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ANTI-HYPERLIPIDEMIC EFFECT OF ACACIA HONEY (DESI KIKAR) IN CHOLESTEROL – DIET INDUCED HYPERLIPIDEMIA IN RATS

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ABSTRACT

Introduction: In this study, the hypolipaemic effect of acacia honey (desi kikar) on cholesterol diet induced hyperlipidaemia in rats has been observed.

Materials and Methods: The study was carried out on 60 male wistar rats weighing 200 – 250g. Rats were divided into five groups, each having 12 animals. Initially all four groups (B to E) were given cholesterol diet (2%) 2 gms cholesterol in 98 gms diet for 2 months to induce hyperlipidaemia except Group A, which served as (control). Group B received no therapy after establishing hyperlipidaemia. Groups C received 20 mg/kg of body weight acacia honey and group D received 10 mg/kg body weight of simvastatin orally for 8 weeks after establishing hyperlipidaemia, while group E received combination of 20 mg/kg and 10 mg/kg body weight of acacia honey and simvastatin respectively orally for 8 weeks. Rats having normal lipid profile were included in this study.

Results: Blood samples were taken on 60th day after giving antihyperlipidaemic therapy. Group B showed increase in serum LDL, TGs, cholesterol but decrease HDL levels. The level of these parameters decreased in group C which was given acacia honey reached near towards normal level. On the other hand with simvastatin, in group D, these levels reached near normal level. In group E given combination of acacia honey and simvastatin the levels reached towards normalcy. In conclusion, acacia honey has an antihyperlidaemic effect against cholesterol diet induced hyperlipidaemia in rats.

Key Words: Acacia honey (desi kikar), hyperlipidaemia, simvastatin, cholesterol – diet.

INTRODUCTION

Coronary heart disease (CHD) refers to the failure of coronary circulation to supply adequate circulation to cardiac muscle and surrounding tissue. It is already the most common form of disease affecting the heart and an important cause of premature death in Europe, the Baltic States, Russia, North and South America, Australia and New Zealand. It has been predicted that all regions of the world will be affected by 2020.[1] People of Indo – Asian origin have one of the highest susceptibilities to coronary artery disease (CAD) in the world,[2,3] and it is therefore not surprising that CAD is now the leading cause of death in the Indo – Pakistan subcontinent.[4]

Hyperlipidaemia is one of the important factors associated with atherosclerosis, others being hypertension, smoking, diabetes mellitus, and other factors. To retard or prevent the formation of atherosclerosis comes hyperlipidaemia on one of the present therapeutic challenges.[5] Elevated plasma cholesterol levels have long been established as risk factors for CHD, and lowering cholesterol levels, particularly low – density lipoprotein cholesterol (LDL – C), has been the focus of the prevention of CHD and its sequelae for almost 25 years. However, the complex mechanisms by which these molecules act are only beginning to be appreciated. Evidences suggest that lipid – lowering modes of therapy also reduce inflammation, which may reduce the risk of cardiovascular events, even for individuals with LDL – C levels in the normal range (< 130 mg/dL) based on the National Cholesterol Education Program (NCEP) Adult Treatment Panel III (ATP III) guidelines.[6]

Several drugs are used to decrease LDL cholesterol such as "-statins" (HMG – CoA reductase inhibitor), bile acid sequestrants, nicotinic acid and gemfibrozil.[7] The most effective and widely used drugs for the treatment of Hyperlipidaemia are the "-statins". Their primary site of action is in the liver where they inhibit HMG – CoA reductase; the metabolic pathway that produces cholesterol and isoprenoids.[8]

Honey has been used since long both in medical and domestic needs, but only recently as an antioxidant. The fact that antioxidants have several preventive effects against different diseases, such as cancer, coronary diseases, inflammatory disorders, neurological degeneration, and aging, led to search for food rich in antioxidants. In addition, the Prophet (PBUH) said: 'Honey is a remedy for every illness of the body and the Qur'an is a remedy for all illness of the mind, therefore I recommend to you both remedies, the Qur'an and honey.[9]

The purpose of the present study was to see the effect of *acacia honey* (desi kikar) on cholesterol diet – induced hyperlipidaemia when given together to wistar rats.

MATERIALS AND METHODS

Animals

The present study was carried out on 60 male wistar rats, each weighing 200 – 250 gms and were divided into 5 groups (12 each). Each group was kept in a separate cage, in the same room and under similar physiological conditions in animal house of National Institute of Health (NIH) Islamabad. Initially all groups were fed on normal rat chow (consisting of wed, starch, choline, methionine, vitamin, mineral mixture and fat) and water for a period of one week for acclimatisation before starting the experiment.

Inclusion Criteria

Adult rats having normal lipid profile were included in this study.

After this period of one week acclimatisation, cholesterol – diet was started by adding 2% cholesterol (2 gms cholesterol in 98gms of diet) in the diet of 4 out of 5 groups of rats (normal controls not included).[10] Each group consisting of 12 male wistar rats, kept in separate cages in the same room.

Plant Materials

Acacia honey (desi kikar) was purchased from local market famous honey dealer.

Experimental Procedure

After acclimatisation all the animals were divided randomly into five groups labelled as A, B, C, D, and E. The rats of group A were fed on normal diet for eight weeks and recieved no drug. Group B received cholesterol – diet orally daily for eight weeks and were fed on standard diet. Group C (experimental group 1) received acacia honey 20 mg/kg once a day using feeding syringe for eight weeks.

Group D (experimental group 2) received simvastatin 10 mg/kg once daily orally for eight weeks. Group E (experimental group 3) received combination of acacia honey 20 mg/kg/day and simvaststin 10 mg/kg/day using feeding syringe for eight weeks.

Blood Sample Collection

Timings: (day 0, day 60th, day 120$^{th)}$. Blood samples were taken at day 0 to establish that all rats had normal lipid profile. The animals were anaesthetised under ether vapours and their blood samples were collected by performing cardiac puncture in sterile vacuotainer with gel. Serum samples were separated from the clot after centrifugation at 3000 rev / min for 5 min, using bench top centrifuge. Serum samples were separated into sterile eppendorf tubes and stored at –20°C until used for biochemical estimation. All analyses were completed within 24 hours of sample collection.[11]

Biochemical Analysis

Serum cholesterol, triglyceride, HDL, LDL were estimated by commercially available kit (Randox).[12]

Statistical Analysis

The data was entered and analysed using SPSS 17.0 (Statistical Package for Social Sciences). All data are shown as mean ± S.E.M. One way ANOVA was applied to observe group mean differences. Post Hoc Tukey test was applied to observe mean differences among the groups. A p-value of <0.05 was considered as statistically significant.

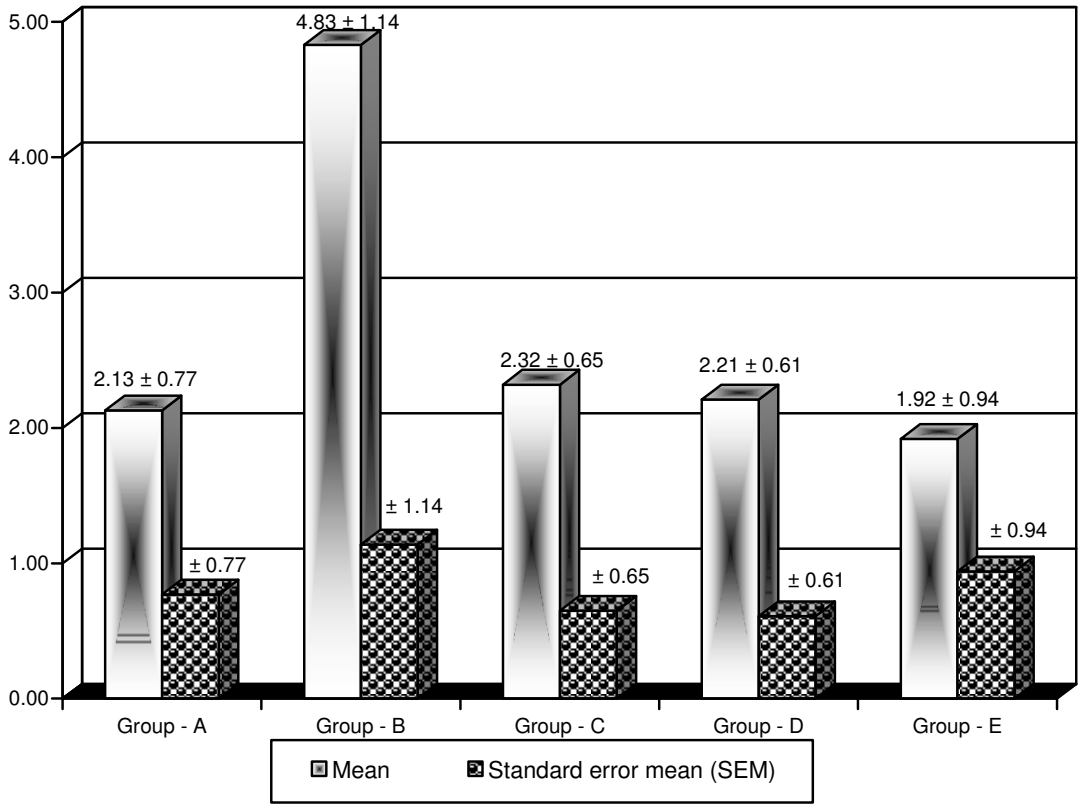
RESULTS

There was a significant increase in the serum TGs levels of group B (cholesterol) rats as compared to group A (control), on the other hand treated group C (acacia honey) group D (simvastatin) and group E (acacia honey plus simvastatin) significantly reduced the levels of TGs as compared elevated levels in group B (cholesterol diet induced). There was a significant increase in the serum LDL levels of group B (cholesterol) rats as compared to group A (control), on the other hand treated group C (acacia honey) and group D (simvastatin) and group E (acacia honey plus simvastatin) significantly reduced levels of LDL as compared to elevated levels in group B (cholesterol diet induced). There was a significant

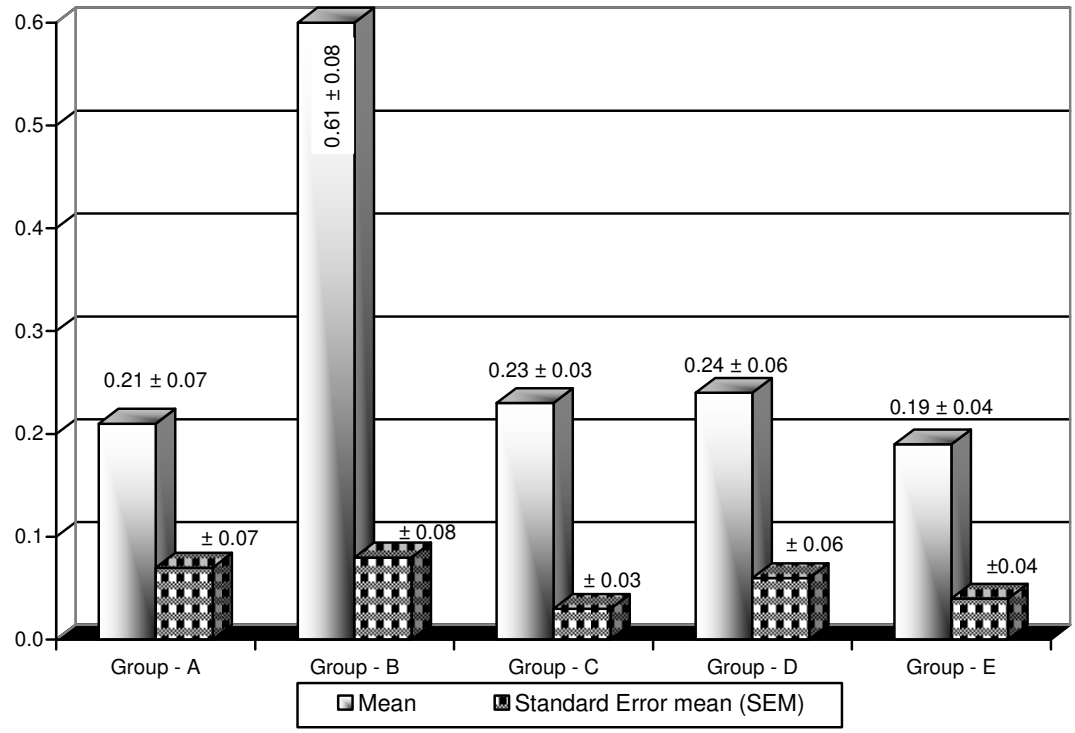
Table 1: *Mean ± SEM values of different parameters in all groups.*

	Group A	*Group B*	*Group C*	*Group D*	*Group E*
Serum TGs (m.mol/L)	2.13 ± 0.77	4.83 ± 1.14**	2.32 ± 0.65*	2.21 ± 0.61*	1.92 ± 0.94*
Serum LDL (m.mol/L)	0.21 ± 0.07	0.61 ± 0.08**	0.23 ± 0.03*	0.24 ± 0.06*	0.19 ± 0.04*
Serum cholesterol (m.mol/L)	1.78 ± 0.27	3.38 ± 0.67**	1.89 ± 0.43*	1.91 ± 0.76*	1.74 ± 0.34*
Serum HDL (m.mol/L)	1.28 ± 0.11	0.67 ± 0.21**	1.09 ± 0.23*	1.06 ± 0.16*	1.32 ± 0.34*

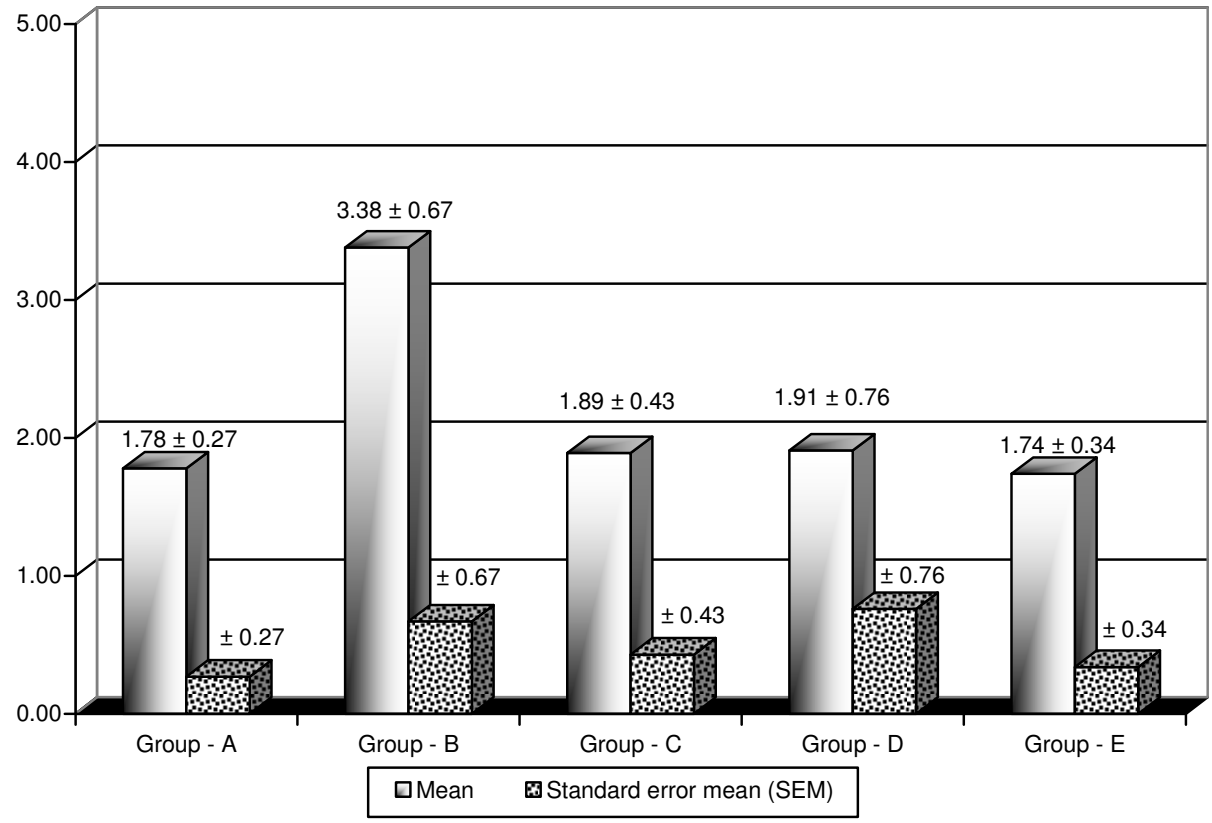
*$p < 0.05$ **$p < 0.01$



Appendix I: *Comparision of Serum TGs levels among five groups (Mean ±S.E.M)*



Appendix II: *Comparision of Serum LDL levels among five groups (Mean ± S.E.M).*



Appendix III: *Comparision of Serm Cholesterol levels among five groups (Mean ± S.E.M).*

increase in the serum cholesterol levels of group B (cholesterol) rats as compared to group A (control), on the other hand treated group C (acacia honey) and group D (simvastatin) and group E (acacia honey plus simvastatin) (significantly reduced the levels of cholesterol as compared elevated levels in group B). There was a significant decrease in the serum HDL levels of group B (cholesterol) rats as compared to group A (control), on the other hand treated group C (acacia honey) and group D (simvastatin) and group E (acacia honey plus simvastatin) significantly increased the levels of serum HDL as compared elevated levels in group B.

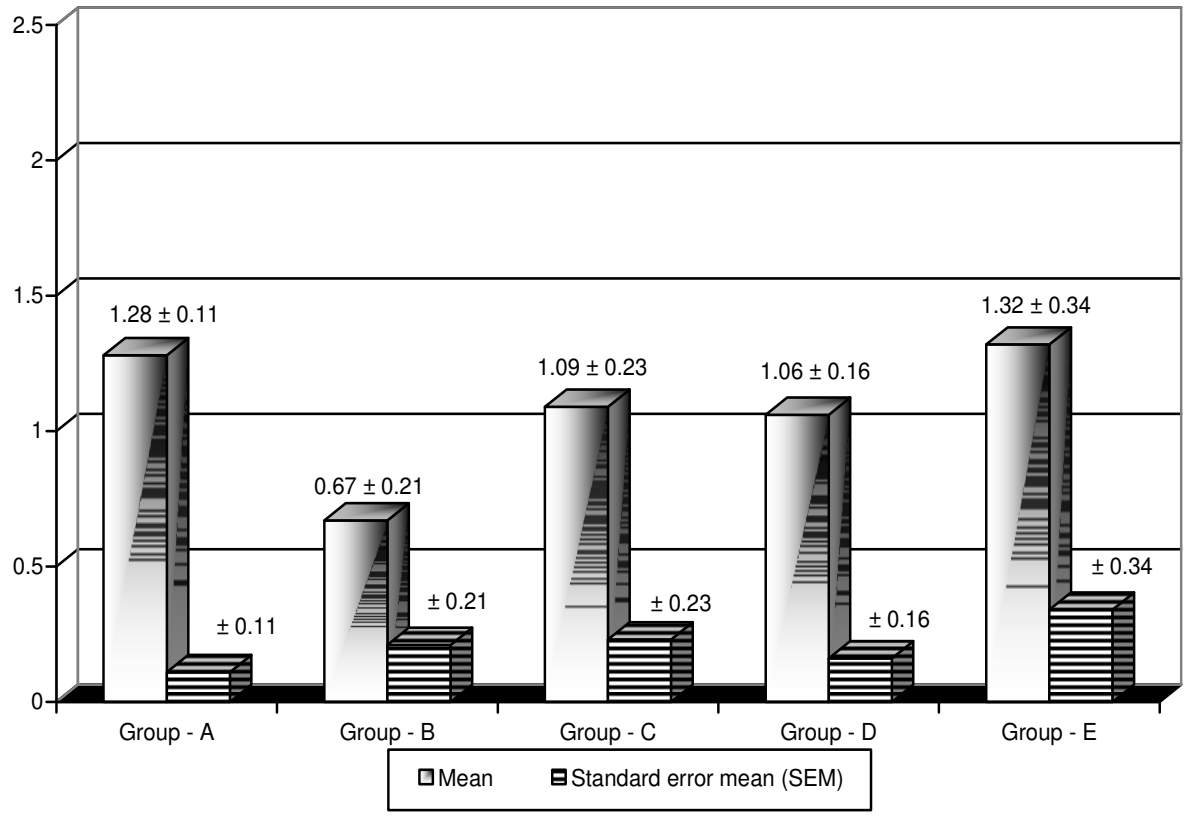
DISCUSSION

In this study, we observed the antihyperlipidaemic effect of acacia in honey in cholesterol diet induced hyperlipidaemia in rats. It was observed that there was a significant elevation in the levels of serum LDL, TGs, total cholesterol. Decreased HDL in group B which received 2% cholesterol diet each respectively as compared to group A rats who received no medicines. Elevated levels of these parameters in serum are presumptive markers of hyperlipidaemia in serum.

Co-administration of acacia honey orally with cholesterol diet in group C, who received 20 mg/kg body weight of acacia honey along with 2% cholesterol diet, oral dose each respectively, maintained the levels of these diagnostic parameters near towards normalcy as compared to group B rats. This was most likely due to the antioxidant effect of acacia honey constituents.

Group D rats, on the other hand received 10 mg/kg of body weight of simvastatin along with the above mentioned dose of cholesterol diet, biochemical levels decreased and reached near to normal. Group E rats who received a combination of acacia honey and simvastatin decreased the biochemical levels towards normal.

There was a significant increase in the serum TGs levels of group B (cholesterol) rats as compared to group A (control). On the other hand treated group C (acacia honey) group D (simvastatin) and group E (acacia honey plus simvastatin) (significantly reduced the levels of TGs as compared elevated levels in group B (cholesterol diet induced) (Table 1). Our results were supported by those of previous studies.[13-15] There was a significant increase in the serum LDL levels of group B (cholesterol) rats as



Appendix. IV: *Comparision of Serm HDL levels among five groups (Mean ± S.E.M).*

compared to group A (control). On the other hand treated group C (acacia honey), group D (simvastatin) and group E significantly reduced the levels of LDL as compared to the elevated levels in group B. These results were similar to the previous studies.[13-15]

There was a significant increase in the serum cholesterol levels of group B (cholesterol) rats as compared to group A (control). On the other hand treated group C (acacia honey) and group D (simvastatin), group E significantly reduced the levels of cholesterol as compared elevated levels in group B. These results were similar to the previous studies.[13-15]

There was a significant decrease in the serum HDL levels of group B (cholesterol) rats as compared to group A (control). On the other hand treated group C (acacia honey), group D (simvastatin) and group E significantly increased the levels of serum HDL as compared elevated levels in group B. Our results were in accordance with the previous studies.[13-15]

Statins exhibit actions beyond lipid – lowering activity that may be helpful for the prevention and treatment of CAD. These actions of statins are sometimes defined as pleiotropic effects. Statins act on various stages of atherogenesis to stabilise the atherosclerotic plaque; these include inhibiting remodeling of the arterial wall and improving endothelial function. Statins also decrease the serum concentration of C-reactive protein (CRP) and regulate the secretion of pro-inflammatorycytokines, such as tumour necrosis factor - (TNF), interleukin-1 (IL-1) and interleukin-6 (IL-6).[16]

The fact that antioxidants have several preventative effects on different diseases, such as cancer, coronary diseases, inflammatory disorders, neurological degeneration, and aging, led to search for food rich in antioxidants. Chemoprevention uses various dietary agents rich in phytochemicals which serve as antioxidants. With increasing demand for antioxidant supply in the food, honey had gained vitality since it is rich in phenolic compounds and other antioxidants like ascorbic acid, amino acids and proteins. Some simple and polyphenols found in honey, namely, caffeic acid (CA), caffeic acid phenyl esters (CAPE), Chrysin (CR), Galangin (GA), Quercetin (QU), Kaempferol (KP), Acacetin (AC), Pinocembrin (PC), Pinobanksin (PB), and Apigenin (AP), have evolved as promising pharmacological agents.[17]

Pakistani acacia honey (desi kikar) was prefer-

red because it is cheaper and most important of all its antihyperlipidaemic effect has not been studied earlier. Therefore, in our study flavonoids in acacia honey might have a role in the recovery in cholesterol – diet induced hyperlipidaemia in rats.

In conclusion the combination of statins plus acacia honey, showed better results than when given separately, which was evident from biochemical analysis. The overall antihyperlipidaemic effect of *acacia honey* is probably due to a counteraction of free radicals by its antioxidant flavonoids.

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