# ANTIHYPERLIPIDEMIC PROPERTIES OF *Carthamus oxyacantha*

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**ABSTRACT:** The plants not only provide shelter, food and fuel but also cure(s) us from many diseases. *Carthamus oxyacantha,* locally used as a medicinal plant in the some areas of Pakistan, belongs to family *Asteraceae*. Antihyperlipidemic properties of aqueous and alcoholic extracts of different parts (leaves and seeds) of *Carthamus oxyacantha* were studied in albino rats of Sprague Dawley Strain. Rats were divided in to different groups depending upon the treatment given. Hyperlipidemic was induced by known standard method and hyperlipidemic state was confirmed on 30$^{th}$ day. A normal control group and hyperlipidemic control group were used in the present study. Normal control group and all the treated groups were compared with hyperlipidemic control group. The antihyperlipidemic effects were evaluated by estimating the serum and liver total cholesterol, total triglycerides, HDL cholesterol and LDL cholesterol. The results showed that aqueous and alcoholic extracts of seeds of *Carthamus oxyacantha* possess significant antihyperlipidemic properties ($p<0.01$). The isolation and characterization of active reagent(s) is under investigation.

**Key words:** *Antihyperlipidemia, Asteraceae, Carthamus oxyacantha*

## INTRODUCTION

The rich diversity assembled by the plants for their sustenance and different means adopted by them for their preservation and conservation are remarkable (Trivedi, 2002). Indigenous knowledge is as old as human civilization but the term ethonobotany was first applied by an American Botanists, Harshberger in 1895 to the study of plants used by the primitive and aboriginal people (Pie, 1995).

The World Health Organization (WHO) recognized traditional medicine or herbal medicine about 20 years ago and started exploring the possibilities to improve or popularize the herbal medicine already used by the people in developing countries of the world for thousands of years (Akerle *et al.,* 1991). Drugs of plant origin are now being increasingly used all over the world (Edwards *et al.,* 1995 & Fischbach, 1984).

Hyperlipidemia refers to an increased lipid level in the body, mainly in blood. Lipids are heterogeneous group of compounds that are carried in body fluids as soluble protein complexes known as lipoproteins (Lewis & Elvin Lewis, 1997). Four major groups of lipoproteins have been identified. These include chylomicrons, very low density lipoproteins (VLDL), low density lipoproteins (LDL) and high density lipoproteins (HDL) (Said, 1996). Chylomicrons initially arise from intestinal absorption of triglycerides. LDL is derived from VLDL and carries cholesterol from liver to all other parts of the body whereas HDL carries cholesterol towards the liver. Oxidation of LDL cholesterol causes many complications like arteriosclerosis resulting in heart problems. The present study attempts to investigate and establish antihyperlipidemic properties of *C. oxyacantha* a commonly used medicinal plant for *Asteraceae* family (Ahmad and Zahoor, 2008).

## MATERIALS AND METHODS

*C. oxyacantha* (Voucher No.58) leaves and seeds were collected from the fields and were identified at the taxonomy section, Department of Plant Sciences, Faculty of Biological sciences, Quaid-i-Azam University, Islamabad, Pakistan. Leaves and seeds were washed, dried and powdered mechanically with a china herb grinder. The powder was kept in dry, clean, air tight glass jars and stored at 4°C until used.

**Preparation of Plant Extracts (aqueous/alcoholic):** The aqueous and alcoholic

extracts were prepared by cold extraction method (Masuda *et al.*, 2002) using the prepared powder. The extracts obtained were dried and stored in a refrigerator until used. The respective extract was dissolved in aliquot of 50% ethanol just before treatment to the respective group of rats. The extract was given as intraperitoneal injection.

**Experimental animals:** Healthy young adult male albino rats of Sprague Dawley strain weighing between 180-300g were obtained from the animal house, Department of Biological Sciences, Quaid-i-Azam University, Islamabad, Pakistan. The animals were handled according to European Community guidelines (EEC Directive of 1986; 86/609/EEC).

**Grouping:** The animals were randomly divided into groups of 5 rats each. The grouping was as under. A normal control group (group-1) and hyperlipidemic control group (group-2) were used in the present study. Normal control group and all the treated groups were compared with hyperlipidemic control group.

**Group-1: Normal control:** The animals in this group were kept on standard feed with clean water

**Group-2: Hyperlipidemic control:** The animals in this group were kept on high lipid feed and ethanol (10 % v/v) in drinking water for 30 days whereafter aliquots of 50 % ethanol was given till slaughter.

**Group-3: Treated: aqueous extract (*C. oxyacantha* leaves):** The animals in this group were kept on high lipid feed and ethanol (10 % v/v) in drinking water for 30 days whereafter plant extract 400 mg/kg body weight from 30$^{th}$ day till slaughter.

**Group-4: Treated: alcoholic extract (*C. oxyacantha leaves*):** The animals in this group were kept on high lipid feed and ethanol (10 % v/v) in drinking water for 30 days whereafter plant extract 400 mg/kg body weight from 10$^{th}$ day till slaughter.

**Group-5:Treated: aqueous extract (*C. oxyacantha* seeds):** The animals in this group were kept on high lipid feed and ethanol (10 % v/v) in drinking water for 30 days whereafter plant extract 400 mg/kg body weight from 30$^{th}$ day till slaughter.

**Group-6: Treated: alcoholic extract (*C. oxyacantha* seeds):** The animals in this group were kept on high lipid feed and ethanol (10 % v/v) in drinking water for 30 days whereafter plant extract 400 mg/kg body weight from 30$^{th}$ day till slaughter.

All rats were slaughtered on 60$^{th}$ day. Serum was preserved in the eppendorf tubes at 20°C in freezer until analyzed. Serum samples collected from different groups were analyzed for respective lipid profile estimation using packed kits made by Far (Italy) and sigma (USA). All values are given ±SE. Statistical analyses were made by means of computer program SPSS. A "p" value of 0.01 was taken as level of significance.

## RESULTS AND DISCUSSION

Table-1 represents the initial body weight, final body weight, difference in body weights, and percent body weight gain / loss in different groups. It is obvious from table-1 that there is weight gain in all groups. Weight gain in group-2 (hyperlipidemic control group), group-3 (treated with aqueous extract of leaves) and group-4 (treated with alcoholic extract of leaves) are significantly higher as compared to the group-1 (normal control), group-5 (treated with aqueous extract of seeds) and group-6 (treated with alcoholic extract of seeds). This shows that aqueous and alcoholic extract of the seeds significantly lowered body weight ( $p< 0.01$). While both the aqueous and alcoholic extracts of leaves have no positive effects on the body weight. The improvement in body weight may be a result of improved lipid profile. Similarly final percent liver weight/body weight in group-2, group-3 and group-4 is significantly higher as compared to the group-1, group-5 and group-6 reflecting an increased lipid uptake / biosynthesis in hepatocytes of group 2, 3 and 4.

Table 2 shows the serum lipid profile of different groups of rats. A two fold increase in serum total cholesterol, triglycerides, four fold increases in serum LDL cholesterol and two fold decreases in HDL cholesterol is observed in groups 3, 4 and 5 as compared to the normal control group. Almost same pattern is found in liver lipid profile (table 3). This clearly suggests that ethanol along with a high fat diet caused hyperlipidemia.

There is a resurgence of interest in herbal medicine for the treatment of various ailments, chiefly because of the prohibitive cost of allopathic drugs, their unavailability in remote areas and the popular belief that naturally occurring products are without any adverse side-effects (Hungard, 1988).

Lipids are essential components of biological membranes, fuel molecules and metabolic regulators that control cellular functions, metabolism and homeostasis. Liver plays the central role in regulating lipid metabolism and whole body lipid homeostasis. Sterols, bile acids and fatty acids are the important products of liver (Chyang, 2005).

Ethanol is a powerful inducer of hyperlipidemia in both animals and humans (Avogaro & Cazzolatu, 1975). It also causes a change in the metabolism of lipoproteins. Remla *et al.,* (1991) reported that administration of ethanol to rats causes changes in the metabolism of serum and tissue lipids. The lipid abnormalities seen after alcohol consumption include alterations in the level of cholesterol, fatty acid esters, cholesterol esters and particularly the fatty acyl composition of membrane phospholipids. Therefore in the present study ethanol along with high lipid diet is used to create a state of hyperlipidemia. Augusti *el al.,* (2001) and Tanaka *et al.,* (2001) have reported a three to four fold increase in serum total cholesterol, triglycerides and LDL cholesterol level after feeding the rats an atherogenic diet containing 2 % cholesterol and 1 % cholic acid. However in the present study a two fold increase in total cholesterol, triglycerides, four fold increase in the LDL cholesterol and a two fold decrease in HDL cholesterol level was observed. The difference may be due to the difference in the diet. In our study the high lipid diet consists of butter and vegetable fat purchased from the local market (Latif and Habib Ghee products).

Serum lipid profile in groups 5 and 6 is significantly improved than the hyperlipidemic control group. The decrease in plasma cholesterol level in seeds extract treated groups (5 and 6) showing antihyperlipidemic effects is in accordance with the previous findings of Qureshi *et al.,* (1995). However Qureshi *et al.,* (1995) studied the hypolipidemic effects of tocotrienols. Cholesterol, an essential constituent of cell membranes is also a substrate for the production of vitamins and steroid hormones (Devlin, 1997). The cholesterol pool of the body is derived from absorption of dietary cholesterol from intestine and biosynthesis primarily in liver. When the amount of dietary cholesterol is reduced, cholesterol synthesis is increased in liver and intestine to meet the needs of other tissues and organs (Devlin, 1997). The liver is the only organ capable of excreting significant amounts of cholesterol. This occurs either by biliary cholesterol secretion or by prior conversion of cholesterol to bile acids (Hoffman *et al.*, 1996).

Clinical studies have shown that increased levels of LDL in plasma contribute significantly to the pathogenesis of atherosclerosis (Goldstein & Brown 1977). Diets rich in saturated fatty acids such as butter and vegetable fat raise plasma cholesterol and LDL-cholesterol compared with diets rich in polyunsaturated fatty acids (Cox *et al.*, 1998). Many classes of hypolipidemic drugs, of both natural and synthetic origin, are effective in inducing a negative sterol balance across the liver. Cholestyramine and colestipol are bile salt-binding drugs that promote excretion of bile salts in the stool. This in turn increases the rate of hepatic bile salt synthesis and of LDL uptake by the liver. Lovastatin, an inhibitor of

**Table-1: Effect of *C. oxyacantha*, leaves and seed extracts on body and liver weight.**

| Group No. | GROUP | Initial body weight (g) | Final body weight (g) | Difference in body weight (g) | % body Weight Gain/ loss | Weight of liver | % liver/ final body weight |
|---|---|---|---|---|---|---|---|
| 01 | Normal control | 180.1 ±10.3 | 188.3 ±12.2 | 8.2 ±2.7 | 4.55 ±0.4 [a] | 6.5 ±0.5 | 3.45 ±0.8 [a] |
| 02 | Hyperlipidemic control | 199.3 ±14.5 | 220.4 ±15.4 | 21.1 ±5.2 | 10.6 ±3.5 [b] | 11.3 ±1.6 | 5.2 ±1.3 [b] |
| 03 | Treated(aqueous extract of leaves) | 182.7 ±13.6 | 205.6 ±14.2 | 22.9 ±5.7 | 12.5 ±4.2 [b] | 10.2 ±3.6 | 5.3 ±0.7 [b] |
| 04 | Treated (alcoholic extract leaves) | 203.4 ±12.1 | 224.8 ±15.8 | 21.4 ±4.6 | 10.4 ±2.9 [b] | 11.3 ±4.6 | 4.9 ±1.4 [b] |
| 05 | Treated (aqueous extract seeds) | 192.8 ±10.1 | 209.3 ±14.2 | 16.5 ±4.3 | 8.6 ±1.7 [b] | 7.9 ±0.8 | 3.5 ±0.8 [c] |
| 06 | Treated (alcoholic extract seeds) | 185.3 ±11.6 | 200.7 ±13.4 | 15.4 ±6.1 | 8.3 ±2.5 [b] | 7.8 ±0.3 | 3.7 ±0.6 [c] |

Values with same superscript in a column differ nonsignificantly while with different superscript differ significantly at $p<0.01$ Values given are mean ±SE of 5Hyperlipidemic control is compared with normal control. Treated is compared with hyperlipidemic control

**Table 2: Effect of *C. oxyacantha* leaves extracts on serum and liver lipid profile**

| OBSERVATION | Normal control | Hyper-lipidemic control | Treated aqueous extract | Treated alcoholic extract |
|---|---|---|---|---|
| *Serum lipid Profile (mg/dl)* | | | | |
| Total cholesterol | 76.3±8.9 [a] | 114±12.7 [b] | 119.3±14.7 [b] | 113.1±8.3 [b] |
| Triglycerides | 78.5±13.4 [a] | 174.3±12.8 [b] | 182.4±14.2 [b] | 188.2±12.4 [b] |
| HDL-cholesterol | 54.2±5.8 [a] | 25.7±4.1 [b] | 22.7±3.4 [b] | 17.9±5.2 [b] |
| LDL-cholesterol | 25.9±5.7 [a] | 113.6±8.4 [b] | 129.8±10.3 [b] | 127.5±13.8 [b] |
| *Liver lipid profile (mg/dl)* | | | | |
| Total cholesterol | 7.1±1.2 [a] | 14.5±1.8 [b] | 15.3±3.4 [b] | 14.8±2.9 [b] |
| Triglycerides | 4.3±0.09 [a] | 8.7±0.7 [b] | 8.6±1.7 [b] | 9.2±2.1 [b] |
| HDL-cholesterol | 3.6±0.08 [a] | 2.1±0.02 [b] | 2.8±0.08 [b] | 2.8±0.07 [b] |
| LDL-cholesterol | 2.6±5.7 [a] | 4.5±0.04 [b] | 4.3±0.6 [b] | 4.1±0.8 [b] |

Values with same superscript in a column differ non significantly while with different superscript differ significantly at $p<0.01$ Values given are mean ±SE of 5Hyperlipidemic control is compared with normal control. Treated is compared with hyperlipidemic control

**Table 3: Effect of *C. oxyacantha*, seed extracts on serum and liver lipid profile**

| OBSERVATION | Normal Control | Hyper-lipidemic control | Treated aqueous extract | Treated alcoholic extract |
|---|---|---|---|---|
| *Serum lipid Profile (mg/dl)* | | | | |
| Total cholesterol | 76.3±8.9 [a] | 114±12.7 [b] | 99.4±9.7 [c] | 95.2±10.6 [c] |
| Triglycerides | 78.5±13.4 [a] | 174.3±12.8 [b] | 134.8±12.5 [c] | 118±12.4 [c] |
| HDL-cholesterol | 54.2±5.8 [a] | 25.7±4.1 [b] | 35.4±6.2 [c] | 42.1±6.9 [c] |
| LDL-cholesterol | 25.9±5.7 [a] | 113.6±8.4 [b] | 99.7±8.6 [c] | 88.3±13.7 [c] |
| *Liver lipid profile (mg/dl)* | | | | |
| Total cholesterol | 7.1±1.2 [a] | 14.5±1.8 [b] | 10.7±0.2 [c] | 8.0±0.9 [c] |
| Triglycerides | 4.3±0.09 [a] | 8.7±0.7 [b] | 6.5±0.05 [c] | 5.1±0.3 [c] |
| HDL-cholesterol | 3.6±0.08 [a] | 2.1±0.02 [b] | 2.4±0.06 [c] | 3.3±0.04 [c] |
| LDL-cholesterol | 2.6±5.7 [a] | 4.5±0.04 [b] | 2.8±0.6 [c] | 2.9±0.07 [c] |

Values with same superscript in a column differ non significantly while with different superscript differ significantly at $p<0.01$ Values given are mean ±SE of 5Hyperlipidemic control is compared with normal control. Treated is compared with hyperlipidemic control.

HMG CoA reductase as well as a limiting enzyme in cholesterol synthesis, decreases endogenous synthesis and stimulates uptake and LDL via the LDL receptor (Devlin, 1997).

In most of the cases the cholesterol lowering effect of medicinal plants is attributed to the inhibition or suppression of mevalonate pathway (Sindurani and Rajamohan 200). *In vivo* studies by Clegg *et al.,* (1982) on monoterpenes, Moreno *et al.*, (1995) on carotenoids and *in vitro* studies by Pearce *et al.,* (1992) on tocotrienols showed that these compounds in HepG2 cells, post-transcriptionally downregulate enzyme activity of 3-hydroxy-3-methylglutaryl (HMG)-CoA reductase, a key enzyme in the biosynthetic pathway of isoprenoids and cholesterol. Another mechanism by which plants extract decrease the level of serum cholesterol and LDL cholesterol is that plants sterols have a structure similar to the cholesterol and hence they may reduce the cholesterol absorption from the intestine by competition. One our previous studies on chemical composition of *C. oxyacantha* showed that seeds of this plant are rich in crude fat (Bukhsh *et al.,* 2007).

Hypertriglyceridemia is common in high lipid diet intake, perhaps due to hydrolysis of lipids in the adipose tissue and may contribute for vascular complications. In the present study a significant decrease in the serum triglycerides level is observed in group 5 and 6 as compared to the group 2. This decrease may be due to the suppression of lipolysis by the plat extract in the adipose tissue. Diaz *et al.,* (1997), reported that oxidative modification of lipids specifically LDL is one of the possible mechanisms leading to the cardiovascular disease. The antiatherogenic action of antioxidants is commonly linked to the inhibition of lipoprotein peroxidation (Heinecke, 1998). The present study suggests that both aqueous and alcoholic extracts of *C. oxyacantha* seeds not only decrease LDL cholesterol in the serum and liver but may also act as inhibitors of LDL oxidation resulting in the prevention of hyperlipidemia as well as atherosclerosis.

**ACKNOWLEDGEMENTS:** The study was fully funded by Higher Education Commission Pakistan.

## REFERENCES

Ahmad, S. S. and S. N.Zahoor. Medicinal Flora of Kallar Khar. *Pak. J. Bot.,* (2008).

Akerle, O., V. Heywood and H. Synge. (Eds.). *The conservation of medicinal plants.* Cambridge University Press, Cambridge. pp 255 (1991).

Augusti, K.T., A. Narayanan, L.S. Pillai .Effects of extract of garlic (*Allium sativum Linn*)on rats fed with diets containing cholesterol and either of the oil seeds,coconuts or groundnuts. Ind. J. Exp. Bio. 39: 660 –667 (2001).

Avogaro, P. and G. Cazzolatu. Changes in the composition and physiochemical characteristics of serum lipoproteins during ethanol induced lipidemia in alcohol subjects. Metab.Clin.Exp., 219:1231-1242 (1975).

Bukhsh, E., S.A. Malik and S.S. Ahmed. Estimation of nutritional value and trace elements content of *Carthamus oxyacantha, Eruca sativa* and *Plantago ovata.* Pak. J. Bot., 39(4): 1181-1187 (2007).

Chyang, J.Y. Nuclear receptor regulation of lipid metabolism: potential therapeutics for dyslipidemia, diabetes, and chronic heart and liver diseases. Curr opinion in investigational drugs. 6(10): 994-1001 (2005).

Clegg, R.J, B. Middleton, G.D. Bell and D.A. White. The mechanism of cyclic monoterpene inhibition of 3-hydroxy-3-methylglutaryl coenzyme A reductase in vivo in the rat. Journal of Biological Chemistry 257: 2294 –2299 (1982).

Cox, C., W. Sutherland, J. Mann, S. de Jong, A. Chisholm and M. Skeaff. Effects of dietary coconut oil, butter and sawflower oil on plasma lipids, lipoproteins and lathosterol levels. Eur. J. of Clin. Nutr. 52: 650-654 (1998).

Devlin, T.M. Textbook of Biochemistry With Clinical Correlation. John Wiley and Sons, Inc. New York. 4th Edition. Pp. 415-416 (1997).

Diaz, M.N., B. Frei, J.A. Vita and J.F. Keaney. Antioxidants and atherosclerotic heart disease. The New England Journal of Medicine 337: 408 –416 (1997).

Edwards, C.R.W., J.D. Baired, B.M. Frier, J. Shephered and A.D. Toft. Ischaemic heart disease, In: Davidsons Principles and Practice of Medicine, by Edwards, C. R. W., Boucher, J. A. D., Haslett, C. and Chilvers, E., 17th ed., ELBS, Churchill Livingstone, London, pp., 245-66 (1995).

Fischbach, F.T. *Chemistry Studies,* In: A Manual of Labortary Diagnostic tests, 2nd ed., J.B. Lippincott Company, Philadelphia, USA, pp., 223-358 (1984).

Goldstein, J.L., M.S. Brown. The low density lipoprotein pathway and its relation to atherosclerosis. Annu. Rev. Biochem. 46: 897-900 (1977).

Heinecke, J.W. Oxidants and antioxidants in the pathogenesis of atherosclerosis: implications for the

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## REFERENCES

Ahmad, S. S. and S. N.Zahoor. Medicinal Flora of Kallar Khar. *Pak. J. Bot.,* (2008).

Akerle, O., V. Heywood and H. Synge. (Eds.). *The conservation of medicinal plants.* Cambridge University Press, Cambridge. pp 255 (1991).

Augusti, K.T., A. Narayanan, L.S. Pillai .Effects of extract of garlic (*Allium sativum Linn*)on rats fed with diets containing cholesterol and either of the oil seeds,coconuts or groundnuts. Ind. J. Exp. Bio. 39: 660 –667 (2001).

Avogaro, P. and G. Cazzolatu. Changes in the composition and physiochemical characteristics of serum lipoproteins during ethanol induced lipidemia in alcohol subjects. Metab.Clin.Exp., 219:1231-1242 (1975).

Bukhsh, E., S.A. Malik and S.S. Ahmed. Estimation of nutritional value and trace elements content of *Carthamus oxyacantha, Eruca sativa* and *Plantago ovata.* Pak. J. Bot., 39(4): 1181-1187 (2007).

Chyang, J.Y. Nuclear receptor regulation of lipid metabolism: potential therapeutics for dyslipidemia, diabetes, and chronic heart and liver diseases. Curr opinion in investigational drugs. 6(10): 994-1001 (2005).

Clegg, R.J, B. Middleton, G.D. Bell and D.A. White. The mechanism of cyclic monoterpene inhibition of 3-hydroxy-3-methylglutaryl coenzyme A reductase in vivo in the rat. Journal of Biological Chemistry 257: 2294 –2299 (1982).

Cox, C., W. Sutherland, J. Mann, S. de Jong, A. Chisholm and M. Skeaff. Effects of dietary coconut oil, butter and sawflower oil on plasma lipids, lipoproteins and lathosterol levels. Eur. J. of Clin. Nutr. 52: 650-654 (1998).

Devlin, T.M. Textbook of Biochemistry With Clinical Correlation. John Wiley and Sons, Inc. New York. 4th Edition. Pp. 415-416 (1997).

Diaz, M.N., B. Frei, J.A. Vita and J.F. Keaney. Antioxidants and atherosclerotic heart disease. The New England Journal of Medicine 337: 408 –416 (1997).

Edwards, C.R.W., J.D. Baired, B.M. Frier, J. Shephered and A.D. Toft. Ischaemic heart disease, In: Davidsons Principles and Practice of Medicine, by Edwards, C. R. W., Boucher, J. A. D., Haslett, C. and Chilvers, E., 17th ed., ELBS, Churchill Livingstone, London, pp., 245-66 (1995).

Fischbach, F.T. *Chemistry Studies,* In: A Manual of Labortary Diagnostic tests, 2nd ed., J.B. Lippincott Company, Philadelphia, USA, pp., 223-358 (1984).

Goldstein, J.L., M.S. Brown. The low density lipoprotein pathway and its relation to atherosclerosis. Annu. Rev. Biochem. 46: 897-900 (1977).

Heinecke, J.W. Oxidants and antioxidants in the pathogenesis of atherosclerosis: implications for the

oxidized low-density lipoprotein hypothesis. Atherosclerosis 141: 1 –15 (1998).

Hoffman, A., M. Schmalz and M. Leineweber. Cholesterol lowerng action of HOE 402 in the normolipidemic and hypercholesterolemic Golden Syrian hamster. Biochimica et Biophysica Acta. 1299: 95-102 (1996).

Hungard, B.L., D.B. Goldstein, F. Villegas and T. Cooper. The ganglioside GM 1 reduces ethanol induced phospholipase activity in synaptosomal preparation from mice. Neurochem Int. 25: 321-325 (1988).

Lewis, H.W., M.P.F. Elvin Lewis. Medical botany. New York: John Willy and Sons. pp. 217-218 (1977).

Masuda, T., Y. Oyama, Y. Inaba, Y. Toi, T. Arata, Y. Takeda, K. Nakamoto, H. Kuninaga, S. Nishizato and A. Nonaka . Antioxidant-related activities of ethanol extracts from edible and medicinal plants cultivated in Okinawa, Japan. J.Jap. Soc. Food Sci. Technol. 49(10): 652-661 (2002).

Moreno, F.S., M.R. Rossiello, S. Manjeshwar, R. Nath, P.M. Rao, S. Rajalakshmi and D.S. Sarma. Effect of beta-carotene on the expression of 3-hydroxy-3-methylglutaryl coenzyme A reductase in rat liver.Cancer Letters 96: 201 –208 (1995).

Pearce, B.C., R.A. Parker, M.E. Deason, A.A. Qureshi and J.J. Wright. Hypocholesterolemic activity of synthetic and natural tocotrienols. J. of Medicinal Chem. 35: 3595 –3606 (1992).

Pie, S. Ethanobotany and sustainable use of plant resources in HKH mountain region. Planning workshop on Ethanobotany and its application to conservation and community development in the Hidu Kush Himalayan region, Nepal (1995).

Qureshi, A.A., B.A. Bradlow and L. Brace. Response of hypercholesterolemic subjects to administration of tocotrienols. Lipids 30: 1171–1177 (1995).

Remla, A., P.V.G. Menon and P.A. Kurup. Effect of ethanol administration on metabolism of lipids in heart and aorta in isoproterenol induced myocardial infarction in rats. Ind. J. Exp. Biol. 29: 244-248 (1991).

Said, H.M. Medicinal Herbal. Karachi: Baital Hikmah at Madinatal-Hikmah, pp. 215-217 (1996).

Sindurani, J. A. and T. Rajamohan. Effects of different levels of coconut fiber on blood glucose, serum insulin and minerals in rats. Indian J Physiol Pharmacol; 44:97-100 (2000).

Tanaka, M., S. Nakaya, T. Kumai, M. Watanabe, N. Matsumoto and S. Kobayashi. Impaired testicular function in rats with diet induced hypercholesterolemia and/or streptozotocin-induced diabetes mellitus. Endocrine Research. 27: 109–117 (2001).

Trivedi, P.C. Ethanobotany. Jaipur 16th ed. Aavishkar pub., Jaipur pp. 456 (2002).