J. Agric. Sci. Mansoura Univ., 32 (1): 711 - 723, 2007

THE POSSIBLE ROLE OF *Pulicaria incisa, Diplotaxis harra* AND *Avicennia marina* AS HYPOCHOLESTEROLEMIC AGENTS BY USING MALE ALBINO RATS.

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ABSTRACT

An experiment was carried out by using 30 male albino rats divided into five groups after an adaptation period (7 days). The negative control (6 rats) was fed on basal diet, while positive control was fed on a hypercholesterolemic diet. The three treated groups were fed on hypercholesterolemic diets supplemented with 8 % of each of *Pulicaria incisa, Diplotaxis harra* and *Avicennia marina*, respectively. The duration period of the experiment was nine weeks, at the end of which the body weight gain, liver, kidneys functions and lipid pattern were determined. It was found that the addition of *P. incisa* gave the highest significant decrease in total lipid, total cholesterol, and triglycerides. Other plants also significantly decrease all these parameters but at lower different degrees. HDL-cholesterol, LDL-cholesterol, VLDL-cholesterol, chylomicronae- mia, TG/LDL-cholesterol ratio, HDL-cholesterol/Pooled lipoprotein, atherogenic index and ratio of HDL-cholesterol/LDL-cholesterol were also determined in rat's serum. Most organs functions were improved and approached nearly the negative control levels of S.G.O.T., S.G.P.T., S.G.O.T./S.G.P.T. ratio, s. albumin, s. total protein, s. globulin, s. total bilirubin, albumin/globulin ratio, s. uric acid and blood urea. Also, s. glucose was nearly returned the negative control group.

Keywords: *Pulicaria incisa, Diplotaxis harra*, *Avicennia marina*, hypercholesterolemia, chylomicronaemia, HDL, LDL.

INTRODUCTION

The progress of civilization, intake of great amounts of fatty diets, lack of exercise, smoking habits and mental stress participate in hypercholesterolemia and the resultant arteriopathies. Although these mentioned exogenous factors play an important role in increasing the incidence of these biochemical phenomena, yet others significant endogenous conditions participate in its etiology. Idiopathic hypercholesterol-emia is a term employed to signify the increase in cholesterol haematological levels without pinpointing its actual pathogenesis. Hypercholesterolemia is a clinical syndrome associated with an abnormally high plasma cholesterol concentration that increased total cholesterol (TC) and low-density lipoprotein cholesterol (LDL) and elevated high-density lipoprotein cholesterol (HDL). It must be understood, however, that current though implicates LDL otherwise known as the “bad” cholesterol as being especially “bad” when it becomes oxidized. Many drugs and some natural supplements with results that vary considerably are used in an effort to lower LDL levels. (Sheehan, 2001). The excessive uses of synthetic chemicals have posed great danger to human health and ecosystem in recent years (Shu, 1998), there has been an

upsurge in the clinical use of indigenous drugs. Such herbal plants, originally used in the traditional system of medicine, are now being effectively tried in a variety of pathophysiological states (Shashi *et al.,* 1998). Non-specific mechanisms like restora-tion of normal physiological milieu and generalized increase in resistance against infections are proposed and the role of immune system in these drug effects was suggested (Shu, 1998*).* So, the aim of the present study is to evaluate the possible role of administrating *P. incisa, D. harra* and *A. marina* as hypercholesterolemic agents by using male albino rats.

MATERIALS AND METHODS

Leaves of wild marine plant *A. marina* (Forssk.) were obtained from South Sinai, *P. undulate* Täckholm (1974), whole plant of *P. incisa* (the new name, Bouls, 1995) and whole plant of *D. harra* (Forssk.) Boiss., were obtained from North Sinai in May 2003 and identified by Herbium of Desert Research Institute. Starch, Cotton seed oil and buffalo fat were purchased from local market. Casein was obtained from Edwic, Egypt. Cholesterol was obtained from Prolab France. Bile salts (gall bladder extract) were obtained from slaughtered cow. Adult male's albino rats (90 $\pm$10g) were obtained from Faculty of Veterinary Medicine, Zagazig University.

Experimental Animals and Diet Composition

Thirty male albino rats were divided randomly into 5 groups, 6 rats each, housed in cages with screen bottom in a controlled environment. Diet and water were available *ad libitum* for nine weeks period. Rats were weighted every week. All groups were fed on the basal diet for 7 days adaptation period (table1) according to Ghali *et al.*, (2000), vitamin mixture was prepared according to A.O.A.C. (1990), and minerals mixture was prepared according to Hegsted *et al.*, (1941). After the adaptation period, one group continued feeding on the basal diet and served as negative control. Another group was fed on high fat diet (1% cholesterol + 0.25% bile salts) without any supplementation and served as a positive control. The other three groups were allowed to feed on hypercholesterolemic diet supplemented with the three studied wild herbs separately (Table 1).

Blood Sampling

At the end of the experiment blood samples were collected by sacrificing all groups by decapitation after overnight fast and part of blood samples were collected into tubes with EDTA as an anticoagulant. The other part of blood samples were left to coagulate at 37 ºC for 30 min. both samples were then centrifuged at 3000 r.p.m. for 15 minutes to obtain plasma and serum respectively, which were kept frozen until analysis.

Plasma lipid assay

Total cholesterol (TC), high-density lipoprotein cholesterol (HDL) and triglycerides (TG) were carried out according to the method of Naito and Kaplan (1984a), and (1984b) and Bucolo and David (1973), respectively, using kits obtained from SPINREACT, S. A. Ctra. Santa Coloma, Spain.

Low-density lipoprotein cholesterol (LDL) was done with the formula of Friedewald according to the method of Naito and Kaplan (1984b).

LDL = TC -TG/5 – HDL

Calculation of very low density lipoprotein cholesterol (VLDL)

VLDL = Triglycerides/5

According to Friedewald *et al.*, (1972).

Atherogenic index

Atherogenic index =(VLDL + LDL)/HDL

According to the formula of Kikuchi *et al.*, (1998).

Risk ratio.

Risk ratio = HDL/LDL

According to the formula of Ghali *et al.*, (2000).

Chylomicronaemia ratio

Chylomicronaemia ratio = TC/TG.

According to the formula of Gray and Howorth (1983).

Total lipid (TL) was carried out according to the method of Estadella *et al.*, (2004*).*

Liver functions assay and serum glucose

Serum glutamate pyruvate transaminase (SGPT) and serum glutamate oxaloacetate transaminase (SGOT), serum total protein, serum albumin and serum glucose were determined according to Reitman and Frankel (1957), Henry (1964), Doumas *et al.*, (1971) and Trinder (1969), respectively, using kits obtained from DIAMOND diagnostics Egypt.

Serum globulin was calculated as a difference between total protein and albumin according to Reinhold (1953).

Kideny functions assay

Serum uric acid and blood urea were carried out according to Gochman and Schmitz (1971) and Patton and Crouch (1977) using kits obtained from SPINREACT, S. A. Ctra. Santa Coloma, Spain and DIAMOND diagnostics Egypt, respectively.

Statistical Analysis

The statistical analysis of results was calculated according to Bailey (1994).

RESULTS AND DISCUSSION

Rats fed on high fat diet, i.e. the positive control, (Table 2) showed the highest daily gain in body weight (1.84 ±0.03 g), while rats fed on the same diet but supplemented with *D. harra* diet showed a highly significant lower daily gain in body weight (-0.54 ±0.04 g). Addition of *P. incisa* to high fat diet in 8 % highly significantly increased body weight gain from 37.36 ±1.21 g in negative control to 68.03 ±3.14 g after 9 weeks. In addition, daily gain in body weight increased in the same period from 0.57 ±0.02 g in negative control to 1.04 ±0.05 g. The same trend was nearly shown for rats fed on *A. marina*, where the body weight gain was increased from 37.36 ±1.21 g to 44.57 ±3.91 g and daily gain in body weight from 0.57 ±0.02 g to 0.66 ±0.03 g. *D. harra* followed an opposite trend, where gain in body weight and daily gain in body weight was decreased from 37.36 ±1.21 g to –34.82 ±2.80 g and from 0.57 ±0.02 g to -0.54 ±0.04 g, respectively.

T1-2

Its clear that the addition of such studied plants has a beneficial effect in daily gain in body weight and it may be due to its content of polyphenols antioxidants which has a hypocholesterolimic effect (Hashem and Saleh, 1999 and Rizk *et al.*, 1982).

TL, TC, TG and other lipid parameters were investigated. Also liver and kidneys functions were determined. The amount in lipids profile, liver and kidneys functions in positive control were given the arbitrary value 100 and the increase and all other treatments were related to 100. TL showed an increase from 400.71 ±7.06 mg/dl in negative control to 1551.12 ±43.76 mg/dl in positive control. By given the value 100 to positive control, the decrease in TL was 25.83 % for negative control.

On other hand, TG and TC were also decreased by 17.85 % and 38.7 %, respectively, (Table 3) as compared with positive control; all these values are highly significant. The effect of *P. incisa, D. harra* and *A. marina* administration for 9 weeks on lipid pattern is recorded in Table 3. The levels of TL, TG and TC were highly significant for all treatments expect *D. harra* group which was non significant and the groups were decreased from 100 % to 40.82 %, 25.51 % and 42.75 % for TL, decreased from 100 % to 40.62 %, 21.9 % and 43.58 % for TG and decreased from 100 % to 52.61 %, 31.66 % and 56.63 %, for TC after 9 weeks of feeding on the three studied wild plants respectively. The obtained results agreed with those of Iizuka *et al.*, (1998) they found that Dia-saiko-Hu-Tang (traditional herb medicine) has inhibitory effects on the development of atheromatous plaque formation in spontaneous familial hypercholesterolemia model in rabbits.

On other hand, LDL and VLDL were decreased by 15.05 % and 17.79 %, respectively, compared with positive control. Administration of *P. incisa*, *D. harra* and *A. marina* for nine weeks were highly significant decreased the levels of LDL and VLDL from 100 % to 34.217 %, 20.04 % and 41.38 % for LDL and from 100 % to 40.5 %, 21.83 % and 43.45 % for VLDL, respectively.

On other hand HDL in negative control showed an increase to 194.05 % compared with positive control and there were an increase from 100 % to 166.67 %, 117.23 % and 158.3 %, for the same componant on the studied wild plants, respectively. Our results agreed with those of Crouse *et al.,* (1999) who found that soy contains isoflavones, naturally occurring plant components that are believed to be soy's main cholesterol-lowering ingredients. Soy preparations containing high amounts of isoflavones effectively lowered TC and LDL. Rodriguez *et al.,* (2002) found that vitamins C and E have an antioxidants properties and its supplementation have a clear beneficial effect in hypercholesterolemia derived vascular.

Data in Table 4 reveal the effect of administration *P. incisa*, *D. harra* and *A. marina* on atherogenic index, risk ratio, chylomicronaemia, HDL/PC* (where PC* = VLDL+LDL+HDL) and TG/HDL of hypercholesterolemic rats. The risk ratio defined as the ratio of HDL/LDL cholesterol is a good indication for hypercholesterolemia. This ratio was 1317.64 % in negative control while in positive control it was 100 %. It increased to 500 %, 600 % and 394.11 %, in rats fed the studied plants, respectively. These results are highly significant and agreed with those of Newman *et al.,* (1992) who found significant lowering of LDL, while HDL increased in chickens fed rice bran diet.

T3-4

Ghali *et al.,* (2000) found that the addition of rice bran to hypercholesterolemic rats can improve the risk ratio. Atherogenic index which is considered as the predictor of atherogenesis was calculated after the experimental period to asses the effect *P. incisa, D. harra* and *A. marina* supplementations on the hypercholesterolemic rats.

The atherogenic index was 100 % for the positive control group and it was decreased to 7.88 % for the negative control group. While it were 21.02 %, 17.22 % and 26.27 %, for the studied wild plants, respectively. Once again it be concluded that supplementation with our studied wild plants is very important to rats fed on hypercholesterolemic diet to prevent free radicals formation and consequently atherogenesis. These results are agreed with those of Montano *et al.*, (1998) and Shige *et al.,* (1998). They mentioned that the role of vitamin C in decreasing plasma LDL might be due to the increase of receptor numbers. However vitamin E suppress the acyl-CoA cholesterol acyltransferase activity in the cell lysate with no effect on hydrolysis of cholesterol ester, which resulted in reducing the uptake of modified LDL and less cholesterol esterification in macrophage.

Chylomicronaemia (WHO Type I lipoprotein lipase deficiency) a rare disease, is due to deficiency of extrahepatic lipoprotein lipase. TC levels may be very high and the serum at 4 ºC shows an upper floating layer of chylomicrons above a clear layer. The Chylomicronaemia ratio is usually less than 0.2 (normally above 3). The Chylomicronaemia ratio was 3.10 ±0.11 for the negative control group and it was decreased to 1.42 ±0.04 for the positive control group, while it were 1.85 ±0.06, 2.07 ±0.24 and 1.77 ±0.04, for the studied wild plants respectively. So, we can say negative, positive groups and all treatment groups were out of its range and not have that disease. This result was agreed with those of Gray and Howorth (1983).

The ratio of HDL/PC* was calculated. It was 0.647 ±0.004 for the negative control group and it was decreased to 0.128 ±0.008 for the positive control group, while it were 0.409 ±0.015, 0.458 ±0.004 and 0.357 ±0.009, for the studied wild plants respectively, they were highly significant affected by the treatments compared with the control group. This result was agrees with those of Kestin *et al.*, (1990), Kahlon *et al.*, (1996) and Ghali *et al.*, (2000) who reported that the ratio of HDL/PC* was highly significantly increased.

The TG/HDL ratio was 0.499 ±0.02 for the negative control group and it was increased to 5.445 ±0.36 for the positive control group, while it were 1.324 ±0.08, 1.014 ±0.08 and 1.496 ±0.05 for the studied wild plants, respectively. This result was agreed with those of Ghali *et al.*, (2000) who found that the addition of rice bran to hypercholesterolemic rats can improve the ratio TG/HDL, respectively, for the studied wild plants.

Table 5 shows the effect of administrating *P. incisa, D. harra* and *A. marina* diets on the liver function of hypercholesterolemic rats. Hyper-cholesterolemia was characterized by a significant increase in serum GPT. The values were 100 % and 44.23 % in positive and negative control groups, respectively, while it were 65.24 %, 65.24 % and 67.93 %, for the three studied plants, respectively. The increase in serum GPT activity indicates liver cell necrosis and hepatic injury. Treatment with *P. incisa, D. harra* and *A. marina* induced a significant decrease in the high activity of serum GPT

and the levels were decreased compared to the positive control group after nine weeks administration. Also, hypercholesterolemia was characterized by an increase in serum GOT. The increase of serum GOT activity was more specific for cardiac injury. The values of serum GOT were 100 % and 38.33 % in positive and negative control groups, respectively, while it were 41.28 %, 45.74 % and 71.77 % for the studied plants, respectively. Percentage of serum GOT/GPT ratio in Table 5 refers to high ratio in positive control and *A. marina* groups it were 100 % and 105.75, respectively, while it were 63.31 %, 84.17 % for *P. incisa* and *D. harra,* respectively. Serum GOT, GPT and GOT/GPT ratio were high significant and significant, respectively, as shown in Table 5 and the data were agreed with those of Hawcroft (1978) and Daher *et al.*, (2006).

Bilirubin is an end product of haem degradation in mammals Maines (1988). Total serum bilirubin for the negative control group was 44 % of the positive control which it were 100 %, 52 % and 81.33 %, for the studied plants respectively. This result was agreed with Neuzil and Stocker (1993) who decided that, *in vitro*, bilirubin is a powerful scavenger of peroxyl radicals and singlet oxygen perhaps the jaundice of premature babies has some physiological role provided it does not occur in excess. Bilirubin bound to albumin can protect both the protein and albumin-bound fatty acids against free-radical damage. However, there is little direct evidence that bilirubin is an important antioxidants *in vivo.*

Hypercholesterolemic state was accompanied by a high significant increase in total serum protein, albumin and globulin for negative control. It were 74.34 %, 87.05 % and 56.33 %, respectively. There were remarkable increases at total protein and globulin with the group treated with *P. incisa* and *A. marina.* The values were 80.71 % and 86.23 %, respectively for total protein and were 92.76 % and 104.29 %, respectively, for globulin. Serum total protein and globulin were decreased to 44.62 % and 47.05 %, respectively, for the group treated with *D. harra*. Albumin content was decreased to 87.05 % in negative control relative to positive control, while the treated groups values were 68.29 %, 41.27 % and 68.66 %, respectively. There were a high significant decrease in serum albumin, globulin and albumin/globulin ratio and non significant decrease in globulin with the group treated with *D. harra*, and these results agreed with Strove (1989). This decrease may be due to the starvation status, which caused from the bad taste of this plant for rats.

Hypercholesterolemic state was accompanied by a high significant increase in serum glucose it was 78.54 % for negative control group and it were 72.79 %, 73.44 % and 72.55 % with the studied plants, respectively, (Table 6). This result was agreed with Labib *et al.,* (1991) who reported that theincrease of serum glucose might be due to impaired function of liver which is reported to play an important role in the regulation of serum glucose. Alternatively, it may be due to the lowered circulating insulin level caused by the high level of serum glucose Serum uric acid was 85.05 % for negative control and increased to 85.35 %, 91.92 % and 86.24 % with the studied plants, respectively.

T5-6

Blood urea was decreased to 56.30 % for negative control and it were 51.32 %, 108.21 % and 90.03 % with the studied plants, respectively. We can notice a high significant increase in *D. harra* which was 108.21 %. The high significant increase may be due to starvation status caused by *D. harra* administration, which caused from the bad taste of that plant for the rats.

Conclusion

Improved knowledge of wild medicinal plants would assist in the efforts to achieve the curing effect of these plants. Obtaind data about its role as a hypocholesterolemic agent gives us valuable information. The *P. incise* and *A. marina* plants can play a hypocholesterolemic role against hyper-cholesterolemic disease at a concentration of 8 % of diets. The *D. harra* may exerce the same effect but in low dose. The possible role of such plants may originate from its contents of active components which may act as antioxidants, attack free radical, improve lipid pattern and organs function. So that these plants are considered as a new non-conventional supply for the pharmaceutical industries and for edible purposes.

REFERENCES

A.O.A.C. (1990). Association of Official Analytical Chemists. Official Methods of Analysis 15th ed. A.O.A.C., Washington D.C.

Bailey, N. T. J. (1994). Statistical methods in biology, 3rd Edd. Cambridge University Press (UK).

Bouls, L. (1995). Flora of Egypt Checklist. Al-Hadara Publishing. Cairo. Egypt: 479, 1836.

Bucolo, G. and H. David (1973). Quantitative determination of serum triglycerides by use of enzyme. Clin Chem.; 19(5): 476-482.

Crouse, J. R.;T. Morgan and J. G Terry (1999). A randomized trial comparing the effect of casein with that of soy protein containing varying amounts of isoflavones on plasma concentrations of lipids and lipoproteins. Arch. Intern. Med. 159: 2070-2076.

Daher, C. F.; F. G. Baroody and M. G. Baroody (2006). Effect of *Urtica dioica* extract intake upon blood lipid profile in the rates. Fitoterapia 77(3): 183-188.

Doumas, B. T.; W. A. Watson and H. G. Biggs (1971). Albumin standards and the measurement of serum albumin with bromocresol green. Clin. Chem. Acta. 31: 87-96.

Estadella, D.; L. M. Oyama; A. R. Dâmaso; E. B. Ribeiro; M. Claudia and D. N. Oller (2004). Effect of palatable hyperlipidic diet on lipid metabolism of sedentary and exercised rats. Nutrition. 20: 218-224.

Friedewald, T .W.; I. R. Levy; and S. D. Fredrickson (1972). Estimation of the concentration of low-density lipoprotein cholesterol in plasma, without use of the preparative ultracentrifuge. Clinical Chemistry, Vol. 18(6): 499-502.

Ghali, Y; R. Laila; A.A. Moustafa; M. M. Wasef and N. S. Abd El-Rahman. (2000). Cholesterol-Lowering effect of various rice bran diets on hypercholesterolemic rats. The Egyptian J. of Biochemistry. 18:213-228.

Gochman, N. and J. M. Schmitz. (1971). Automated determineation of uric acid with use of uricase-peroxidase system, Clin. Chem. 17(12): 1154-1159.

Gray, C. H. and P. J. N. Howorth (1983). " Clinical chemical pathology" Ninth edition, The English language book society and Edward Arnold (publishers) Ltd. P. 144.

Hashem, F. A. and M. M. Saleh (1999). Antimicrobial components of some *Cruciferae* plants (*Diplotaxis harra* Forssk. and *Erucaria microcarpa* Boiss.). Phototherapy Res. J. 13(4): 329-332.

Hawcroft, D. (1978). "Diagnostic enzymology" John Wiley and Sons, New York.

Hegsted D. M.;R.C. Mills; C. A. Elvehjem and E.B. Hart (1941). Choline in the nutrition of chicks. J. Biol. Chem., 138: 459-466.

Henry, R.J. (1964). Clinical Chemistry, Harber and Row Publisher, New York. p. 181.

Iizuka, A.; T. I. Osamu; Y. Fumihiko; M. Bunsho; A. Sakae; K. Yasuhiro; K. Kazuo; M. Akiyo and I. Hiroshige (1998). Inhibitory effects of Dai-saiko-to (Da-Chai-Hu-Tang) on the progression of atherosclerotic lesions in Kurosawa and Kusanagi-hypercholesterolemic rabbits. J. of Ethnopharmacology 63:209-218.

Kahlon, T. S.; M. M. Chow; C. A. Hudson and R. N. Sayre (1996). Cholesterol-lowering by rice bran and rice bran oil unsaponifiable matter in hamsters. Cereal Chem. 73(1): 69-74.

Kestin, M.; R. Moss; P. M Clifton and P. J. Nestel (1990). Comparative effects of three cereal brans on plasma lipids, blood pressure and glucose metabolism in mildly hypercholesterolemic men. Am. J. Clin. Nutr., 52: 661.

Kikuchi, H. H.; N. Onodera; S. Matsubara; E. Yasuda; O. Chonan; R. Takahashi and F. Ishikawa (1998). Effect of soy milk and bifidobacterium fermented soy milk on lipid metabolism in aged ovariectomized rats. Bioscience, Biotechnology and Biochemistery. 62(9): 1688-1692.

Labib, S.; M. Sitohy; R. El-Massry and S. El-Saadany (1991). Lipid metabolism in rats fed either butter or butter products as the fat source.Fasc. 42(4): 255-260.

Maines, M. D. (1988). Heme oxygenase: function, multiplicity, regulatory mechanisms and clinical application. FASEB J. 2, 2557.

Montano, C. E.; M. L. Fernandez and D. J. McNamara (1998). Regulation of apolipoprotein B-containing lipoproteins by vitamin C level and dietary and fat saturation in guinea pigs. Metabolism Clinical and Experimental. 47(7): 883-891.

Naito, H. K. and A. Q. Kaplan (1984a). Cholesterol. Clin. Chem. The C.V. Mosby Co. St Louis. Toronto. Princeton; 437:1194-11206.

Naito, H. K. and A. Q. Kaplan (1984b). High-density lipoprotein (HDL) cholesterol. Clin. Chem. The C.V. Mosby Co. St Louis. Toronto. Princeton; 437:1207-1213.

Neuzil, J. and R. Stocker (1993). Bilirubin attenuates radical-mediated damage to serum albumin, FEBS Lett. 331, 281.

Newman, R. K.; A. A. Betsehart; C. W. Newman and P. J. Hofer (1992). Effect of full-fat or defatted rice bran on serum cholesterol. Plant Foods Human Nutr., 42(1): 37-43.

Patton, C.G. and S.R. Crouch. 1977. Enzymatic determineation of urea. Anal. Chem., 49:464-469.

Reinhold, J.G. (1953). Submitted by, to Standard Methods in Clinical Chemistry, Editor Reiner, M., Volume I, Academic Press, New York, P. 88.

Reitman, S. and S. Frankel (1957). Determination of serum glutamate oxaloacetate and glutamate pyruvate transaminases. Am. J. Ciln. Path., 28: 56- 60.

Rizk, A. M.; F. M. Hammouda and L. Hussein (1982). 21st International Horticultural Congress (Hamburg. W. Gremany, Aug. 29th - 4th Sep.), Abstract collection, 2148.

Rodriguez, A. J.; G. Andres; E. Ezequiel; N. Beatriz; P. Maitane; A. Roberto; S. B. Maria; A. P. Jose and M. Diego (2002). Dietary supplementation with vitamins C and E prevents down regulation of endothelial NOS expression in hypercholesterolemia *in vivo* and *in vitro*. Atherosclerosis 165: 33-40.

Shashi, G. P. K.; M. K. R. Gupta; A. S. Somvanshi and V. K. Vijjan (1998). Toxicity of azadirachtin (neem) based formulation in rates. Indian J. Environ. and Toxicol., 8 (2):77 – 80.

Sheehan, A. (2001). Cholesterol Update: Oxidized and nonoxidized LDL-cholesterol, do we have to worry about both? Dynamic. Chiropractic., May 21, Volume 19, issue 11.

Shige, H.; T. Ishikawa; M. Suzukawa; M. Nishiwaki; K. Nakajima; T. Ito; K. Higashi; M. Ayaori; A. Yonemura; P. Nestel and H. Nakamura (1998). Vitamin E reduces cholesterol esterification and uptake of acetylated low-density lipoprotein in macrophages. Lipids. 33:(12) , 1169-1175.

Shu, Y.Z. (1998). Recent natural products based drug development : A pharmaceutical industry perspective. J. Nat. Prod., 61: 1053-1071.

Strove, E. A. (1989). Biochemistry. Mir Publishers, Moscow. P. 422.

Täckholm, V. (1974). Students flora of Egypt. second edition, Cairo University. Beirut: 479, 562, 871.

Trinder, P. (1969). Determination of glucose in blood using glucose oxidase with an alternative oxygen acceotor. Ann. Clin. Biochem. 6 : 24-27.

الدور المحتمل لتأثير نباتات شاى الجبل وفجل الجبل والمنجروف كعوامل خافضة للكوليسترول باستخدام ذكور الفئران البيضاء

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[2]المعهد القومي للتغذية-القاهرة

لقد أصبح دور الليبيدات في الخطوة الأولي لحدوث تصلب الشرايين دورا هاما حيث انه يدخل فى تركيب الليبوبروتينات والتي يمكن بطرق مختلفة أن يحدث لها تحوير وبالتالى يحدث تغير فى ارتباطها مع المستقبلات الخاصة على جدر الخلايا، لذلك اهتمت العديد من الأبحاث فى السنوات الأخيرة بدراسة النباتات الطبية والعطرية البرية وتأثيراتها البيولوجية فى خفض كوليسترول الدم وبالتالى الحماية من تصلب الشرايين، ومن ثم يأتى دور هذه النباتات بما تحتوى من مركبات طبيعية ذات تأثيرات كيميائية وحيوية كمضادات للأكسدة وبالتالى خفض الكوليسترول بالدم وحدوث تصلب الشرايين.

أجريت هذه الدراسة لمعرفة تأثير استخدام وجبات عالية الدهن مدعمة بـ 8 % من نباتات شاى الجبل وفجل الجبل والمنجروف كعوامل خافضة للكوليسترول وذلك باستخدام ذكور الفئران البيضاء، حيث أجريت تجربة بيولوجية قسمت فيها الفئران إلى 5 مجموعات، تم تغذية كل المجموعات علي الوجبة القياسية خلال فترة الأقلمة و التى استمرت أسبوع، ثم تم تغذية المجموعة الأولي (6 فئران) على الوجبة المثالية طوال فترة التجربة والتي استمرت 9 أسابيع، والمجموعة الثانية (6 فئران) تم تغذيتها على وجبات مرتفعة الدهن ومحتوية علي 1 % كوليسترول طوال مدة التجربة، أما المجاميع الثلاثة الأخرى فتم تغذيتها على وجبات مرتفعة الدهن وتحتوى علي 1 % كوليسترول ومضافا إليها 8 % من مطحون أحد النباتات الثلاثة بصورة منفصلة، وفى نهاية التجربة تم تقدير كل من الزيادة النهائية وكذلك الزيادة اليومية فى وزن الجسم لكل المجاميع، حيث أظهرت المجموعة التى تم تغذيتها علي شاى الجبل أعلي نسبة انخفاض فى كل من الليبيدات الكلية و الكوليسترول وباقى قياسات الدهون ووظائف الكبد والكلى والجلوكوز بالدم وذلك عند مقارنتها بمجموعة الكنترول عالية الدهن بينما كانت مجموعة المنجروف هى التالية فى النتائج والتأثير بالمقارنة مع مجموعة الكنترول عالية الدهن.

أظهرت مجموعة نبات فجل الجبل (الحارة) أعلى انخفاض ملحوظ إلا أن هذا الانخفاض كان غير حقيقى وقد يرجع ذلك لعزوف فئران تلك المجموعة عن الأكل بصورة ملحوظة (فربما يكون طعم النبات غير مستساغ الطعم بالنسبة الفئران) وهو ربما ما أدى إلى ذلك الانخفاض، وقد ظهر ذلك واضحا في انخفاض أوزان الفئران عند نهاية التجربة عن الأوزان التى بدأت بها التجربة وبصورة كبيرة، ويظهر ذلك أيضا فى قياسات كل من الألبيومين والبروتين الكلى واليوريا حيث انخفضت قياسات كل من الألبيومين والبروتين الكلى بصورة كبيرة بينما ارتفعت قياسات اليوريا بصورة كبيرة ويعزى هذا إلى عملية الجوع الشديدة التى عانت منها فئران هذه المجموعة على الرغم من توفر الغذاء نظرا لعدم استساغتها للنبات المضاف.

وبذلك يمكن التوصية باستخدام نباتى شاى الجبل والمنجروف كعوامل خافضة للكوليسترول.

Table 1. Diet composition as g/100g According to Ghali *et al.* (2000)

Constituents diet	Percentage of											
	Starch	Casein	Cotton seed oil	Buffalo fat	Salt mix.	Vitamin mix.	Cellulose	Cholesterol	Bile salts	*Pulicaria incisa*	*Diplotaxis harra*	*Avicennia marina*
Basal diet (Negative control)	70.00	10	10	-	4	1	5	-	-	-	-	-
Hypercholesterolemic diet (Positive control)	63.75	10	-	15	4	1	5	1	0.25	-	-	-
***Pulicaria incisa* (group 1)**	55.75	10	-	15	4	1	5	1	0.25	8	-	-
***Diplotaxis harra* (group 2)**	55.75	10	-	15	4	1	5	1	0.25	-	8	-
***Avicennia marina* (group 3)**	55.75	10	-	15	4	1	5	1	0.25	-	-	8

Table 2. Body weight gain of rats feed on basal diet (negative control), hypercholesterolemic diet (positive control), hypercholesterolemic diets-supplemented with *Pulicaria incise*, *Diplotaxis harra* and *Avicennia marina.*

Treatment	Initial body weight	Final body weight	Body weight gain	Daily body weight increase
Negative control	82.68 ±1.41	119.69 ±1.15	37.36 ±1.21	0.57 ±0.02
Positive control	86.31 ±1.46**	205.60 ±2.04**	119.34 ±2.16**	1.84 ±0.03**
Pulicaria incisa	86.38 ±1.37**	155.15 ±4.51**	68.03 ±3.14**	1.04 ±0.05**
Diplotaxis harra	89.21 ±1.76**	54.59 ±3.20**	-34.82 ±2.80**	-0.54 ±0.04**
Avicennia marina	91.69 ±1.90**	139.81 ±3.01**	44.57 ±3.91**	0.66 ±0.03**

**** P< 0.01= highly significant**

Table 3. Effect of feeding on hypercholesterolemic diet supplemented with different medicinal wild plants for nine weeks period on total lipids, total cholesterol, triglycerides, HDL-cholesterol, LDL-cholesterol and VLDL-cholesterol.

Type of lipid pattern	Total lipids (TL)		Total cholesterol (TC)		Triglycerides (TG)		HDL-cholesterol		LDL-cholesterol		VLDL-cholesterol	
	mg/dl	%	mg/dl	%	mg/dl	%	mg/dl	%	mg/dl	%	mg/dl	%
Negative control	400.71 ±7.06	25.83	148.80 ±4.29	38.70	48.07 ±1.61	17.85	96.25 ±1.88	194.05	42.83 ±1.51	15.05	9.61 ±0.32	17.79
Positive control	1551.12 ±43.76**	100.00	384.40 ±7.24**	100.00	269.15 ±7.26**	100.00	49.60 ±3.18**	100.00	284.52 ±10.25**	100.00	53.99 ±1.51**	100.00
Pulicaria incisa	633.22 ±22.23**	40.82	202.26 ±5.22**	52.61	109.35 ±3.56**	40.62	82.67 ±2.97**	166.67	97.34 ±3.52**	34.21	21.87 ±0.71**	40.50
Diplotaxis harra	395.74 ±40.43 (N.S.)	25.51	121.73 ±6.25**	31.66	58.97 ±5.03**	21.90	58.15 ±1.72**	117.23	57.03 ±1.78**	20.04	11.79 ±1.01**	21.83
Avicennia marina	663.17 ±15.89**	42.75	217.72 ±4.24**	56.63	117.31 ±2.97**	43.58	78.54 ±3.14**	158.30	117.76 ±5.25**	41.38	23.46 ±0.59**	43.45

**** P< 0.01= highly significant** **(N.S.) = non significant**

Table 4 . Effect of feeding on hypercholesterolemic diet supplemented with the studied medicinal wild plants on the parameters of atherogenic index, risk ratio, chylomicronaemia ratio, HDL-cholesterol/pooled lipoprotein cholesterol (PC*) and TG/HDL-cholesterol.

Treatment	Atherogenic index		Risk ratio		Chylomicronaemia ratio		HDL/PC* ratio		TG/HDL ratio	
		%		%		%		%		%
Negative control	0.54 ±0.01	7.88	2.24 ±0.06	1317.64	3.10 ±0.11	218.30	0.647 ±0.004	505.46	0.499 ±0.02	9.16
Positive control	6.85±0.45**	100.00	0.17 ±0.01**	100.00	1.42±0.04**	100.00	0.128±0.008**	100.00	5.445 ±0.36**	100.00
Pulicaria incisa	1.44±0.09**	21.02	0.85 ±0.06**	500.00	1.85±0.06**	130.28	0.409±0.015**	319.53	1.324 ±0.08**	24.31
Diplotaxis harra	1.18±0.02**	17.22	1.02 ±0.02**	600.00	2.07±0.24**	145.77	0.458±0.004**	357.81	1.014 ±0.08**	18.62
Avicennia marina	1.80±0.08**	26.27	0.67 ±0.03**	394.11	1.77±0.04**	124.64	0.357±0.009**	278.90	1.496 ±0.05**	27.47

Atherogenic index = VLDL-Cholesterol + LDL-Cholesterol / HDL-Cholesterol **Risk ratio = HDL-Cholesterol / LDL-Cholesterol**
Chylomicronaemia ratio = Serum Total Cholesterol/Triglycerides **PC*= VLDL-Cholesterol + LDL-Cholesterol + HDL-Cholesterol**
**** P< 0.01= highly significant** **(N.S.) = non significant**

Table 5. Serum contents of total bilirubin, serum protein fractions and transaminase activities in rats fed on basal diet, high fat diet and diet supplemented with *Pulicaria incisa* , *Diplotaxis harra* and *Avicennia marina*.

Investigation	S.G.O.T.		S.G.P.T.		S.G.O.T./ S.G.P.T. ratio		Total bilirubin		Total protein		Albumin		Globulin		Albumin/ Globulin ratio	
Treatment	**u/l**	**%**	**u/l**	**%**		**%**	**gm/dl**	**%**	**gm/dl**	**%**	**gm/dl**	**%**	**gm/dl**	**%**		**%**
Negative control	6.75± 0.21	38.33	5.60± 0.24	44.23	1.20± 0.03	86.33	0.33± 0.02	44.00	7.13± 0.22	74.34	4.64± 0.24	87.05	2.49± 0.30	56.33	1.86± 0.19	153.71
Positive control	17.61± 0.32**	100.00	12.66± 0.24**	100.00	1.39± 0.02**	100.00	0.75± 0.04**	100.00	9.59± 0.41**	100.00	5.33± 0.23**	100.00	4.4.2± 0.18**	100.00	1.21± 0.05**	100.00
***Pulicaria incisa* group**	7.27± 0.43*	41.28	8.26± 0.41*	65.24	0.88± 0.05**	63.31	0.75± 0.05**	100.00	7.74± 0.48**	80.71	3.64± 0.15**	68.29	4.10± 0.24**	92.76	0.91± 0.09**	75.20
***Diplotaxis harra* group**	9.64± 0.32**	54.74	8.26± 0.37*	65.24	1.17± 0.06*	84.17	0.39± 0.04**	52.00	4.28± 0.13**	44.62	2.20± 0.26**	41.27	2.08± 0.19(N.S)	47.05	1.06± 0.15**	87.60
***Avicennia marina* group**	12.64± 0.29**	71.77	8.60± 0.49*	67.93	1.47± 0.06**	105.75	0.61± 0.07**	81.33	8.27± 0.37**	86.23	3.66± 0.22**	68.66	4.61± 0.28**	104.29	0.79± 0.08**	65.28

**** P< 0.01= highly significant** *** P< 0.05= significant** **(N.S.) = non significant**

Table 6. Serum glucose, urea and uric acid in rats fed normal diet, high fat diet and high fat diet supplemented with *Pulicaria incisa, Diplotaxis harra* and *Avicennia marina*.

Investigation Treatments	Glucose		Urea		Uric acid	
	mg/dl	**%**	**mg/dl**	**%**	**mg/dl**	**%**
Negative control	**88.28 ±2.12**	**78.54**	**18.36±0.62**	**56.30**	**5.84 ±0.53**	**85.05**
Positive control	**112.40±4.28****	**100.00**	**32.61 ±2.28****	**100.00**	**6.69 ±0.22****	**100.00**
***Pulicaria incisa* group**	**81.82 ±3.25****	**72.79**	**29.78 ±3.80****	**51.32**	**5.71±0.51(N.S.)**	**85.35**
***Diplotaxis harra* group**	**82.55 ±3.93****	**73.44**	**35.29 ±2.15****	**108.21**	**6.15±0.49 (N.S.)**	**91.92**
***Avicennia marina* group**	**81.55 ±4.37****	**72.55**	**29.36 ±1.35****	**90.03**	**5.77±0.51(N.S.)**	**86.24**

**** P< 0.01= highly significant** **(N.S.) = non significan**

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