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CARTHAMUS TINCTORIUS (SAFFLOWER) A NATURAL REMEDY FOR DYSLIPIDEMIAS IN DIABETIC PATIENTS

NASREEN QAZI[1], RAFIQUE ALAM KHAN[2], ALI RAZA MEMON[3], JAMIL AHMED LAGHARI[1], IRSHAD HUSSAIN GHANGHRO[*4]

Department of Pharmacology , Liaquat University of Medical & Health Sciences (LUMHS) Jamshoro[1], Department of Pharmacology, Faculty of Pharmacy, University of Karachi[2] , Department of Biochemistry, Liaquat University of Medical & Health Sciences (LUMHS) Jamshoro[3], Water Testing & Surveillance Laboratory, Liaquat University of Medical & Health Sciences (LUMHS), Jamshoro[4]. Institute of Biochemistry, University of Sindh, Jamshoro[5]

[*]Corresponding Author: Irshad Hussain Ghanghro: E-mail: irshadghanghro@yahoo.com; Contact: +923043733104

ABSTRACT

Diabetic patients are more prone to develop hyperlipidemias which further worsen the condition thus there are increased chances of coronary artery disease and stroke. 35 male albino rabbits were divided into 5 groups with seven animals in each group. Diabetes was induced by giving 150 mg of alloxan intraperitoneally. Group A and B served as normal and diabetic controls while group C received Glibenclamide and group D and E received 200 and 300mg/kg methanolic extract of carthamus tinctorius. Total cholesterol, triglycerides, HDL and LDL were estimated on day 15 and 30 0f the study. Total antioxidant effect of Carthamus tinctorius was also evaluated and it was compared to Trolox standard control at the end of the study, it was found that the mean TAS levels were within the established normal range of 1.30 - 1.77 mmol/l Plasma given by the Randox protocol.

Key Words: Diabetes, *carthamus tinctorius*, albino rabbits, Dyslipidemias

INTRODUCTION

Diabetes Mellitus in adults is associated with two to four fold increased risk of vascular disease as compared to individuals without diabetes. CVD (Cardiovascular Disease) is the primary cause of death among people with insulin dependent and non insulin dependent diabetes mellitus (1,2).

Diabetes Mellitus is independent and the most powerful risk factor for stroke, coronary artery disease and peripheral artery disease (3,4). When there is hyperglycemia it tends to increase the risk of diabetic complications and leads to increased vascular alterations thus speeding up atherosclerosis.

One of the important mechanisms responsible for increased atherosclerosis is Maillard or browning reaction which results in more enzymatic reaction between glucose and protein or lipoproteins in the walls of the arteries. The reversible early glycosylation products with reactive amino acid groups of circulating or vessel wall proteins (Schiff bases) with glucose subsequently are re arranged to form more stable amadosi-type early glycosylation products. The equilibrium between levels of Amadosi products (the best known of which is HbAIC) and Schiff base is reached within hours and weeks (5,6).The advanced glycosylation end products (AGEs) are formed as some early glycosylation products and continue to undergo series of chemical rearrangements on vessel wall collagen of long –lived proteins, these AGEadducts are virtually irreversible and stable if once formed. Among the large number of AGEs carboxymethyllysine is found abundantly in vivo (7).

Tissue microenvironment redox potential is another factor in the formation of AGEs. If oxidative stress increases AGEs formation increases substantially (8).

In patients of Insulin resistance the metabolic tracer studies have documented that there is over production of triglycerides VLDL (Very Low density lipoproteins) with hypertriglyceridemia (9). Recent studies has also reported that there is increase secretion of apolipoprotein (apo) B in type 2 diabetes(10). The increase in apo B – containing lipoproteins is may be due to free fatty acids (FFA) flux towards the liver (11), and due to lack of sensitivity to suppress effects of insulin on apo B in insulin resistant persons (12). The increase in apo-B containing lipoprotein particles and increased levels of triglycerides can drag the metabolic process that result in reduced smaller and dense HDL cholesterol particles.It is very necessary that aggressive management of all CV risk factors, including dyslipidemias,

should be initiated early in the course of the disease before developing complications (6). In previous study carthamus tinctorius was known to have a significant glucoselowering effect along with promising effect on insulin levels so this study was performed with the aim to observe its lipid lowering properties.

MATERIALS AND METHODS

Collection and preparation of crude extracts

Dried safflowers (petals of carthamus tinctorius) were obtained from a local herbal dealer at Peshawar, Pakistan and were identified by Professor Dr. Ghazala H. Rizwani, Department of Pharmacognosy, Faculty of Pharmacy,university of Karachi. The dried flowers were soaked in ethanol for eight weeks, filtered and then evaporated in rotary evaporator at 40^0 C followed by freeze drying at -30^0 at HEJ Research Institute of Chemistry. Extract yield was approximately 10 gm from 100 gm.

The animals (Rabbits) were placed in separate cages under controlled conditions of humidity (50-60%) and temperature (22 ± 2^0C) and twelve hours light and dark cycles, were provided with diet and water ad labitum. Animals were kept under observation for a week in animal house of Pharmacology Department before using them in the experiment.

Induction of diabetes mellitus in rabbits

Rabbits were chosen as animal model. Studies has demonstrated that they have ideal characteristics required in laboratory animal model including strain specific, longer life span, convenient size and are easily handled and are also reasonably priced (13).

Rabbits were weighed at start and end of the study. Alloxan monohydrate was provided by Sigma Aldrich Company. After dissolving in distilled water, it was injected through intraperitoneally at a dose of 150mg/kg (14). Alloxan is commonly used chemical for induction of diabetes mellitus and a well-known diabetogenic agent which is widely used to induce Type 2 diabetes in animals (15).

Alloxan along with its reduction product dialuric acid starts up a redox cycle with the formation of superoxide radicals. When these radicals undergo dismutation to hydrogen peroxide, after that highly reactive hydroxyl radicals are formed by fenton reaction. There is rapid destruction of beta cells after action of reactive oxygen species with a concurrent enormous increase in cytosolic calcium concentration (16).

Cholesterol profile

Overnight fasted blood samples were collected to the tubes with no anticoagulant,

Serum separation was done by centrifugation at 3000 rpm for 15 minutes. Total cholesterol, Triglycerides, Low density lipoproteins, high density lipoproteins levels were measured in mg/dl by using automatic analyzer by Hitachi model 902, configured as console type automatic analyzer with analytical and operation units integrated and based on colorimetric (absorbance method) principle.

Total antioxidant status (TAS)

A new-generation Daytona automatic chemical analyzer was used to assess TAS. It was performed according to directions provided by the Randox Company. The protocol quantified absorbance of plasma samples at 600 nm. The Trolox standard and the control plasma sample were run at the same time. The results were expressed as mmol Eq Trolox/L of plasma (Pl).

ml blood was taken by cardiac puncture technique. Plasma was separated by centrifugation at 3000 rpm for 15 minutes in 14K Humax centrifuge, Plasma samples were stored without further preparation at -20^{o}C until use for TAS measurement The TAS assaywas conducted applying Randox TAS kit components with Trolox as an equivalent standard (17).

RESULTS AND DISCUSSION

Results in table 1 reveals the comparison of cholesterol, triglycerides, LDL-C and HDL-C after 15 days of treatment with CTE among control, Diabetic, Glibenclamide treated , and two CTE treated groups (200 and 300mg/kg), Cholesterol, triglycerides, and LDL values were increased significantly whereas HDL values were significantly decreased in diabetic rabbits (p- value≤ 0.05).

Animals of group D who received 200m/kg of CT extract, showed a highly significant (p-value ≤0.01) reduction in total cholesterol (94.29±4.9 versus 121.00± 6.83) and LDL-C (14.86 ±2.34 versus 34.00 ±4.32). However there was a significant decrease in triglycerides (p value ≤0.05) levels (118.43 ±4.65 versus 124. ±4.32) while there was a non-significant change in HDL-C (25.50±3.90 versus 25.50±1.91) as compared to diabetic group.

There was more promising results in animals of group E at a CT extract 300mg/dl, total cholesterol and LDL-C reduced to 57.00± versus 121.00±6.83 and 11.67±1.86 versus 34.00±4.42 respectively which is highly significant (p value 0.01), whereas triglycerides were also reduced significantly to 99.00±4.85 as compared to diabetic control. There was a significant increase in HDL-C to 40.17±4.75 as compared to

diabetic control group i-e 25.50±1.91 which is beneficial. Glibenclamide a standard drug reduced cholesterol significantly to 109.29±12.29 as compared to diabetic control ie 121.00 ± 6.83, triglycerides to116.00± 3.41L mg/dl and LDL-C to 17.43 ± 2.44 when compared to diabetic control group which is highly significant, On the other hand it increased HDL–C significantly33.14 ± 3.97 mg/dl versus diabetic control group.

Data in table 2 reveals the hypolipidemic effect of carthamus tinctorius at 200 and 300mg/kg after 30 days of dosing. Levels of cholesterol, triglycerides and LDL-C were significantly increased in diabetic rabbits as compared to control group.

At 200m/kg dose of CT there was a highly significant reduction (p value ≤0. 01) in total cholesterol 88.71 ± 2.87 versus 127.50 ± 9.88mg/d and LDL-C (12.57± 1.39 versus 28. ±4.32) and significant decrease in triglycerides (p value ≤0.05) as compared to diabetic control group. However HDL-C levels increased significantly as compared to diabetic controls.

When results were compared for group E in which animals were treated with 300mg/kg of CT extract for 30 days, total cholesterol decreased to 52.83 ± 5.81mg/dl which is highly significant, however triglycerides and LDL –C were significantly reduced to 50.17 ± 4.21 and10.67 ± 31.58mg/dl respectively as compared to diabetic control group while HDL values increased significantly (p≤0.05).

Glibenclamide a standard drug reduced triglycerides to 88.57 ± 5.74mg/dl and LDL-C to 9.43 ± 1.51 when compared to diabetic control group which is highly significant, On the other hand it increased HDL–C significantly to 39.00 ± 4.33mg/dl versus diabetic control which was 26.00±4.32.

When antioxidant effect of carthamus tinctorius was compared to Trolox standard control at the end of the study, and results were compared in diabetic rabbits treated with carthamus tinctorius at 200mg/kg and 300mg/kg dose. It was found that the mean TAS levels were within the established normal range of 1.30 - 1.77 mmol/l Plasma given by the Randox protocol.

Table1: Effect of Carthamus tinctorius on Lipid Profile in alloxan-induced Diabetic rabbits after 15 days (n = 31)

Groups	Cholesterol	Triglyceride	LDL-C	HDL –C
Control [a]	86.86 ± 7.90	53.14 ± 3.84	15.57 ± 2.14	33.29 ± 4.27
Diabetic [b]	121.00 ± 6.83	124.00± 4.32	34.00 ± 4.32	25.50 ± 1.91
G. Treated [c]	109.29±12.29† [b]	116.00± 3.41† [b]	17.43 ± 2.44†† [b]	33.14 ± 3.97† [b]
CT 200mg/kg [d]	94.29 ± 4.19 ** [b]	118.43 ± 4.65*b	14.86 ± 2.34** [b]	25.71 ± 3.90
CT 300 mg /kg [e]	57.00 ± 4.64 ** [b]	99.00 ± 4.85 * [b]	11.67 ± 1.86 ** [b]	40.17 ± 4.75* [b]

Values are expressed as Mean ± SEM

***P < 0.05:** (significant) or **P<0.01**(highly significant) as compared to group b

† p value < 0.05(significant), †† p value < 0.01 (highly significant)

Table 2: Effect of Carthamus tinctorius on Lipid Profile in alloxan-induced diabetic rabbits after 30 days(n = 31)

Groups	Total Cholesterol	Triglycerides	LDL -C	HDL -C
Control [a]	86.14 ± 6.56	50.29 ± 4.27	14.14 ± 2.61	32.00 ± 2.82
Diabetic [b]	127.50 ± 9.88	135.50 ± 6.60	28.00 ± 2.82	26.00 ± 4.32
G. Treated [c]	102.71 ± 2.62 † [b]	88.57 ± 5.74†† [b]	9.43 ± 1.51††[b]	35.29 ± 4.42†[b]
CT 200mg/kg [d]	88.71 ± 2.87** [b]	61.00 ± 5.85* [b]	20.67 ± 1.39* [b]	36.43 ± 2.93* [b]
CT 300 mg /kg [e]	52.83 ± 5.81** [b]	50.17 ± 4.21* [b]	12.57 ± 31.58* [b]	39.00 ± 4.33* [b]

Values are expressed as Mean ± SEM
***P < 0.05:**P < 0.01) compared to group b**
† p value < 0.05(significant), †† p value < 0.01 (highly significant)

DISCUSSION

CVD (Cardiovascular Disease) is the primary cause of death among people with Diabetes mellitus (1,2) as insulin dependent and non-insulin dependent Diabetes both act as strong and independent risk factors for CVD. One of the most important reactions in the arterial walls is the non-enzymatic reaction between glucose, proteins and lipoproteins which is called Maillard or browning reaction. Oxidative stress too has been involved in the pathogenesis of atherosclerosis (18).

Safflower has attracted significant attention due to its edibility and medicinal values. The petals of Safflower occupy a very significant place in the field of traditional medicine. Along with its use as a colorant, these petals are also helpful as remedies for many chronic illnesses such as rheumatism coronary heart diseases, hypertension as well as female and male fertility problems. Pleasantly flavored herbal tea is served from safflower blossoms in China (19),and has been a part of folklore medicine since ancient times in China, India, Iran and many parts of the world for its cardiovascular indications. The objective of the study was to observe the hypolipidemic effects of CT in alloxan induced diabetic rabbits. Ethanol extract used in present study not only reduced the total cholesterol, triglycerides, LDL-C but also increased HDL-C, significantly at both doses which makes CT extract promising for the patients with diabetes and dyslipidemias simultaneously. One of the possible mechanism for its improvement in lipid profile might be its hypoglycemic effect as it is a known fact that hyperglycemia promotes atherosclerosis. Safflower contains flavonoids, which decreases blood glucose levels thus rendering alleviation of atherosclerosis.

Querscetin component has also been studied in past and has been found to lower hyperglycemia as well as cholesterol and LDL-C in alloxan induced diabetic rats (20). It is reported that it inhibits glucose transporter GLUT-2 thus decreasing absorption of glucose from intestine (21).Chronic diseases like diabetes, rheumatoid arthritis and hypertension are leading causes of morbidity and mortality

throughout the world. Among the other complications oxidative stress plays a significant role in further worsening of these conditions if these are not reversed can result in loss of cell function and ultimately to apoptosis and necrosis (22).

Medicinal herbs are a source of natural antioxidants (23). Many traditional plants have been used for the treat many diseases throughout the world. It has been explored in detail in a systematic review to provide collective information of natural potential of drugs in future in every part of the world especially those areas who have a rich traditional history and has concluded that natural antioxidants should replace the synthetic ones as these products are rich in antioxidants (24).

Total antioxidant status (TAS) was chosen for estimation of improvement in oxidative stress produced by experimentally induced Type 2DM in rabbits and it was found that CT has highest potential to combat oxidative stress produced during such situations at both doses. When results were evaluated at the end of the study in diabetic rabbits treated with CT it was found that the mean TAS levels were within the established normal range of 1.30 - 1.77 mmol/l Plasma given by the Randox protocol. As CT possesses safflor yellow (20-30% w/w), neocarthamin, cathamidin, carthanin, lignans and polysaccharides. Brilliantly colored flower petals contain an orange red dye (carthamin) which is known for its poly unsaturated fatty acids (linolenic acid 78%) flowers are also rich in vitamin A, iron, phosphorus and calcium which contribute for its potential to be a potent antioxidant (25).

Carthamin has highest antioxidant potential due to its polyunsaturated fatty acid (linolenic acid) which helps in free radical scavenging activity and the flowers used in present study were orange in color (26,27).

Despite recent advances in pharmacotherapy in every field traditional remedies still work well when no other help is available. Herbal medicines often labeled as unscientific and anachronistic has proved to be equally effective in this pace of advanced technology. Results of present study are quite promising for the patients of type 2 DM associated with dyslipidemia and CT can prove to be helpful as adjunctive or sole agent for such patients since it contains flavonoids glycosides C-glucosylquinocalcone uniquely present in it (28).

CONCLUSION

It was concluded from study that total antioxidant effect of *Carthamus tinctorius* was found to be effective and TAS levels

were within the established normal range of 1.30 - 1.77 mmol/l Plasma given by the Randox protocol.

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