidemic drug.

It is well known that HDL-Cholesterol levels have a protective role in Coronary artery disease¹⁹. The increased level of HDL- cholesterol and decreased cholesterol level along with its LDL and VLDL fraction which is evident from the results could be due to a decreased oxidation of lipid so that it cannot breakdown to form plaque and block the artery so the atherosclerosis doesn't exists which is mainly due to the presence of flavonoids which act as antioxidant²⁰ and scavenge the free radical between endothelial cells. Thus the decreasing harmful lipid levels in the body under the

influence of plant *Quisqualis indica* linn could have stop the formation of more oxygen between endothelial spaces.

The antihyperlipidemic activity of *Quisqualis indica* extracts (100 and 200 mg/kg) against passive smoking showed significant activity comparable to Atorvastatin treated groups in a dose dependant manner. The result positively suggests that the antihyperlipidemic activity of these herbal plants could be attributed to the presence of the valuable flavonoids and other components in the extracts simultaneously it also reduce cholesterol and triglycerides which strongly strengthen the hypolipidemic activity of the plant.

CONCLUSION

The present study established that the passive smoking raise the lipid and cholesterol level with reducing the HDL level which cause hyperlipidemia as well as hypercholesterolemia existing heart disease such as heart attack, heart stroke etc in future. Epidemiological studies suggest that increased dietary intake of antioxidants reduces the risk of coronary artery disease and the plant extracts showing positive indication as it contains flavonoids and phenolic compounds that helpful in CVD. The present investigation shows that the methanolic extracts of aerial parts of QI had markly reduced the raised lipid level LDL, VLDL and cholesterol due to passive smoking induced, thus it acts as hypolipidemic at dose dependent manner.

Thus, our present study showed that administration of Methanolic and aqueous extract of 100 and 200 mg/kg of *Quisqualis indica* was effective to manage hyperlipidemia in which Methanolic extract of plant at dose 200 mg/kg was found to be more effective. The active ingredients

present in plant extracts may recover the disorders in lipid metabolism noted in hyperlipidemic state.

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REFERENCES

1. Beltina.org encyclopedia of health, 1999, <http://www.beltina.org/health-dictionary/hyperlipidemia-symptoms-treatment-definition.html>.
2. Hubert Chen, VeriMed Healthcare Network, San Francisco 2005. www.healthscout.com/ency/1/366/main.html
3. Barnoya J, Glantz SA, Cardiovascular effects of secondhand smoke: nearly as large as smoking. *Circulation*, 111, 2005, 2684-2698.
4. Mitchell, B, Hawthorne VM, Vinik AI., Tobacco Use and Cessation: The Adverse Health Effects of Tobacco and Tobacco-Related Products, *Primary Care: Clinics in Office Practice*, 26(3), 1999, 463-98.
5. Vadnere GP, Patil AV, Jain SK, Wagh SS., Investigation on in-vitro Antioxidant activity of whole plant of *Cassia occidentalis* Linn. (Caesalpiniaceae), *IJPRIF*, 3, 2011, 1985-1991.
6. Engler M. M, Engler M. B., Malloy M.J., Chiu E. Y., Schloetter M. C., Paul S. M. , Stuehlinger M, Lin Ken Y., Cooke John P., Morrow Jason D., Ridker Paul M, Rifai Nader, Miller Elizabeth, Witztum Joseph L., Snyder Michele Mietus, Antioxidant Vitamins C and E Improve Endothelial Function in Children with Hyperlipidemia: Endothelial Assessment of Risk from Lipids in Youth (EARLY), American Heart Association, 2003, 0009-7322.
7. Yadav Yashraj, Mohanty PK, S B Kasture, Anti-inflammatory activity of hydroalcoholic extract of *Quisqualis indica* Linn. flower in rats, *International Journal of Pharmacy & Life Sciences*, 2, 2011, 977-981.
8. Yadav Yashraj, Mohanty PK, S B Kasture, Evaluation of immunomodulatory activity of hydroalcoholic extract of *Quisqualis indica* Linn. flower in wistar rats, *IJPLS*, 2011, 2, 689-686.
9. Singh Nitu, Khatri Pankaj, Samantha K.C., Damor Reena, Antipyretic activity of methanolic extract of leaves of *quisqualis indica* linn, *IJPRD*, 2,2010, 122-126.
10. Jahan Fatima N, Rahman Mohammad S. , Rahman M. Mukhlesur, Gibbons Simon, Mohammad M. Masud, Sadhu Samir k., Hossain Mahboob, Hasan Choudhury M., Mohammad a. Rashid, Diphenylpropanoids from *quisqualis indica* linn. And their anti-staphylococcal activity, *latin american journal of pharmacy*, 28 (2), 2009, 279-83.
11. Kaiser Md. Abul, Islam Mohammad Rashedul, Mohammad Sarifur Rahman, Md. Khalid Hossain , Mohammad A. Rashid, Total Phenolic Content, Free Radical Scavenging Activity and Reducing Power of *Quisqualis indica* Linn", *Dhaka Univ. J. Pharm. Sci.*, 8(2), 2009, 173-175.
12. Wetwitayaklung Penpan, Limmatvapirat Chutima, Thawatchai Phaechamud, Sindhchai Keokitichai, Kinetics of Acetylcholinesterase Inhibition of *Quisqualis indica* Linn. Flower Extract, *Silpakorn U Science & Tech J*, 1(2), 2007, 20-28.
13. Bagchi Manashi, Mark Milnes Casey Williams, Jaya Balmoori, Xumei Ye, Sidney Stohs, Acute and chronic stress-induced oxidative gastrointestinal injury in rats, and the protective ability of a novel grape seed proanthocyanidin extract, *Nutrition Research*, 19 (8), 1999, 1189–1199
14. Paech D., Tracey M. V., *Modern Methods of Plant Analysis*, Berlin, Springer- Verlag, 4, 1995, 373-374.
15. Ecobichon DJ., *The basis of toxicity testing*. CRS Press, New York. 1997, 43-49.
16. Kapral A, Lavernel L.S., *Lipid metabolism*, In *clinical Chemistry: interpretation and techniques*, Lea & febiger, Philadelphia, 2nd ed, 1983 333-336.
17. Third report of the National Cholesterol Education Program (NCEP) Expert Panel on Detection, Evaluation and Treatment of High Blood Cholesterol in Adults (Adult Treatment Panel III). Executive Summary. Available at: <http://www.nhlbi.nih.gov/guidelines/cholesterol/atp3xsum.pdf> Accessed April 16, 2001.
18. Warnholtz A, Mollnau M, Oleze.M, Wendt, Munzel.T, Antioxidants and endothelial dysfunction in hyperlipidemia. *Curr. Hytertens. Rep*, 3, 2001, 53-60.
19. Wilson P.W., Abbott R.D, Castelli W.P., High density lipoprotein cholesterol and mortality, *The Framingham heart study*. *Arteriosclerosis*, 8, 1988, 737-740.
20. Zhang Y. Influence of smoking on cholesterol concentrations in serum lipoprotein of healthy subjects, *Chung-Hua Liu Hsing Ping Hsueh Tsa Chih*. 13, 1992, 97-100.

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