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HYPOGLYCEMIC AND HYPOLIPIDEMIC POTENTIAL OF HERBAL MIX

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ABSTRACT:

Researchers have reported that consumption of high fructose may cause hyperinsulinemia, hyperglycemia and hyperlipidemia which can be treated by using natural herbs. Antidiabetic potential of herbal mix (carrot, bitter gourd, garlic, cinnamon) was evaluated using (*in vitro)* total phenol and antiamylase activity and values were found 267.18 mg % and 11.5 % (400µg) inhibition respectively. The result confirmed a moderate antidiabetic potential of herbal mix. For in *vivo* studies 24 albino rats were divided in four groups and were fed (G-I control diet, G-II control + herbal mix, G-III high fructose diet and G-IV high fructose + herbal mix) for eight weeks followed by physiological, blood and tissue analysis. Fructose fed group have shown increase in plasma insuline, glucose, total lipid, triglyceride, VLDL level significantly ($P<0.05$) which was decreased by supplementation of herbal mix. A developed herbal mix show hypoinsulinemia, hypoglycemic, hypolipidemic effect of developed herbal mix. Value for body weight, plasma total cholesterol, liver triglyceride were increased in high fructose fed group which were decreased non significantly by supplementing herbal mix. In contrast there was No positive effect of herbal mix on kidney weight, liver weight, plasma LDL, HDL and liver cholesterol.

KEY WORDS: Hypoglycemic, Hypolipidemic, Potential, Herbal Mix.

INTRODUCTION:

Diabetes is fast becoming the epidemic of the 21st century. According to international diabetes federation (IDF), there are 246 million people worldwide with 46 % of all those affected in 40-59 years age group. Major causes of diabetes are inheritance, viral infection, age, emotional stress, smoking, obesity and diet (1).

Recently the consumption of dietary fructose, which has increased in some countries, has been associated with the increasing prevalence of overweight and obesity (2).High fructose corn syrup (HFCS) is now used extensively in carbonated beverages and other sweetened drinks, backed goods, candies, canned fruits, Jam, Jellies and dietary product and mixes well in many food (3).

Fructose feeding has been shown to induce insulin resistance in rats, associated with hyperinsulinemia, hyperglycemia, hypertriglyceridemia, high glyogen content and hypertension (4).

A number of antidiabetic regimens are available in the market, but some of these produce undesirable side effects after long term consumption (5). Consequently , there is a critical need for new therapeutic agents that target the underlying pathogenic mechnisms and are able to arrest or reverse the progression of type-2 diabetes.

In field of phytomedicine , much interest has been focused on the development pf alternative medicinal herbal preparation, which includes screening of natural bioactive compounds with the ability to cure and delay the progression of diabetes through different unknown mechanism(6). These compounds may provide safer drug for treatment of diabetes. A plethora of literature is available to document the antidiabetic effect of plants and their preparation (7-8)

In series of studies to determine the antidiabetic effect of a number of plant and herbal preparation , a new herbal formuation containg four plant extracts, viz Momardica charatia (bitter melon), Garlic(allium sativum), Cinanmon (cinnamon Zellanisum), Carrot(dacus carot) was developed at P.G. Depatment of Home Science, Sardar Patel University, Vallab Vidyanagar during period of November 2007 to April 2008.

In content to above, the present investigation was planned to evaluate the hyporglycemic and hyporlipidemic, effect of a herbal mix prepared using carrot, bitter gourd, garlic and cinnamon with following objective.

1. To evaluate the in *vitro* antiamylase activity of herbal mix.
2. To evaluate in *vivo* hypoglycemic and hypolipidemic effect of a herbal mix in fructose induce diabetes rats.

MATERIALS AND METHOD:

Herbal mix preparation

Bitter gourd, carrot, garlic and cinnamon used for herbal mix. Bitter gourd and carrot were washed, cut into thin sliced , garlic dehusked and dried in oven at 60 C. The dried product and cinnamon were grind into fine powder. All four powders were mixed in the ratio of 40:25:25:10 (carrot: bittergurd:garlic:cinnamon) to make herbal mix. Extract the liquid from hebal mix. This extraction used to measure the total phenol and antiamylase activity.

Total Phenolic Compounds

Total phenolic compound was estimated according to the method described by Malik and Singh (1971).(9)

0.2 ml of diluted herbal mix extract [HME] was taken and volume was made up to 1 ml with distilled water (5 time dilution). To this, 1 ml each of Folin ciocalteaus reagent (diluted 1:2) and 1 ml of 35% sodium carbonate (Na_2CO_3) were added with immediate cyclomix. Incubate it for 10 minutes at room temperature then 2 ml of distilled water was added and intensity of the colour was

recorded at 620 nm in UV spectrophotometer (Hitachi 220S) against the reagent blank. For blank 1 ml of distilled water was taken .

Amylase inhibition assay

The anti-amylase (α-amylase inhibition disc assay) was followed by starch agar plate method by Carieia, 2003

400 µl and 800 µl of methanolic extract of sample, 200 µl of α-amylase (sigma) were add and volume made up to 1 ml with 20 mM phosphate buffer (PH 6.9) in 2ml vials. Cyclomix it and incubated it in deep freezer for 15 minutes. Repeated this procedure at every 15 minutes till 1 hour. For control 200 µl of an α-amylase (Sigma) was taken and treated same as sample.

For preparation Starch agar plate:1 gm soluble starch (potato starch) and 1 gm agar were added to 100 ml of distilled water in sterilized flask and boiled it to dissolved. The sterile starch agar gel is transferred in sterile clean empty glass Petri plates, allowed it to solidity. Place a sterile whatman filter paper dice (1/2 inch) in the centre of the plates. 50 ul of each sample was applied on the paper dice. Allowed to diffuse and incubated at 37º C in an incubator for 48 hours. After incubation iodine solution was added in the plates and drained it after 5 minutes. The clear zone was measured and percent inhibition of enzyme activity was calculated.

Animals and treatment schedule

24 albino rats (16 female and 8 male) of Wistar Strain of 8 to 10 week were divided equally into four groups. Group-I→control, Group-II→ control+herbalmix, Group-III→ high fructose, Group-IV→high fructose+ herbal mix. Each group consists of six animals (four females and two males). The initial body weight of females ranged from 152 to 188 gms, while the males rats weighed between 210 to 258 gms. The animals were housed under condition of light 12 hours and dark cycle. Animals were fed control as well as experimental diet, for the period of 8 weeks. The diet used for this experiment is as per the composition given by AIN -93 M (Philip et al.,1993). The animals were fed ad libitum and free access to water. The detailed composition of a diet for different groups is given in table No. 1

Autopsy procedure:

At the end of experimental period (8 weeks), the rats (fasted for 16 hours) were weighed and sacrificed under mild anesthesia. Blood was collected from the eyes directly into clean, dry test tubes by using anticoagulant (EDTA). The blood in the tubes was centrifuged at 5000 rpm, for 10 minutes under refrigeration for plasma separation. Plasma was used for analysis of the insulin, glucose, total cholesterol, HDL cholesterol , LDL, VLDL, triglycerides, total lipid.

Tissue Processing

Liver was made blood free using saline solution, blotted on filter paper, cleaned of extraneous tissues and weighed on a preweight alluminium foil.

Table No : 1Composition of diets fed to different groups

Ingredients	Group- I	Group-II	Group-III	Group-IV
Corn starch(gm)	450.692	432.06	171.27	15.64
Casein (≥ 85% protein) (gm)	140	140	140	140
Dexteinized corn starch(gm)	140	133.8	47	40.8
Sucrose(gm)	100	100	100	100
Ground oil(gm)	70	70	70	70
Fiber(gm)	50	50	50	50
Mineral mix*(gm)	35	35	32	35
Vitamin mix*(gm)	10	10	10	10
L-cystine(gm)	1.8	1.8	1.8	1.8
Choline bitartarate(gm)	2.5	2.5	2.5	2.5
Tert-butylhyroquinone(gm)	0.008	0.008	0.008	0.008
Fructose(gm)	--	--	372.41	372.41
Herbal mix(gm)	--	24.82	--	24.82

A known amount of liver tissue was homogenized with phosphate buffer [PH 7] and volume was made up to 10% level. This tissue homogenate was used for analysis of liver cholesterol, triglycerides.

A small portion of muscle from thigh was obtained and known amount was used for the estimation of glycogen.

Plasma Insulin and Glucose

The plasma insulin was tested in endocrine laboratory of ahemdabad. ADVIA centaur insulin assay two site sandwich immunoassay using direct chemiluminescent technology. Plasma glucose was estimate by GOD/POD kit method using the glucose kit supplied by Eve's diagnosis, Baroda, India.

Blood and liver tissue lipid.

The total cholesterol, triglyceride(TG) and HDL estimated by CHOD/POD method using the cholesterol kit supplied by Span Diagnostic, Surat, India and LDL cholesterol and VLDl cholesterol were calculated by using Friedewald's equation. (10)

Liver total cholesterol:

Determination of total cholesterol was carried out according to Verley ,1969 (11).

About 0.4 to 0.5 gm of fresh liver was taken in 1 ml of phosphate buffer (PH 7) solution. The liver sample homogenized and volume made up to 10 ml with acetone: ethanol (1:1) mixture. The mixture was centrifuged at 3000 rpm for 10 minutes. Supernatant was collected and volume made

up to 10 ml with acetone: ethanol (1:1) mixture. A known amount (1 ml and 2 ml) were taken into test tube and evaporated completely. 3.0 ml of glacial acetic acid was added and tube placed in boiling water bath for 1 minute. After coolig, 2 ml of colour reagent was added and reading was taken at 570 nm. For blank 3 ml of acetic acid was treated as for sample.

Liver triglycerides

Weight amount of liver was taken and lipid was extracted according to method given by Hock.

1 gm of liver was homogenate in phosphate buffer (PH 7) and volume made up to 10 ml with distilled water. From that 1 ml of liver homogenate was taken and added 2 ml of chloroform: methanol (2:1) mixture and 1 ml of distilled water. The content was cyclomixed and centrifuged (low speed) for 10 minutes. Supernatant was taken in test tube and evaporated completely at 60º C. In dry test tube 4 ml of isopropyl alcohol was added and followed by 400 µg of activated alumina. The tube was cyclomixed for 15 minutes and centrifuged at 300 rpm, for 10 minutes. 2 ml of clear supernant was taken in other test tube and added 0.6 ml of saponifying reagent. The table was placed in incubation at 60º C for 15 minutes was cooled and added 1 ml of sodium metaperiodate. After addition of 0.5 ml acetyl acetone again tube was incubated at 30 minutes at 50° C. The colour was developed which was read at 405 nm. For blank 4 ml of isopropyl alcohol was taken and then treated as sample.

Statistical Analysis

All result are presented as mean ± S.D. Differences between variable were tested for significance by using a one way analysis of variance, Duncan using a level of significance of $P< 0.05$ (SPSS for windows 10.0)

RESULT AND DISCUSSION:

Table No. 1 Shown the mean value of Total phenol and antiamylase inhibition. The mean value was 267.18 mg % in herbal mix.)

Phenolic compound have antiamylase activity as phenol known to interact with protein and can inhibit enzymatic activity of α-glucosidse and α-amylase

Amylase is an enzyme which breaks down starch. The inhibition α-amylase is effective in controlling the disease such as diabetes and obesity

Table no-1 Total phenol, Anti-amylase of methalonic extract of herbal mix

Parameter	Mean
Total phenol (mg %)	267.18 ±48.77
Anti-amylase	
a) 400 µg/ml	11.5%
b) 800 µg/ml	23.0%

The mean value of antiamylase activity was 11.5 % and 23 % for 400 µg/ml and 800 µg/ml concentrated sample respectively. The obtained result indicated a moderate anti amylase activity

Effect on physiological parameters

Table no 2 shown the value of initial, final, and weight gain by animals and depicts the liver and kidney weight / 100 gm body weight.

Table no-3 Initial body weight, Final body weight and Weight gain, Tissue (Liver and Kidney) weight/100 gm body weight of control and experimental animals

Parameter	Group-I	Group-II	Group-III	Group-IV	F Value
Initial Body weight (gm)	192.17^{a} ±37.58	191.50^{a} ±35.27	191.83^{a} ±33.04	190.67^{a} ±29.82	0.02
Final Body weight (gm)	262.33^{a} ±50.01	258.50^{a} ±48.93	269.33^{a} ±59.43	257.00^{a} ±43.96	0.070
Weight gain (gm)	70.17^{a} ±20.27	67.00^{a} ±16.04	77.50^{a} ±26.79	66.33^{a} ±15.32	0.387
Liver Weight (gm)	3.08^{b} ±0.24	$2.81^{a,b}$ ±0.31	2.51^{a} ±0.19	2.91^{b} ±0.40	4.018*
Kidney Weight (gm)	0.59^{a} ±0.03	0.60^{a} ±0.03	0.55^{a} ±0.03	0.56^{a} ±0.11	0.931

- Values are Mean ± SD of six animals from each group.
- Mean values with the difference superscript within a row are significantly different ($P \leq 0.05$).
- * indicate significant difference at $P \leq 0.05$.

Weight gain of animal fed high fructose diet (Group-III) was higher than those of all other group. Weight gain was lower in the herbal mix fed control (Group-II) as well as high fructose diet along with herbal mix fed group (Group-IV), although change were non significant.

Liver weight ranged from 2.51 gm to 3.08 gm and they were statistically significant. Liver weight of animals fed high fructose diet (Group-III) was lower compared to the normal control (Group-I) and high fructose along with a herbal mix supplemented group (Group - IV).

Kidney weight / 100 gm body weight did not differ significant in herbal mix supplemented groups (Group-II and Group-IV) and high fructose diet fed group (Group-III) compared to normal control (Group-I).

Table no-3 Plasma insulin and glucose, Total Lipid, Triglycerides, Cholesterol, LDL, VLDL, HDL, of control and experimental animals

Parameter	Group-I	Group-II	Group-III	Group-IV	F value
Insulin (U/ml)	65.83^{a} ±5.42	64.67^{a} ±5.47	90.67^{c} ±8.29	80.00^{b} ±7.07	20.727*
Glucose (mg%)	50.13^{a} ±10.95	$52.45^{a,b}$ ±5.04	66.77^{b} ±13.33	$59.97^{a,b}$ ±15.12	2.4888*
Total Lipid (mg/dl)	144.20^{a} ±13.78	135.64^{a} ±19.00	203.22^{b} ±14.57	161.48^{a} ±35.90	10.556*
Triglycerides (mg/dl)	113.29^{a} ±10.77	114.20^{a} ±11.05	138.39^{b} ±7.71	116.58^{a} ±10.80	8.247
Cholesterol (mg/dl)	85.07^{a} ±7.86	72.79^{a} ±28.74	87.66^{a} ±6.40	82.02^{a} ±9.85	0.984
LDL (mg/dl)	29.54^{a} ±13.16	27.58^{a} ±12.18	31.01^{a} ±9.59	35.53^{a} ±10.10	0.532
VLDL (mg/dl)	23.99^{a} ±3.07	22.83^{a} ±2.21	29.01^{b} ±3.11	23.32^{a} ±2.16	6.810*
HDL (mg/dl)	31.54^{b} ±8.51	31.22^{b} ±5.62	27.44^{ab} ±5.12	22.72^{a} ±1.72	3.056

- Value are mean ± SD of six animal from each group
- *indicates a significant difference at P≤ 0.005
- Mean values with the different superscript within a raw are significantly different (P≤0.005)

Table no. 3 depicts the plasma insulin levels in control and experimental animals.

The **plasma insulin** level was 65.83. µU/dl in control animals (G-I). This was not affected when animals fed with control diet along with a herbal mix (G-II). The plasma insulin level was increased significantly (37.73 %) in G-III where animals were fed high fructose diet and value was significantly decrease in high fructose along with herbal mix compare to G-III. It shows positive effect of a herbal mix of plasma insulin level.

The increased level of plasma insulin could be due to hypertriacylglycerolemic among the fructose induced animals (12). It was reported that insulin resistance and reduced insulin binding in hypertriacyglycerolemic person, this may be one mechanism by which fructose diet promote insulin resistance.(13). Another potential mechanism leading to insulin resistance could involve decreased production of the adipocyte protein, adiponectin, because reduced circulating concentration of this hormone are associated with insulin resistance independently of body adiposity (14-15)

The plasma insulin has shown a positive and significant relationship with plasma glucose, (0.574), triglyceride (0.660) and VLDL (0.633), total lipid (0.750) and liver triglycerides.

The mean value fasting **plasma glucose** was 50.13 mg % in control group (G.I.). This was not affected when animals fed with control diet along with a herbal mix. The plasma glucose was significantly increased (37.73 %) in fructose fed group (G-III) and it was decrease significantly fructose with herbal supplementation (G-IV). Mechanism for increased in plasma glucose level is due to depletion of chromium among the high consumption of fructose in diet. Chromium is important in helping glucose pass from the blood stream into cell. Another mechanism was reported that fructose does not stimulate the production of 2 key enzymes insulin and leptin, which are involved in the long-term regulation of energy homeostasis. Thus if fructose intake is very high, lead to excessive energy consumption, it does have an adverse effect on glucose regulation. (2)

The plasma glucose levels showed a significant and positive relationship with plasma insulin level (0.574), liver triglyceride (0.453) and weight gain (0.349).

In the present study, the positive effect of a herbal mix on insulin and glucose is due to presence of variety of phenolic compound, in four different raw material used to developed to a herbal mix.

The results of plasma total cholesterol, HDL, LDL, VLDL, triglycerides and total lipid are depict in **table No 3.**

High intake of fructose cause dyslipidemia characterized by high triacylglycerol concentration and low concentration of HDL (16), which increased small dense, LDL (12), decreased circulating concentration of adiponection hormone (14).

The **plasma total lipid**, **triglyceride** and **VLDL** values of control animals (G-I) were 144.20 mg/dL 113.29 mg/dl and 23.99 mg/dl respectively . These were decreased (5.93 %, 22.15 %, 4.83%) when animals fed with control diet along with herbal mix (G-II). The plasma total lipid, triglyceride and VLDL were increased significantly (40.93 %, **30.13 %,** 20.92 %) in high fructose fed group (G-III). Which confirmed the lipogenesis and hypertriglyceridemia action of high fructose diet. The values were decrease significantly in high fructose along herbal supplementation (G-IV).

The lipogenic properties of high fructose feeding due to insulin and glucose are known to directly regulate the lipid synthesis and secretion. Insulin control hepatic sterol regulatory element binding protein (SREBP) expression, which is a key transcription factor responsible for regulating fatty acid and cholesterol biosynthesis, which was increased due to over consumption of fructose, so more cholesterol and fatty acid synthesis. Lead to hyperlipedemia. (17)

The higher level of TG and VLDL in blood may be due to the uncontrolled fructose metabolism in liver. Glucose metabolism is negatively regulated by phosphofructokinase. Fructose can continuously enter the glycolytic pathway without any disturbance of phosphofructokinases,

therefore fructose can uncontrollably produce glycerol molecules, will promote the over production of TG. (18)

The plasma TG shown positive relationship with insulin (0.660) VLDL (0.772) total lipid (0.808) and weight gain (0.364).

In the present study positive effect of herbal mix might be due to the presence of components such as s-allyl cystein sulfoxide (garlic), mamordicine (Bitter gourd), insoluble fiber rich fraction (carrot) and 3, 4 dihydroxy cinnamic acid also called caffic acid (cinnamon)

The mean values of plasma **total cholesterol and LDL** were found to be 85.07 mg/dl and 29.54 mg/dl control animals (G-I). Which were non significantly increase when animal fed with high fructose diet (3.58 %, 4.97 % respectively). The plasma cholesterol level was reduced non-significantly in animal fed with a herbal mix along with control (14.43 %). There was no positive effect on LDL level in fructose diet along with herbal mix . The positive effect of a herbal mix on total cholesterol could be due to the presence of fiber and other bioactive compounds.(4,19,20) when rat fed 60 % fructose, they developed high cholesterol level in blood, which was significantly reduced by supplementing them seasoning spices mix at three different doses (10, 30 or 50 mg/day per rat) (24). The increased level of cholesterol due to continue production of acetyl CoA. When much larger amount of fructose are consumed, fructose continues to enter the glycolytic pathway distal to phosphofructokinage and relatively unregulated sources of aetyl CoA in liver which lead to cholesterol production. (25).

It was studied that elevation level of LDL in blood is due to overproduction of VLDL. These VLDL, reach peripheral tissues where it reduced it triglycerides and designated as IDL. By receptor mediate endocytosis these IDL taken up by liver, where IDL reduced more triglycerol and convert into LDL (Cholesterol rich). So if there is more VLDL production. It leads to more LDL formation. (21)

In present study, we get positive effect of herbal mix on the blood cholesterol. It could be due to various bioactive constituents present in Bitter guard, garlic, cinnamon and carrot. Garlic contain sulpher compound, “S allyl cysteine sulphoxide (SACS)” was reported the lower serum cholestered level effect (22).

The **HDL** cholesterol of control group was 31.34 mg %, which was non significantly reduced (12.99 %) in high fructose group. The similar result was observed by Shalini et al., (2006). They found the significantly reduced in plasma HDL in high fructose fed group compared to control group .Supplementation of a herbal mix did not show any significant effect on plasma HDL level in G-II (Control +herbal mix) and G-IV (high fructose + herbal mix) as compared to control group.

Table No. 4 represents the value of triglycerides and cholesterol

The mean of **liver cholesterol** was 0.41 mg % in G-I (control) which was increased to 31 % and 19.51 % in G-II (Control + mix) and G-IV (Fructose + mix) respectively. The value of high fructose fed group (G-III) was increased (19.5 %) as compared to control group. It showed there was no effect of herbal mix on liver cholesterol.

The mean value of the liver triglycerides of control group (G-I) was 0.42 mg %. This value was increased slightly when animals fed with control diet along with a herbal mix (G-II), and significantly increased (40.47 %) in fructose fed group (G-III). It was decrease significantly (6.77 %) in fructose induced rat along fed with herbal mix (G-IV). Carbohydrate (Fructose) overload results in elevated TG because the large amount of sugar that need to be absorbed so rapidly from the intestine lead to the involvement of other metabolic pathways, such as hexose monophosphate shunt, that favor the syntheses of FFA (Free fatty acid) (23).

Table-4 Triglyceride and cholesterol of control and experimental group

Parameter	Group-I	Group-II	Group-III	Group-IV	F value
Triglyceride (mg%)	0.42^{a} ±0.04	0.43^{a} ±0.04	0.59^{b} ±0.04	0.55^{b} ±0.04	9.049*
Cholesterol (mg%)	0.41^{a} ±0.04	0.44^{a} ±0.04	0.49^{a} ±0.04	0.49^{a} ±0.10	1.217

- Value are mean ± SD of six animal from each group
- *Indicates a significant difference at P≤ 0.005
- Mean values with the different superscript within a raw are significantly different (P≤0.005)

REFERENCES:

1) American Diabetes Association & National Institute of Diabetes. The prevention or delay of type-2 diabetes care 2002, 742-9.

2) Elliott S, Keim N, Stern J, Teff K, Havel P. Fructose, weight gain, and the insulin resistance syndrome. *Am J Clin Nutr* 2002; 76:911-922

3) Crapo P, Scarlett J, Teff K, Havel P/ Fructose, weight gain, and insulin resistance syndrom. *Am J Nutr* 1982;36;256-261

4) Hariom yadav, shalini jain, and PR Sinha. Effect of skim milk and dahi (yogurt) on blood glucose, insulin and lipid fed with high fructose diet. *J Med Food* 2006;9(3): 328-335.

5) Shukla R, Sharma SB, Puri D, Prabhu KM, Murthy PS. Medicinal plants for treatment of diabetes mellitus. *Indian J Clin Biochem*. 2000;15:169-177

6) Shipra R, Agrawal V. Phytoconstituents and worldwide uses of ethomedicinal plants for hypoglycemic activity. *Res J Chem Environ*. 2002;6:63-68

7) Grover JK, Yadav S, Vats V. Medicinal plants of Indian with antidiabetic potencial. *J Ethnopharmacol*. 2002;81:81-100.

8) Yeh GY, Eisenberg DM, Kaptchuk TJ, Phillips RS. Systemic review of herbs and dietary supplementary for glycemic control in diabetes. *Diabetes Care.* 2003;26:1277-1294

9) Malik and Singh. Plant Enzymology and Histology. Kalayni Publication, New Delhi, PP:53.

10) Friedewald WT, Levi RI,Fredrickson DJ, Estimation of the concentration of low denstiny lipoprotein cholesterol in plasma without use of the ultracentrifuge. *Clin Chem* 1972; 18:499-52.

11) Verley H(1967). Blood Sugar determination & lipid in: Practical Clinical Biochemestry. Heineman Medical Books, LTD. New York. pp: 82-85 and 309-310.

12) Reaven G, Chen Y, Jeppesen J, Maheux P, Krauss R. Insulin resistance and hyperinsulinemia in individuals with small, dense low density lipoprotein particles. *J Clin Invest* 1993; 92:141-146.

13) Bieger W, Michel G, Barwich D, Biehl K, Wirth A. Diminished insulin receptors on monocytes and erythrocytes in hypertriglyceridemia. *Metabolism* 1984;33:982–7

14) Havel P. Control of energy homeostasis and insulin action by adipocyte hormones: leptin, acylation stimulating protein, and adiponectin. *Curr Opin Lipidol;*2002;13:51–9.

15) Weyer C, Funahashi T, Tanaka S . Hypoadiponectinemia in obesity and type 2 diabetes. Close association with insulin resistance and hyperinsulinemia. *J Clin Endocrinol Metab* 2001;86:1930–5.

16) Reaven G. Banting lecture 1988. Role of insulin resistance in human disease. *Diabetes* 1988;37:1595–607.

17) Brown M, Goldstein J. The SREBP pathway: regulation of cholesterol metabolism by proteolysis of a membrane bound transcription factor. *Cell* 1997; 89:331-340.

18) Mayes P. Intermediary metabolism of fructose. *Am J Clin Nutr* 1993;58:754–65.

19) Shalini J, Hariom Y, Sinha P. Antidiabetic effect of probiotic dahi containing Lactobacillus acidophilus and Lactobacillus Casri in high fructose fed rats. *Nutrition* 2007;23:62-68

20) Rajamani R, Suganthi M, Ravichndran Y and Anuradha C. Food seasoning spices mixture improve glucose metabolism and lipid profile in fructose fed hyperisulinemia rats. *J Medicinal Food 2005*;8(4): 502-507

21) Hellerstein M, Schwar J, Neese R. Regulation of hepatic de novo lipogenesis in humans. *Annu Rev Nutr* 1996;16:523–57.

22) Swanson J, Laine D, Thomas W, Bantle J. Metabolic effect of dietary fructose in healthy subjects. *Am J Clin Nutr* 1992;55:852-856

23) Sheela C, Augusti K. Antidiabetic effect of S-aayl cysteine sulphoxide isolated from garlic allium sativum. *Ind J Exper Biol* 1992;30:523-526

24) Hirsch J. Role and benefits of carbohydrate in the diet: key issues for future dietary guidelines. *Am J Clin Nutr* 1995; 61:996s-1000s.