Available online at www.journalijmrr.com

INTERNATIONAL JOURNAL OF MODERN RESEARCH AND REVIEWS

International Journal of Modern Research and Reviews
Volume1, Issue 1, pp 35-43 December,2013

ISSN: 2347-8314

ORIGINAL ARTICLE

ROLE OF METHANOLIC EXTRACT OF *Dolichos biflorus* SEEDS IN HYPERLIPIDAEMIC MODELS OF WISTER ALBINO RATS

C. Menaka and *A. Mary Helitha

Department of Zoology, Govt. Arts College for Women, Pudukkottai - 622001

Article History: *Received 18th Nov,2013, Accepted 23th Nov,2013, Published 16th December,2013*

ABSTRACT

The aqueous extract of *dolichos biflorus* seeds was tested for in hyperlipidaemic models of wister albino rats. *dolichos biflorus* seeds exhibited significant protective activity by reducing lipids. The present study shows the Total Cholesterol Triglceride High Density Lipoprotein Low Density Lipoprotein Low Very Density Lipoprotein were altered due to cholesterol induction. After the *dolichos biflorus* seeds extract treatment, the Total Cholesterol Triglceride High Density Lipoprotein Low Density Lipoprotein Low Very Density Lipoprotein were recovered. The present study concludes that the *dolichos biflorus* reduces the hyperlipidaemic models of rats

Keywords: *Dolichos biflorus, Lipid profile Cholestrol, Rats*

1.INTRODUCTION

Herbal Medicine and herbal plant maintains stays of about 75-80% of the world population mainly in the developing countries for primary health care because of better cultural acceptability. The world health organization recently defined tradition medicine (Including herbal drugs) as comprising therapeutic practices that have been in existence after from hundreds of years. Herbal drug constitute a major share of all officially recognized system of health in India more than 80% of the world population depend on traditional plant based medicine for primary health care plant and plant products are being used as a source of medicinal value. Medicinal plants were assuming greatest importance in the primary health care of individual and community in many developing countries.

Plants and herbs in the Indian system of medicine have been reported to be beneficial against various diseases more than hundred of plants possess medicinal value. Indian medicinal plants are rich source of substances that have several therapeutic properties cardioprotective , chemoprotective and other effects. Medicinal plants are assuming greater importance in primary health care of individuals and communities in many developing countries .The medicinal plants are widely used by all section of society whether directly as folk remedies or indirectly as pharmaceutical preparation of modern medicine. The natural products are non toxic have less side effects and safetly available at affordable cost. Now a day there is a retrival of interest with herbal based medicine due to the increasing radization of the health. Hyperlipidemia has been ranked as one of the greatest risk factors contributing to the prevalence and severity of coronary heart diseases (Grundy, 1986).Coronary heart disease, stroke, atherosclerosis and hyperlipidemia are the primary cause of death (Davey Smith, 1993).

Hyperlipidemia is characterized by elevated serum total cholesterol, lowdensity, and very low-density lipoprotein and decreased high-density lipoprotein levels. Hyperlipidemia associated lipid disorders are considered to cause atherosclerotic cardiovascular disease (Saravanan *et al.*, 2003). Among these hypercholesterolemia and hypertriglyceridemia are closely related to ischemic heart disease (Kaesancini and Krauss., 1994). The main aim of treatment in patients with hyperlipidemia is to reduce the risk of developing ischemic heart disease or the occurrence of further cardiovascular or cerebrovascular disease (Smith and Pekkanen., 1992). Currently available hypolipidemic drugs have been associated

Corresponding author:
A. Mary Helitha, Department of Zoology,
Govt. Arts College for Women, Pudukkottai - 622001

with a number of side effects (Brown, 1996). Hyperlipidemia is a highly predictive risk factor for atherosclerosis, coronary artery diseases and cerebral vascular diseases (Wang *et al.,* 1997). Hyperlipidemia is characterized by elevated serum total cholesterol and low density and very low-density lipoprotein cholesterol and decreased high density lipoprotein levels. Hyperlipidemia associated lipid disorders are considered to cause atherosclerotic cardiovascular disease (Saravanan *et al.,* 2003).

Atherosclerosis (Sclrero-hardening) of arteries is a generalized disease of arterial network known as a progressive and silent killer disease characterized by the formation of lesions called atherosclerosis plaques in the walls of large and or medium sized coronary arteries and reduces blood flow to the myocardium called coronary artery diseases (CAD) (Mohale *et al.,* 2008). Hyperlipidemia is classified into primary and secondary type, which indicates the complexities associated with the disease. The primary disease may be treated using anti-lipidemic drugs but the secondary type originating from diabetes, renal lipid nephrosisorhypothyroidism demands the treatment of the original disease rather than hyperlipidemia (Suzuki and Suzuki., 2006). Medicinal plants play a major role in hypolipidemic activity, literature suggests that the lipid lowering action is mediated through, inhibition of hepatic cholesterol biosynthesis and reduction of lipid absorption in the intestine (Gramza and Korczak., 2005).

The seed of the plant can be added to the diet to reduce serum cholesterol concentration. Saponins of the arial parts and leaves are responsible for activity of neutralizing cholesterol to be excreted from the body. The whole plant material contains many important substances including steroidal coumarin, flavonoids rich in value in other medicinal activity. The saponins are involved in reducing the hyperlipidemia. The increase in the blood cholesterol causes the hyperlipidemia. It is one of the risk factor for coronary artery disease. The increase in level of cholesterol causes the disease related to heart like cardio vascular disease, atherosclerosis and fatty streak formation.

The fat protein complexes in the blood are called lipo proteins. The excess LDL cholesterol contributes to blockage of arteries 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. 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. The most serious effect of hyperlipidemia in the obstruction of blood vessels in heart and brain.

Hypercholesteremia is one of the risk factors for coronary artery diseases. The increase in the level of cholesterol causes the diseases related to heart like cardio vascular disease atherosclerosis and fatty streak formation.People who are proposed to a combination of risk factors (Dietary habit, genetic susceptibility etc) are more prone to develop hyper cholesterolemia. Besides stress sedentary habits use of tobacco and alcohol are reported to have an additive effect in contributing to develop hypercholesterodemia (Ashakkumary and Vijayammal, 1993).

Hyperlipidemia along with diabetes hypertension positive family history and smoking may also cause coronary heart disease. Hyperlipidemia occurs as a consequence of several interrelated factors that may be lifestyle genetic metabolic or other conditions that influence plasma lipoprotein metabolism. Hyperlipidemia usually has no noticeable symptoms and tends to be discussed during routine examination or evaluation for atherosclerotic, cardiovascular disease.

Dolichos biflorus Linn (Fabaceae), is commonly known as Kollu in Tamil and horse gram in English. It has been reported to lower lipids in rats (Kottai Muthu *et al.,* 2005). A high fat diet induces oxidative stress in the cells by producing reactive oxygen species. Therefore, in this study, the influence of the *Dolichos biflorus* extract on high fat diet (HFD) induced oxidative stress in rabbits, has been investigated. Whole plants of *D. biflorus* were collected from keeramangalam, pudukkottai district of Tamilnadu, India. Taxonomi identification was made by the Botanical Survey of Medicinal Plant Unit, Siddha, Government of India, Palayamkottai, Tamilnadu.

Four month-old whole plants were dried in the shade, segregated, and pulverized by a mechanical grinder and passed through a 40 mesh sieve. The powder was extracted by methanol in Soxhlet apparatus by continuous hot percolation method. After filtration through Whatmann filter paper No 40, the filtrate was vacuum dried at 35 to 40oC. The extracts were stored in screw cap vials at 4oC until further use. The extractive value of the methanolic extract was 8.13% w/w. The methanolic extract of *D.biflorus* was subjected to preliminary phytochemical screening to find out the presence of active principles. The extracts were suspended in 2% tween 80.

The plant *Phyllanthus amarus* Schumach (Euphorbiaceae) is commonly known as 'Bhuivali' usually occurs in Asam, Maharashtra, Burma, Nicobar, Islands Malesia and America. *Phyllanthus amarus* Schumach is a native to the Americas (Van Holthoon, 1999). The Spanish name of the plant, *Chanca piedra*, means, "Stone breaker". It was named for its effective use by generations of Amazonian indigenous peoples to eliminate gallstones and kidney stones. In India, the plant is used for numerous conditions including colic, diabetes, malaria, dysentery, fever, flu, tumors, jaundice, vaginitis, gonorrhea, and dyspepsia (Kirtikar and Basu, 1935; Burkill, 1966). The juice or extract of its thinner roots and young leaves are taken internally to stimulate the kidney. Heyne recorded its uses in the Dutch Indies (Indonesia) for stomachache, gonorrhea and children's cough. However, no systemic study on antihyperlipidemic activity of the leaves has been reported in the literature. In present investigation, we have screened hydro-alcoholic extract of leaves of *Phyllanthus amarus* Schumach (HAEPAS) for its anti-hyperlipidemic activity (Van Holthoon, 1999).

The blood lipids (total cholesterol, LDL-cholesterol, HDL-cholesterol and triglycerides) have been shown to be related to the development of coronary heart disease (CHD), since these risk factors play an important role in determining atherogenesis and the subsequent space of atherosclerosis. Evidence from clinicopathological and epidemiological studies overwhelmingly confirms cholesterol, the greater the plaque build up (Temple and Burkitt 1994).

Mimosa pudica (Mimosaceae) known as chue Mue, is a stout straggling prostrate shrubby plant with the compound leaves sensitive to touch. It has spinous stipules and globose pinkish flower heads and grows as weed in almost all parts of the country(Ghani, 1998). Leaves and stem of the plant have been reported to contain an alkaloid mimosine; leaves also contain mucilage and the root contains tannins . *Mimosa pudica* is used for its anti-hyperglycemic (Umamaheswari and Prince., 2007), anti-diarrhoeal (Balakrishnan *et al.*, 2006), anti-convulsant(Bum *et al.*, 2004), cytotoxic(Chowdhury *et al.*, 2008) and hepatoprotective properties(Rajendran *et al.*, 2009). The plant also contains turgorins leaves.

Consumption of much fat may lead to the production of extra VLDL, resulting in the formation of large amounts of LDL which may stick to the walls of the blood vessels if the quantity of HDL is insufficient, causing blockages for the normal flow of blood. Therefore, improvement in human diet is highly recommended for disease prevention (Ryan Diet, 2003). Medicinal plants play a major role in hypolipidemic activity. The leaves of *Aleurites moluccana*, *Piper betle* suggests that the lipid lowering action is mediated through inhibition of hepatic cholesterol biosynthesis and reduction of lipid absorption in the intestine (Gramza and Korczak., 2003).

Sapindus emarginatus Vahl family Sapindaceae is a medium-sized deciduous tree found in south india. It is commonly called as soap nut tree. Native to South India *Sapindus emarginatus* is found wild or introduced in tropical and sub-tropical regions, particularly the Indo- Malayan region. Traditionally, *Sapindus emarginatus* is used as anti inflammatory and antipurutic. It is used to purify the blood. The seed is in intoxicant and the fruit rind has oxytropic action. Its powder is used as nasal insufflations. *S.emarginatus* also showed strong antibacterial activity against the tested bacterial strains (Nair *et al.*, 2005). Antifertility and antiandrogenic activities of *S.emarginatus* extract have been reported(Venkatesh *et al.*, 2002).High content of saponins has been reported in the pericarp. Two Pisicidal triterpenoid saponin, acetylated triterpene saponins, hederagenin, sweet acyclic sesquiterpene glycoside, Mukuroziosides IIb have been isolated from the Pericarps of *S. emarginatus*. Flavonoids have been isolated from the pericarp of *Sapindus emarginatus* (Tripetch *et al.*, 2001).

2.MATERIALS AND METHODS

Collection of plant materials

Selected healthy seeds of *Dolichos biflorus* was collected from Keeramangalam Pudukkottai district (Plate – I a). The botanical identity of the specimens was confirmed by using floras of the presidency of madras and flora of Tamilnadu (Kirthikar and Basu, 1935).

Extract preparation

The seeds were dried under shade. The dried seeds were separately subjected to pulverization to get course powder (Plate –I b). Extract with methanol by continuous hot percolation process using soxhlet apparatus. After completion of extraction it was filtered and the solvent was removed by distillation under reduced pressure brown residue was obtained. The extract was then stored in a refrigerator at 4°C. The aqueous extract of the plant material was also prepared by boiling in distilled water. Extract of *Dolichos biflorus* was obtained and used as an herbal medicine (Plate-I c).

Phytochemical screening

Phytochemical test were carried out with the aqueous extract and ethanolic extract of *Dolichos biflorus* using standard procedure to identify the constituents (Sofowara, 1993).

Test for tannins

0.5 ml of aqueous extract was taken and few drops of 0.1% ferric chloride was added and observed brownish green or blue black coloration.

Test for phlobatannins

1 ml of aqueous extract was boiled with 1% aqueous hydrochloric acid deposition of red precipitate indicates the presence of phylobatannins.

Test for saponins

10 ml of the aqueous extract was mixed with 5 ml of distilled water and shaken vigorously for a stable persistant froth. The frothing was mixed with 3 drops of olive oil and shaken vigorously then observed for the formation of emulsion.

Test of flavanoids

5 ml of dilute ammonia solution was added to aqueous filterate extract. Followed by addition of concentrated sulphuric acid. The yellow color indicates the presence of flavonoids.

Test for steroids

To 0.5 ml of ethanolic extract of *medicago sativa* 1ml of acetic anhydride and 2ml of sulphuric acid was added. The change of color from violet to blue or green indicates the presence of steroids.

Test for terpenoids

To 5 ml of ethanolic extract 2 ml of chloroform and 3ml of concentrated sulphuric acid was added to form a layer. The formation of reddish brown color at the interface indicates the presence of Terpenoids.

Teat for cardiac glycosides

To 5 ml of ethanolic extract 2 ml of glacial acetic acid and one drop of ferric chloride solution was added followed by 1ml of concentrated sulphuric acid. The formation of brown ring indicates the presence of deoxy sugars.

Purchasing of animals

Male albino rats weighing 100-150 g were obtained from Tamil university animal house. Animals were housed in well ventilated standard cage with normal daynight (12 h light dark cycle) light on at 07:005. Fresh, dry husk was used as bed material. They were fed with commercial pellet diet obtained from Sai Durga feeds and foods from Bangalore and they had free access from tap water.

Experimental designing of animals

The animals were divided into three groups containing Four rats in each group;**Group-I** Animals received standard diet and act as a control;**Group-II** Animal received cholesterol diet (one full egg yolk) for 7 days; **Group-III** Animals received *Dolichos biflorus* extract along with cholesterol diet.

Cholesterol induction

The cholesterol in rat was induced by egg yolk. The boiled egg yolk was given to group-II and group-III rats for a period of 7 days.

Egg yolks are nutrition rich food containing 17 different vitamins and mineral including omega -3 fatty acids, folate and vitamin E, which may be associated with protection from some of the risk factors for coronary heart disease.

Egg yolks provide about half the dietary cholesterol in a typical western diet. The British nutrition bulletin suggests that consumption of up to 1 egg per day is unlikely to have substantial overall impact of cholesterol level. The normal egg yolk contains about 215 mg of cholesterol (Sharon natoli, 2001)

Herbal drug administration

Herbal drug was administrated through oral route. Before drug administration Group - III animals were allowed to starve for 2 hours to enhance the intestinal absorption of drug. The drug was administered at 0.5 ml /kg and the experiment was carried out for 7 days (Plate – IV).

Assessment of biochemical parameters

Collection of blood

After the experiment regimen the animals were demanded to overnight fasting but were permitted for water. From all group of animal 5 ml of blood sample were drawn using heart puncture (Plate-IV).

Serum collection

The collected blood was centrifuged at 3000 rpm for 10 minutes. The serum was collected and stored in cool temperature it was used for assessing the various biochemical parameters such as Total cholesterol,Triglyceride,High density lipoprotein,Low density lipoprotein and Very low density lipoprotein

Total Cholesterol

The amount of cholesterol was estimated by Zatskis, Zak and Boyle's method (1970).Take 0.2 ml of serum add 9.2ml working ferric chloride acetic acid reagent mixed and allowed to stand for 15 min and centrifuge. Take 0.5 ml of supernatant and add 3ml of conc. sulphuric acid and 1 ml of ferric chloride acetic acid reagent.Prepare a series of working standard of cholesterol. prepare a blank with 6 ml of ferric chloride acetic acid reagent. To all the tubes add 5 ml of concentrated sulphuric acid and mix well measure the color which develops at 560 nm after 15 minutes. Values are expressed as mg/dl.

Triglceride

The levels of triglyceride were estimated by the procedure of Rice (1970).To 0.1 ml of serum 8.9ml of isopropanol was added followed by 50 mg of activated alumina. It was mixed well and left for 15 minutes. It was centrifuged and 2 ml of the supernatant was taken for analysis 0.6 ml of alkaline potassium hydroxide was added to all the tube. The tubes were incubated at 60 °C for 10 minutes. The tubes were cooled and 1 ml of sodium metaperiodate reagent was added to the tubes. Followed by the addition of 0.5 ml of acetyl acetone reagent. The tubes were cooled and the color developed was read at 405 nm. Values are expressed in mg/dl.

High Density Lipoprotein

The HDL cholesterol was estimated by the Heparin manganese chloride precipitation (Williams, 1996).To 1 ml of plasma 0.8ml of heparin manganese chloride reagent was added and mixed after standing in ice bath for 30 minutes, the contents were centrifuged at 2500 rpm for 30 minutes to get HDL fraction.An aliquot of the HDL supernatant was used for cholesterol estimation by the methods of Zlatkis zak.Values are expressed in mg/dl.

Low Density Lipoprotein

The value of LDL cholesterol can be calculated as follow if the value of total cholesterol triglycerides and HDL cholesterol are known, LDL cholesterol can be calculated based on Fried walds equation.Values are expressed as mg/dl.

Very Low Density Lipo Protein

The value of VLDL Cholesterol can be calculated as follow. If the value of triglyceride is known, VLDL cholesterol can be calculated based on fried welds equation.

Statistical analysis

The results obtained in the present investigation were subject to statistical analysis like Mean ($\bar{x}$) and Standard Deviation (SD) by Zar (1984).

$$Mean\ (\bar{x}\ ) = \frac{\sum x}{N}$$

The Standard Deviation (SD) was calculated by the following formula.

$$\text{Standard Deviation } (\delta) = \sqrt{\frac{\sum(x-\bar{x})^2}{N-1}}$$

3.RESULTS

Screening of phytochemical compounds

The phytochemical active compounds of *Dolichos biflorus were* qualitatively analyzed and the results were presented in and Table-1. In this study, phytochemical compounds such as saponin, flavanoids, were present, tannins, phlobatannins, steroids, terpenoids and cardiac glycosides were absent in *Dolichos biflorus.*

Table. 1 Screening of phytochemical compounds of *Dolichos biflorus*

S.No	Phytochemical compounds	Observation
1	Tannins	Absent
2	Phlobatannins	Absent
3	Saponin	Present
4	Flavanoids	Present
5	Steroids	Absent
6	Terpenoids	Absent
7	Cardiac glycosides	Absent

Fig.1 Body weight on normal, cholesterol induced and treated

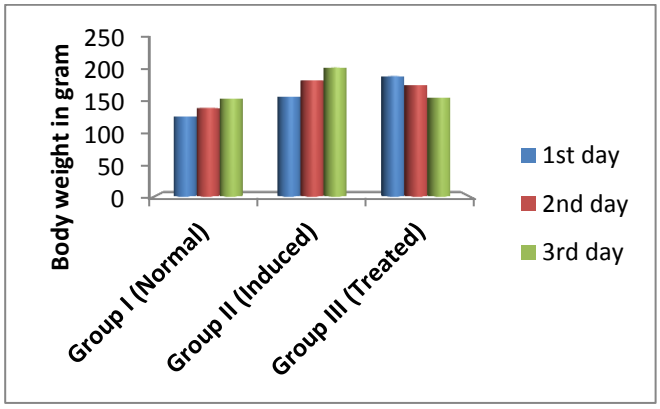
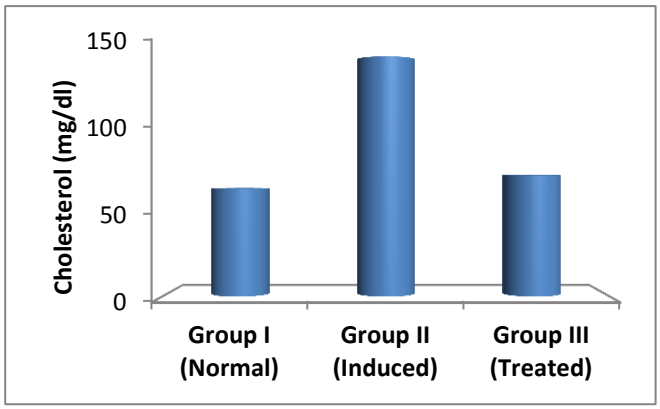


Fig. 2 The Level of Total cholesterol in albino rats before and after treatment of *Dolichos biflorus*



Body weight

The body weight of the experimental rats were measured and the results were presented in Table-2 and Fig-1. The weights are measured in gram.

The body weights are measured upto 7 days. When egg yolk orally administrated rat's body weight significantly increased at the 7th day (159 to 205.25) s compared than control rat (127.25 to 155.75). At the same time cholesterol induced *Dolichos biflorus* plant methanolic extract treated rat's body weight increased in 1st day and decreased after administration of the plant extract at 7th day (191.5 to 157.5). After 7th day treatment ,Group III rats body weight reached to the normal rats.

Fig 3 The Level of Triglyceride in albino rats before and after treatment of *Dolichos biflorus*

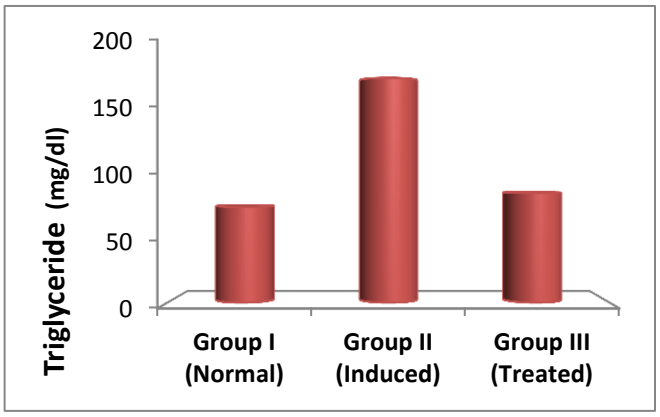
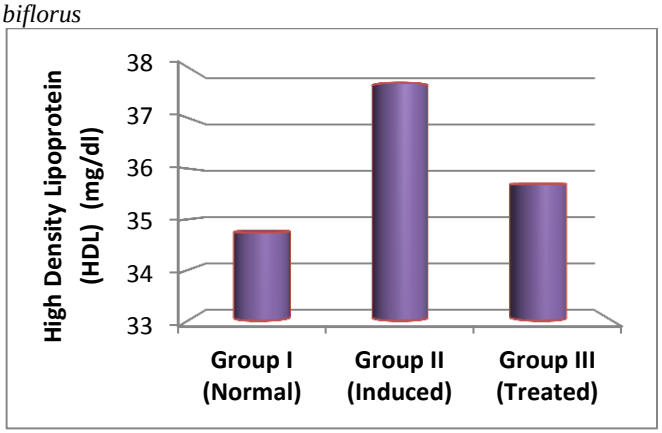


Fig. 4 The Level of High Density Lipoprotein (HDL) in albino rats before and after treatment of *Dolichos biflorus*



Cholesterol level

Anti-cholesterol activities of *Dolichos biflorus* were analysed in the albino rats. Total cholesterol level was estimated in all the three groups. The group-I (Control) rat's cholesterol level was estimated on 7th day was noted 64.31 mg/dl. Similarly the cholesterol level was estimated in group-II (Cholesterolinduced

Fig. 5 The Level of Low Density Lipoprotein (LDL) in albino rats before and after treatment of *Dolichos biflorus*

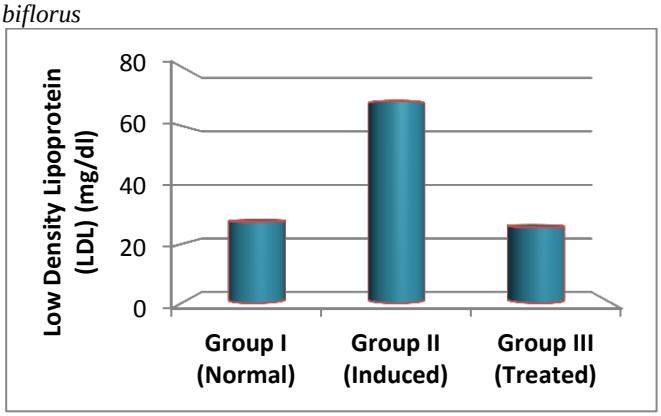
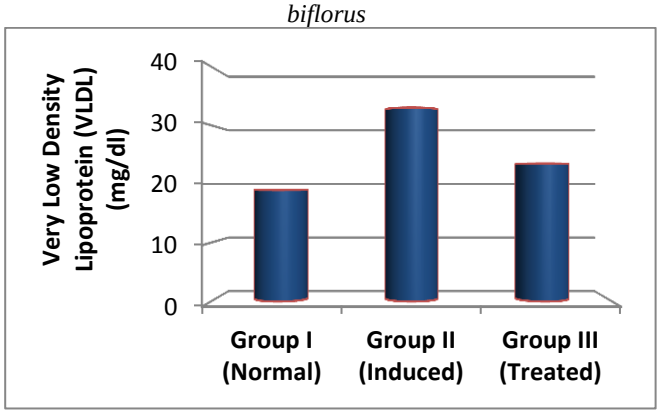


Fig. 6 The Level of Very Low Density Lipoprotein (VLDL) in albino rats before and after treatment of *Dolichos biflorus*



the cholesterol level is gradually increased from 7th days range from 143.41 mg/dl. The plant extract treated rats cholesterol level was reduced (72.38). the results were presented in Table-3 and fig-2.

Triglyceride and HDL

The level of Triglyceride and HDL were analyzed in all experimental group rats and the results were given in the Table-4, 5 and fig-3, 4. In this study both Triglyceride and HDL increased in cholesterol induced rats 174.68 and 37.69 respectively.In the plant extract treated group III (85.16 and 35.70) Triglyceride and HDL level significantly decreased the level reach to group-I (74.42 and 34.74) respectively.

Level LDL and VLDL

LDL level of three experimental group's rats was estimated. The results were presented in Table-6 and Fig-5. The highest level of LDL were showed in group –II (68.49). The level slightly decreased in plant extract administered rats (25.66). The control rat LDL level (3.74 and 27.47).

The VLDL was found to be (18.96) control in rat after cholesterol induction the level was increased to be (32.99) gradually decreased level of VLDL were noted in group III (23.48) which is compared with group I (18.96). The results were presented in Table-7 and Fig-6.

4.DISCUSSION

Horse gram (*Dolichos biflorus*) is indigenous to the Indian Subcontinent; Archaeological investigations have revealed the use of horse gram as food around BC (Mehra 2000). The Brahadaranyaka (5500BC), a Commentary on the Rigveda (8000BC) mentions Khatakula, which is the original Sanskrit name for horse gram. The yajuveda (7000BC) mentions the Sanskrit Kulattha (Achaya 1998) as the name for horse gram. Subsequently Buddhist and Jain literature, and Kautilya's Arthasastra, all mention Kulattha. The original Latin name for horse gram was *Dolichos biflorus*, which was later changed to *Dolichos uniflorus.*

The active phytochemcial compounds were analyzed in *Dolichos biflorus* tannins, saponin, flavanoids, terpenoids were present this phytochemical compounds may be responsible for anticholesterol activity.

The *Dolichos biflorus* seed lectin is a tetramer composed of equal amounts of two subunit types. The subunit types are structurally very similar, yet only the larger subunit exhibits the ability to bind carbohydrate. A cDNA cone representing the entire coding region of the *D. biflorus* lectin mRNA has been sequenced.

Germination induces changes in the Bowman-Birk type proteinase inhibitors (BBIs) of horse gram (*Dolichos biflorus*) at both qualitative and quantitative levels. The original isoinhibitors present in the dry seed almost disappear by the fifth day of germination with the concomitant appearance of two new inhibitor species. The isoinhibitors present in the dry seed are electrophoretically distinct from the isoinhibitors that appear during the early stages of seed development. The two inhibitor species that appear during the process of germination appear to be electrophoretically similar to the inhibitor species found in the flower, leaf and early stages of seed development (Yadahalli Sreerama and Lalitha Gowda, 1998).

In this study body weights are measured upto 7 days. Egg yolk orally administrated rat's body weight significantly increased when compared than control rat At the same time *Dolichos biflorus* plant methanolic extract treated rat's body weight increased initially decreased after administration of the plant extract. Soxhlet extract of seeds of *Dolichos biflorus* and Rhizomes of *Bergenia ligulata* were tested for their invitro antilithiatic/anticalcification activity by the homogenous precipitation method. The extracts were compared with an aqueous extract of cystone (a marketed preparation) for their activities. Also a combination of the extracts of the two plants was tested. Extracts of *Dolichos biflorus* showed activity almost equivalent to cystone while *Bergenia ligulata* showed less activity and the combination was not as active as the individual extracts (Garimella Jolly and Narayanan, 2000).

In this study total cholesterol, triglyceride, LDL, HDL, VLDL, level also increased in fat diet administrated rat. The rats were treated with *Dolichos biflorus* plant extract, after the administration all the parameters were decreased to control level.The similar result was reported as (Muthu *et al.*, 2006) *Dolichos biflorus* Linn in high fat diet fed rabbits were noted.

Methanolic extract of *D. biflorus* administration to high fat diet fed rats showed near to normal levels of body weight and thiobarbituric acid reactive substances. And also methanolic extract of *D. biflorus* showed increased in the level of glutathione and enhance the activities of antioxidant enzymes, which is superoxide dismutase and catalase in liver, heart and aorta of rabbit fed with HFD. Higher dose of plant extract was found to be more effective and showed comparable results with standard drug atorvastatin on these parameters.

High fat diet fed rats showed significant increased levels of plasma and tissue total cholesterol, triglycerides, free fatty acids, phospholipids, plasma LDL cholesterol and decreased level of plasma HDL cholesterol. Methanolic extract of *D. biflorus* administration to high fat diet fed rats showed near to normal levels of the above lipids in plasma and tissues. Higher dose of the extract (400mg/ kg body weight) showed comparable results with standard drug atorvastatin. It is concluded that the methanolic extract of *D. biflorus* possesses hypolipidemic activity in high fat diet fed rats (Kottai muthu *et al.*, 2005).

Total phenolics and the antioxidative properties of two varieties of horse gram (*Macrotyloma uniflorum*) were studied. The raw dry heated seed samples were extracted successively with methanol and 70% acetone separately. After removing the solvents, the extracts were freeze-dried. The black seeds contained relatively high levels of total phenolics and tannins than the brown seeds with respect to the treatments and solvents extraction (Perumal Siddhuraju, and Sellamuthu Manian, 2007).

Kottai muthu *et al.*, (2005) reported that both higher and lower doses of *D. biflorus* extract showed decreased levels of plasma, total cholesterol, triglycerides, free fatty acids, phospholipids, plasma LDL cholesterol and increased HDL cholesterol level in albino rats. Higher doses of the extract (400mg/kg body weight) showed comparable results with drug atorvastatin. From the above results it is confirmed that *Dolichos biflorus* on herbal formulation can be included in diet, to reduce cholesterol level and heart disease and the saponins and flavanoids present in herbal plant may involve in reduction of cholesterol. In my further study the active compound of *Dolichos biflorus* were analysed.

5.ACKNOWLEDGEMENT

The authors are grateful to Head of the Department of Zoloogy, Government Arts College for Women, Pudukkottai to complete the work successfully.

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