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Research Article

Studies on Hypocholesterolemic and Antidiabetic Activity of *Capsicum annuum* Linn on Diet Induced Obese Rats

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ABSTRACT

Introduction: Herbal drugs constitute a major part in all traditional system of medicine. Researchers have no doubt that nature is still the preeminent synthetic chemist and that in plants, particularly; there are almost infinite reserves of chemical constituents with actual and potential effects on human body. The herb *Capsicum annuum* is a culinary herb and its two pharmacological properties i.e. lipid lowering and antidiabetic activity are unexplored. This research paper focuses on hypocholesterolemic and antidiabetic property of capsaicinoids and its further use as an antiobese drug. Experimental Work: Wister strain of albino rats were divided into four groups comprising of six rats each. Group I served as normal control fed with normal pellet chow, group II served as disease control fed with high fat diet /diabetogenic diet, group III and IV animals, received capsaicinoids and standard drug (Atorvastatin / metformin) respectively. The study was carried out for a period of 9 weeks for hypocholesterolemic and 11weeks for antidiabetic activity. Results: Administration of HFD or diabetogenic diet for stipulated days to rats significantly increased the body weight, serum total cholesterol, LDL cholesterol, VLDL cholesterol, triglycerides and glucose levels; and decreased HDL cholesterol as compared to normal control. Treatment with capsaicinoids/ atorvastatin showed a significant reduction in the body weight gain, and the levels of serum triglycerides, total, LDL, VLDL cholesterol and increase in HDL cholesterol . However capsaicinoids not only significantly reduced the cholesterol levels but also reduced the glucose levels which were due to dyslipidemia when compared with the standard. This was true with antidiabetic activity where the blood serum profiles reduced along with glucose levels

Keywords : Hypocholesterolemia, Antidiabetic, *Capsicum annuum*.

INTRODUCTION

Hypercholesterolemia and Diabetes mellitus (DM) are common disorders that affect more than 100 million people worldwide (6% of the population). These are two major disorders involved in the development of cardiovascular disease. Medical treatment and reduction of effects of these conditions are key modalities in the prevention of heart disease. The term diabetic dyslipidemia has been used to describe the pathophysiology surrounding the effect of insulin resistance on abnormal lipid levels. This concept states that defects in insulin action and increase in glucose can lead to higher amounts of lipoproteins in the blood. The subsequent increase in lipids, secondary to the state of glucose intolerance, adds to the progression of atherosclerosis and cardiovascular disease. As well, even slight increases in lipid levels in such diabetic patients are associated with substantial increase in cardiovascular disease. Overweight and obesity is the most frequent nutritional problem resulting mainly from an energy imbalance caused by an increased ratio of caloric intake to energy expenditure. Obesity is a strong risk factor for diseases such as type 2 diabetes, heart disease and stroke. Left unabated, the increasing rates of obesity in the world will place a severe burden on national healthcare systems. Obesity is recognized as the most crucial risk factor for type 2 diabetes. Obesity, in particular intra-abdominal adiposity, is associated with increased fatty acid (FFA) concentrations in blood plasma which exercises a major negative effect on insulin sensitivity in both muscle and liver[2]. Besides insulin resistance, defective insulin secretion is a prerequisite for the development of type 2 diabetes. Both lipotoxoicity and glucotoxicity may initiate and enable a vicious circle dependable for the metabolic impairment. Thus, discovery of new targets and therapeutic agents which can take care of Hypercholesterolemia and Diabetes is a focal point for combating this epidemic. A large section of world's population relies on traditional remedies to treat various diseases. Medicinal herb is an indispensable part of traditional medicine practiced all over the world due to its efficacy, low costs, easy access, ancestral experience and less side effects[3].

A number of plant neutraceuticals are common food constituents. There are a number of sources of neutraceuticals which includes dietary components of plant origin, plant metabolites, synthetic constituents and plant secondary metabolites. Neutraceuticals have shown to treat the underlying cause of illness and as a

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consequence of this many neutraceutical manufacturers and pharmaceutical companies are increasingly investigating the possibility of formulating and marketing plant based neutraceuticals[4,5]. Capsaicinoids obtained from the fruits of *Capsicum annuum* are gaining attention as neutraceutical health supplements today for wide variety of health benefits. Hence, the present study was carried out with an objective to investigate the hypocholesterolemic and antidiabetic activity of *Capsicum annuum* Linn.

Capsicum consists of dried ripe fruits of *Capsicum annuum* Linn var. pandi Family Solanaceae The fruit is yellowish orange to reddish brown, oblong conical with an obtuse apex, about 1-3 cm long and 1cm in diameter at the widest part. The pericarp is shriveled, glabrous, enclosing 10-20 flat reniform seeds either loose or attached to reddish dissepiments[6]. The fruits have been used since ancient times for its cardiovascular, cholesterol lowering, radical scavenging, pain killing, antidiabetic and antiarthritic potential. Capsicum contains mainly alkaloids (Capsaicin, dihydrocapsaicin), fatty acids, flavonoids, volatile oil and carotene pigments. It is also high in vitamin A, C, rutin, betacarotene, iron, calcium and potassium[7]. The experimental evidence for the evaluation of hypocholesterolemic and antidiabetic activities of fruits of *Capsicum annuum* variety pandi; (the Indian variety), could not be traced in the literature. In the present study an attempt has been made to evaluate the hypocholesterolemic and antidiabetic activity of the oleoresins of *Capsicum annuum* Linn.

MATERIAL AND METHODS

Preparation, authentication and evaluation of Capsicum annuum Linn.

The dried fruits of *Capsicum annuum* Linn were procured from local market, authenticated by Dr. Harshad Pandit, at Khalsa College and a voucher specimen CAA/270411A was assigned. The fruits were coarsely powdered and extracted in a Soxhlet extractor using ethanol. The extract was dried on a rotary evaporator to get a thick reddish brown oleoresin containing capsaicinoids[8]. The oleoresin was analysed to quantitate the capsaicin content.

A stock solution of standard capsaicin (1mg/ml) was prepared and it was diluted to prepare a working standard of 100µg/ml. The calibration curve for capscaicin was prepared in the range of 20-100µg/ml. The linearity response for capsaicin was assessed in the range of 0.2 µg/spot to 1ug/spot in terms of slope, intercept and correlation coefficient values. HPTLC was performed on precoated silica gel G plates in the solvent system Toluene: Ethylacetate (7:3) and was scanned at 280nm. A 1:50 dilution of the oleoresin was carried out and 10µl of the oleoresin was co chromatographed with standard capsaicin to determine the content of capsaicin in the oleoresin sample.

Study of Hypocholesterolemic and antidiabetic activity

Experimental Animals

Healthy Wistar strain of Albino rats (4 weeks old) of both sexes weighing 40-45 gm were used for the present study. A random distribution of male and female animals was done in both the groups as no significant difference in both sexes was reported in the literature. The animals were housed in polypropylene cages and maintained under controlled environment (25°±2°C temperature, 60 ± 5% relative humidity, 12hr light/dark cycle) with free access to standard animal pellet diet and water. They were acclimatized to the laboratory conditions for a week. The animal experiments were conducted as per the ethical guidelines of CPCSEA after obtaining necessary clearance from the Institutional Animals Ethics Committee

Experimental Diet

High fat diet was fed to the animals to induce hypercholesterolemia. The high fat diet (HFD) consisted of starch 20g + Coconut oil 25g +Lard 20g + Mineral mixture 3g+ cholic acid 1g + vitamin mixture 5g + cholesterol 1g+ cellulose 25g. All the ingredients were weighed and dispensed in coconut oil. 3gms of the high fat diet was fed to the animals using an oral gavage feeding tube.

A diabetogenic diet was used to induce diabetes in the antidiabetic study**.** The diabetogenic diet included the following- starch 15g + soyabean oil 20g +Lard 20g + Mineral mixture 2g+ sucrose 10g + vitamin mixture 3g + cellulose 10g + Milk Powder 10g + casein 10g. All the ingredients were weighed and dispensed in soyabean oil. 3gms of diabetogenic diet was fed to the animals using an oral gavage feeding tube. These diets were provided in addition to normal pellet chow[9,10].

Experimental Design

Hypocholesterolemic activity

Following one week of acclimatization, the rats were randomly divided into 4 groups with six rats each: Group I: Normal control rats fed on Standard Chow Diet. Group II: Obesity control rats fed on High Fat Diet for 9weeks. Group III: Rats fed with HFD and treated with Atorvastatin 1.2mg/kg bw. Group IV: Rats fed with HFCD and treated with *Capsicum annuum* oleoresin 50mg/kg b.w. Animals of groups II, III and IV were fed with HFD throughout the 9 week study. Group III and IV animals were given treatment from 7th to 9th week (i.e. for a period of 21days).

Antidiabetogenic activity

Following one week of acclimatization, the rats were randomly divided into 4 groups with 6 rats each. Group I-Normal Control rats were fed on Standard Chow Diet. Group II: Negative control, where rats were fed with diabetogenic diet for 11 weeks Group III: Rats fed with diabetogenic diet and treated with Metformin100mg/k.g.b.w. Group IV: Rats fed with diabetogenic diet and treated with capsicum annuum oleoresin 50mg/kg b.w. Animals of groups II, III and IV were fed with diabetogenic diet throughout the 11 week study. After completion of 8 weeks Group III and IV animals were administered respective treatments from 9th to 11th week (i.e. for a period of 21days) along with the diabetogenic diet.

Sample collection and Biochemical Analysis

Blood samples were withdrawn under ether anaesthesia by puncturing retro orbital plexus with the help of heparinised glass capillary into a 5ml test tube and was allowed to coagulate at room temperature for 30 mins. Serum was

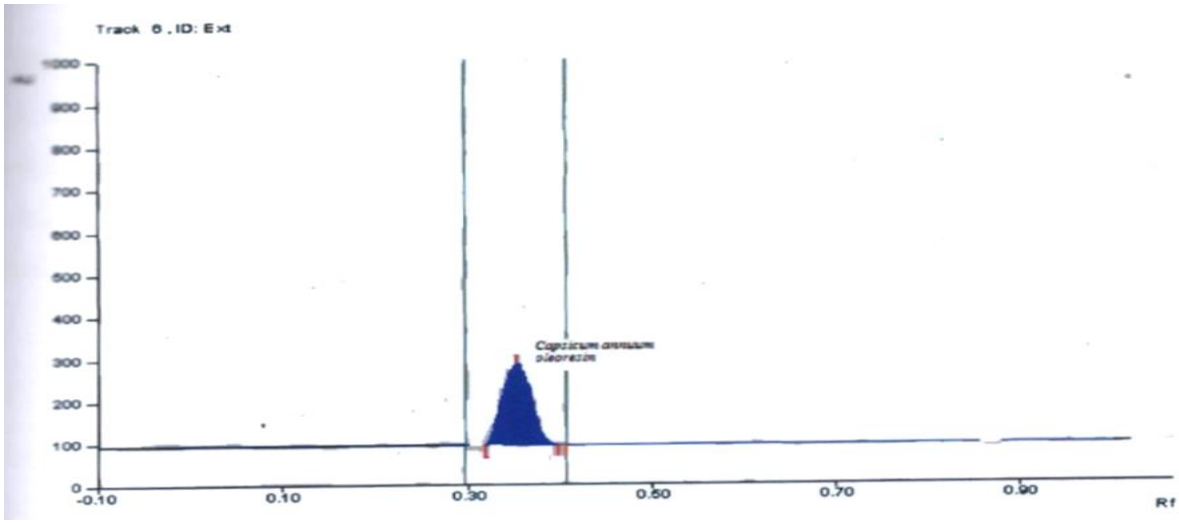

Figure 1: HPTLC Chromatogram of *Capsicum annum* oleoresin at 280nm.

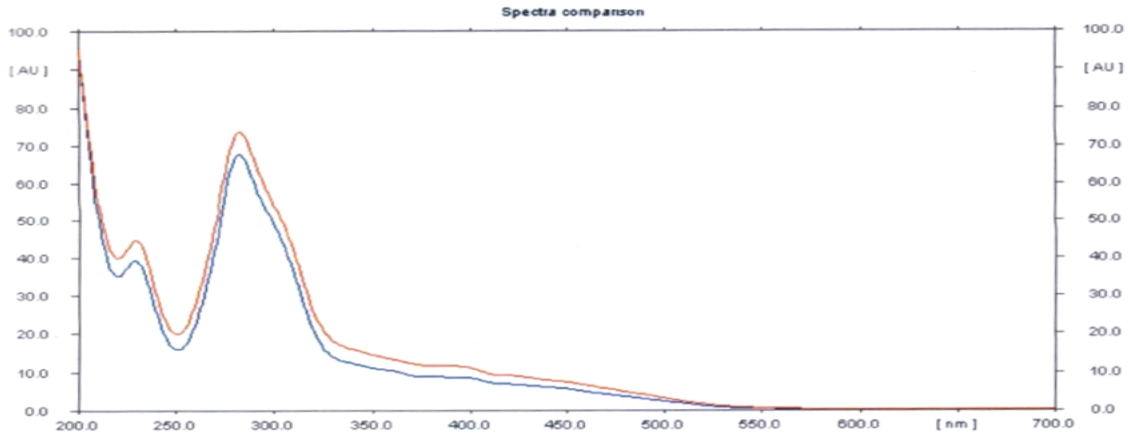

Figure 2: Spectral overlay of standard capsaicin (blue) and capsicum annum oleoresin (Pink).

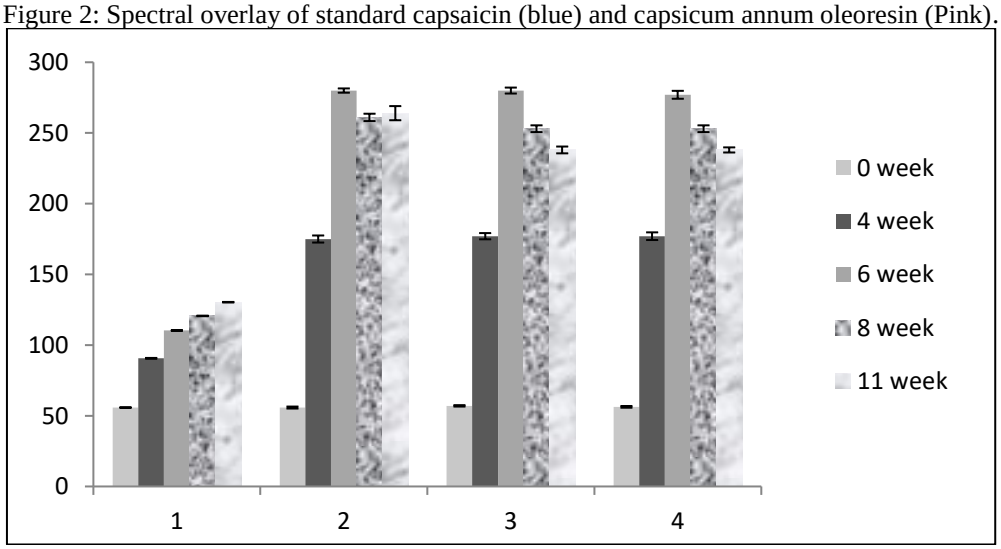

Figure 3: Effect of body weight on rats with diabetogenic diet.

separated by centrifugation at 4000rpm for 20 min at 40˚ C.

The separated serum was analysed for serum cholesterol levels (mg/dl), serum glucose levels (mg/dl), serum triglycerides levels (mg/dl), serum LDL, HDL, VLDL levels (mg/dl). Serum glucose was estimated by the Glucose Oxidase Peroxidase method (GOD-POD), Serum cholesterol levels were estimated by enzymatic Cholesteroloxidase- Peroxidase (CHOD-POD) method, triglycerides by enzymatic Glycerol-3-phosphate oxidase - Peroxidase (GPO-POD) method and HDL cholesterol by phosphotungstic acid (PTA) method.

At beginning of the study i.e. on day 0 fasting blood samples from all the four groups in both the study were

Table 1 : Effect of test samples on the biochemical profiles under hypocholesterolemic property.

		Cholesterol	Triglycerides	VLDL	HDL	LDL	GLUCOSE
Group I	0 week	75.0± 0.3	69.8.2 ±0.2	13.9±0.2	44.1 ± 0.2	17.5± 0.2	79.5 ± 0.4
	6 week	105±0.8**	74.3±0.6**	15.6±0.2**	44.3±0.6**	32.7±0.3**	84.6±0.3**
	9 week	110±0.6***	77.6±0.7***	16.3±0.5***	45.6±0.7***	35.2±0.3***	89.2±0.2***
Group II	0 week	75.0± 0.6	69.3± 0.8	13.9± 0.2	44.1± 0.2	17.5 ± 0.5	78.8± 0.4
	6 week[a,d]	208.1±1.5**	145.80±1**	29.1±0.2**	18.7±0.7**	160.3±1.9**	136.0±1.2**
	9 week[a,d]	290.4±3.9***	167±2.4***	33.4±0.5***	14.9±0.5***	242.1±3.9***	160.±1.4***
Group III	0 week	75.2± 0.5	70.5 ± 0.6	14.3 ± 0.2	44.4 ± 0.4	17.8± 0.4	79.4± 0.4
	6 week[a,c]	202.2 ±1.0**	146.45±1**	29.23±0.2**	18.25±0.5**	154.63±1.1**	132.11±1.8**
	9 week[a,c]	91.07±0.2***	97.49 ± 0.4***	19.5±0.1***	37.8 ± 0.6***	33.68± 0.5***	138.13±0.8***
Group IV	0 week	76.1 ±0.4	69.6± 0.7	15.0 ±0.4	44.4± 0.5	18.0 ± 0.4	79.1 ± 0.3
	6 week[b,c,d]	202.96± 1.4**	144.1±1.1**	29.02±0.2**	17.7 ±0.6**	157.17±1.6**	129.62±2.1**
	9 week[b,c,d]	81.80 ±0.2***	90.42± 0.3***	18.1± 0.1***	40.89 ±0.3***	25.1 ±0.5***	88.13 ±1.2***

Table 2 : Effect of the test samples on biochemical properties under antidiabetic study.

		Cholesterol	Triglycerides	VLDL	HDL	LDL	GLUCOSE
Group I	0 week	76± 0.4	70.2 ±0.1	14.2±0.2	43.7 ± 0.1	17.3± 0.3	79.8 ± 0.3
	9 week	113±0.6	76.5±0.4	19.1±0.2	46.7±0.3	36.3±0.2	86.7±0.1
	11 week	115±0.2***	77.6±0.2***	20.6±0.1***	47.5±0.2***	38.2±0.4***	89.8±0.3***
Group II	0 week	75.5± 0.6	69.3± 0.8	13.9± 0.2	44.1± 0.2	17.5 ± 0.5	78.8± 0.4
	9 week [a,d]	240.47±0.7	141.80±0.3	28.4±0.1	21.0±0.4	190.8±1	306.8±5.9
	11 week[a,d]	274.51±2.2***	159±0.8***	31.8±0.2***	17.7±0.5***	223±2.3***	344.2±8***
Group III	0 week	76.2± 0.4	69.8 ± 0.4	14.5 ± 0.2	44.6 ± 0.2	17.8± 0.2	78.9 0.3
	9 week[a,c]	240 ±0.7	141.33±0.3	28.26±0.1	20.15±0.2	191.49±0.7	305.91±5.6
	11 week[a,c]	192.2±0.8***	143.3± 0.8***	28.66 0.2***	30.23 0.3***	133.28 0.8***	130.37 0.6***
Group IV	0 week	75.1 ±0.4	70.1± 0.3	15.0 ±0.2	45.1± 0.3	17.3 ± 0.5	78.6 ± 0.3
	9 week[b,c,d]	241.7± 1.2	142.2 ±0.5	28.4 ±0.1	21.0 ±0.5	192.2± 1.1	307.4 ±6.2
	11week[b,c,d]	99.4 ±1.2***	91.5± 0.6***	18.3± 0.1***	40.8 ±0.1***	39.7 ±1.2***	121.0 ±1.0***

**P<0.001 statistically significant when compared with 6 week

***P<0.001 statistically significant when compared with 9 week

Data represents mean ± SEM. One way ANOVA followed by Dunnett's test, p<0.001.

a . Positive control group was found to be significantly different from the negative control group

b. Test group was found to be significantly different from positive control group

c. Positive control and Test group were found to be significantly different and the results are comparable

d. Test group found to be significantly different from the negative control group

collected to evaluate the biochemical parameters. Blood samples were then collected at the 4th, 6th and 9th week in case of hypocholesterolemic study and 4th 6th, 8th and 11th week in case of diabetogenic study and biochemical parameters were estimated. The animals were sacrificed after 9 weeks and histopathology of liver was done in case of hypocholesterolemic study. The histopathology of organs i.e. liver, kidney and pancreas was carried out after 11weeks in case of diabetogenic activity.

In addition to the biochemical parameters, the individual body weights of the animals were also recorded. These values were recorded and treated as baseline values. The significant changes in individual body weight was recorded during the period of study.

Statistical Analysis

All the data reported are expressed as mean ± S.E.M. Statistical analysis was performed by one way ANOVA followed by Dunnett`s test . P <0.001 was considered as extremely significant.

RESULTS

Evaluation of Capsicum annuum Linn.

The HPTLC chromatogram with a single peak at Rf 0.35 for capsaicin was obtained and was found to be linear in the range of 0.2ug/spot to 1ug/spot with a correlation coefficient r^2=0.9971. The linear regression equation was y=823.89x+842.81 for capsaicin. The capsaicin content in the fruit was found to be 2.8%w/w, which was found to be within the limits given in literature. The HPTLC chromatogram of Capsicum annuum fruit showing the peak of capsaicin is given in Fig.1. A spectrum overlay of standard capsaicin over capsaicin isolated from *Capsicum annuum* fruit is shown in Fig.2.

Evaluation of hypocholesterolemic and antidiabetic activity

After 7 weeks of HFD administration, wistar rats developed hypercholesterolemia which was associated with elevated levels of cholesterol, triglycerides, LDL,

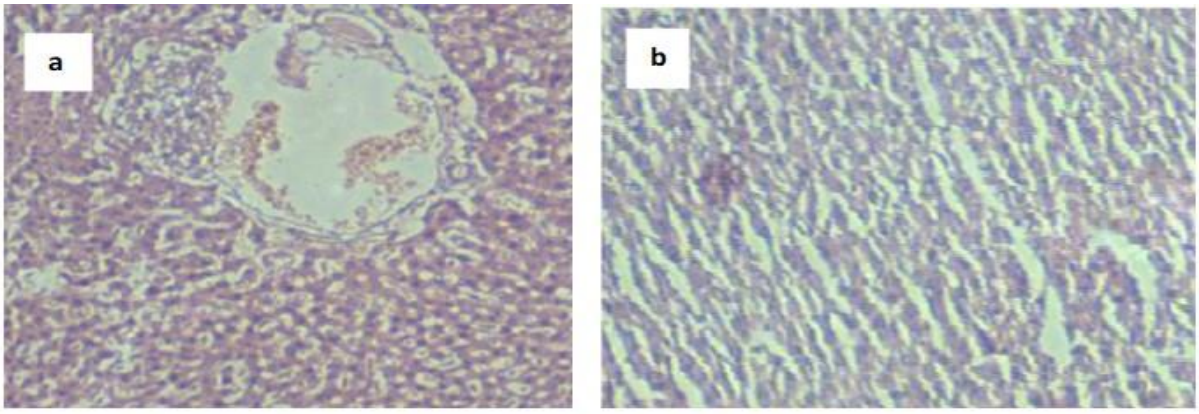

Figure 4 : a. Hyperlipidimic liver of a hypercholesterolemic rat showing fatty degeneration. b. After treatment with capsaicinoids , fatty degeneration was cured.

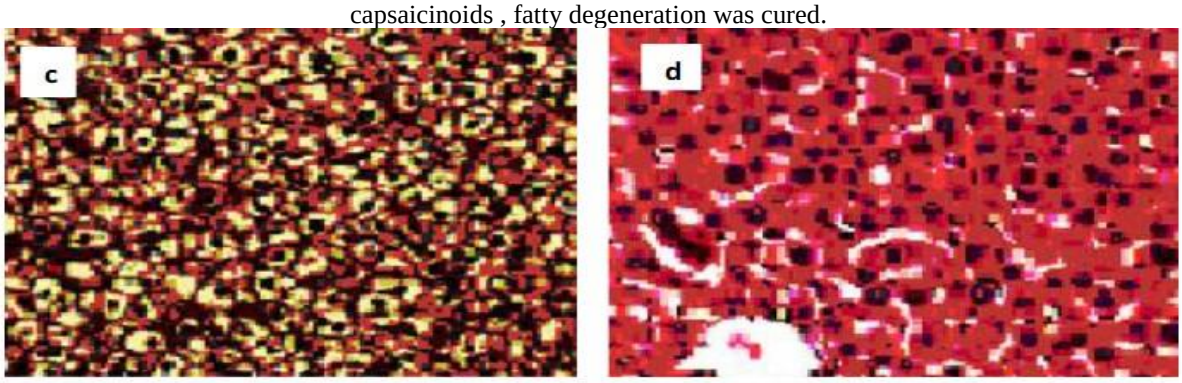

Figure 5: (c). Liver of diabetic rat showing fatty infiltration (d). After treatment with capsaicinoids normal liver tissue.

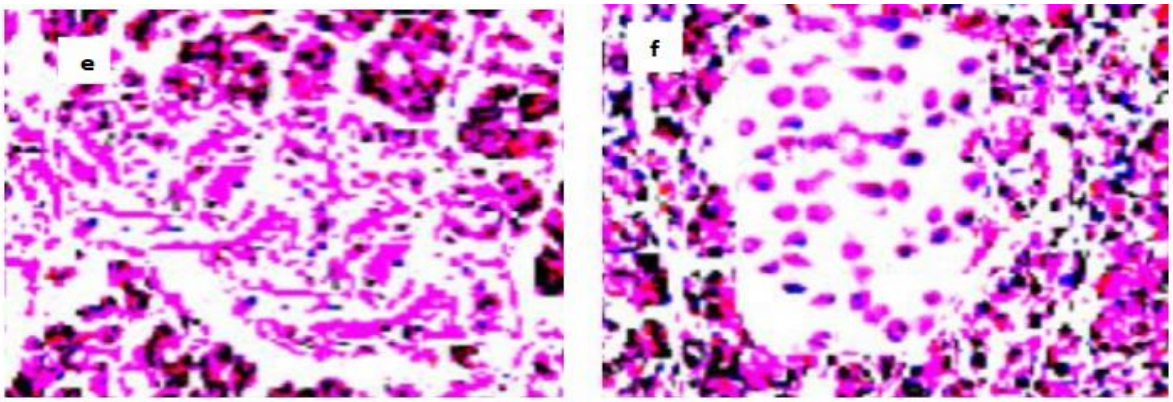

Figure 6: (e) Pancreas of diabetic rat showing depleted islets of pancreas.(f) Normal pancreatic islets after treatment with capsaicinoids.

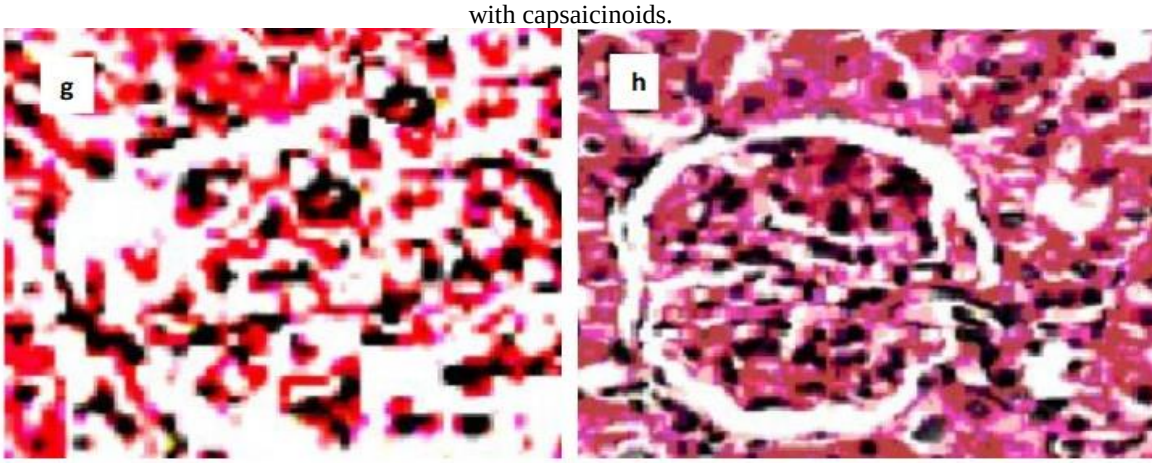

Figure 7: (g) Diabetic rat showing tubular damage, proteinuria and haemorrhage. (h) Capsaicinoids treated rat mild tubular epithelial artopy and congestion of capillaries and almost normal histology.

glucose, and lowering of HDL and also significant increase in animal body weight. Repeated once daily oral administration of atorvastatin and *Capsicum annuum* oleoresin to the groups III and IV produced significant changes ($P<0.001$) in biochemical parameters at the 9th week of the experimental period compared to Group II, the negative group. Treatment with the standard drug atorvastatin showed no significant decrease in the elevated glucose blood levels which is the result of dyslipidemia. Whereas treatment with *Capsicum annuum* oleo resin not only significantly reduced the cholesterol levels but also reduced the glucose levels.(Table-1) The histopathology of a hyperlipidemic liver of a hypercholesterolemic rat (group II) showed fatty degeneration which on treatment (group III and IV) showed normal hepatic structure with no fatty degeneration. (Fig. 4a,b)

After 8 week of administration of diabetogenic diet, the Wister rats developed diabetes which was associated with elevated levels of glucose i.e hyperglycaemia, and high lipid levels. Repeated once daily oral administration of metformin and *Capsicum annuum* oleoresin orally to the groups III and IV produced a significant ($P<0.001$) decrease in serum glucose levels at the 11th week of the experimental period compared to the negative group thus demonstrating the anti diabetic activity of metformin and Capsicum oleoresin (Table 2) .Treatment with *Capsicum annuum* oleo resin not only reduced the glucose levels but also helped in improving dyslipidemia. The lipid profile, glucose levels and body weight of animals reached almost normal values after 21 day treatment (Fig.3). The histopathology of kidney of Group II diabetic rats showed tubular damage, proteinurea and haemorrhage. In treated diabetic rat kidney, glomeruli and tubules without proteinurea were observed. In the section of normal liver tissue, sinusoidal cards of hepatocytes with central vein and portal tracts were observed. The portal tracts show portal triad with portal vein, hepatic artery and bile duct, where as the diabetic rat liver tissue section shows distortion in the arrangement of cells around the central vein, periportal fatty infiltration with focal necrosis of hepatocytes.. The histopathology of pancreas in normal rat showed normal, round exocrine acini and endocrine islets The pancreas of treated group rats showed normal architecture of islets of pancreas (Fig.5-7)

DISCUSSION

The relationship of obesity and associated hypercholesterolemia with type -2 diabetes is well established. Genetic susceptibility, environmental factors, nutrition deficiencies, physical inactivity exposes an individual to greater risk of obesity and its related hypercholesterolemia, which further leads to complications associated with type 2 diabetes. Thus an individual exposed to the risk of obesity and hypercholesterolemia is equally at a risk of acquiring type -2 diabetes and reverse is also true[2]. It is well documented that obesity is associated with serious mortalities like high incidence of type 2 diabetes, hyperlipidemia, hypercholesterolemia, fatty liver, cardiovascular diseases, osteoarthritis and increased risk of many forms of cancer[3]. Medicinal herbs are indispensable parts of traditional medicine and there is a big renaissance of the herbal medicines globally as these drugs are found to be effective and safe[11]. Hence, in the present work the hypocholesterolemic and antidiabetogenic potential of capsaicinoids was investigated.

The hypocholesterolemic activity was studied in rats fed with high fat diet (HFD) and was investigated by analyzing the body weight, blood biochemical profiles and histopathology of liver. Similarly the antidiabetogenic activity was carried out on rats fed with diabetogenic diet and was investigated by analyzing the body weight, blood biochemical profiles and histopathology of liver, kidney and pancreas. Body weight significantly increased in HFD fed and diabetogenic fed rats when compared with normal diet fed rats. This increase in weight might be due to increased energy intake leading to increased fat deposition in tissues and organs. An increase in organ fat causes an increase in lipolysis that leads to an increase in free fatty acids. This acts as precursor to gluconeogenesis or new production of glucose in the liver causing increased hyperglycemia. They are also the building blocks for increased production of very low-density lipoproteins. Increased free fatty acids in the beta cell leads to impairment of beta-cell function causing cell death. This leads to impaired insulin secretion that perpetuates the hyperglycemic state[12]. In HFD fed rats, when treated with capsaicinoids, the gain in body weight decreased significantly. It was also observed that administration of capsaicinoids did not alter food intake in treated rats, indicating that prevention of weight gain induced by this extract was not due to the reduction of energy intake but because capsaicinoids increased the catabolism of lipids in adipose tissue resulting in decrease in mean body weight[13]. Elevated serum concentrations of Total Cholesterol (TC), Low Density Lipoprotein Cholesterol (LDL-C), Triglycerides (TG) along with decreased concentration of High Density Lipoprotein Cholesterol (HDL-C) observed in HFD / diabetogenic diet fed disease control rats are the major risk factors for the development of coronary heart disease and atherosclerosis. Data of the Hypocholesterolemic study suggested that a 21 days administration of capsaicinoids exerts a positive effect on lipid profile ie., Capsaicinoids (50mg/kg bw) showed a significant reduction of lipid levels ($p<0.001$) and improvement of hypercholesterolemia, dyslipidemia and also caused a significant lowering of elevated serum glucose levels when compared with untreated disease control group. The results may partly be caused by the decreased absorption of cholesterol from the diet or by the thermogenesis effect[9]. Data of the Antidiabetogenic study suggested that a 21 days administration of capsaicinoids showed a significant reduction in glucose levels and at the same time also dyslipidemia The significant increase in glucose in diabetogenic diet fed animal can be due to defective insulin synthesis and decreased insulin efficiency[13]. The test drug might have enhanced the secretion of insulin from the β-cells of the islets of pancreas or increased the efficiency of insulin which

facilitates the delivery of glucose from blood to target tissues.

The literature review revealed that high fat diet-induced choleterolemia and abnormal lipid metabolism all collectively are associated with inflammation, congestion, and nonalcoholic fatty liver disease (NAFLD) leading to hepatic failure. On observation of histopathological results, these conditions were completely restored in treated groups.

CONCLUSION

To conclude, the results of the present study depict that administration of capsicum oleoresins regulates serum lipid profiles and reduces the glucose levels in both high fat diet fed rats and diabetogenic diet fed rats. The mechanism induced by oleoresins of *Capsicum annuum* will be further researched.

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