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Preventive role of propolis against hyperglycemia and hyperlipidemia in Sprague dawley rats (*Rattus norvegicus*) animal modelling system

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Abstract: Human diets with functional ingredients showed promising role in management of diseases of modern age like hyperglycemia and hyperlipidemia and even cancer. The study designed to elucidate role of honeybee propolis for management of hyperglycemia and hyperlipidemia states through animal modeling system. Hydroalcoholic extract of propolis was used for development of functional drink with standard recipe and addition of specified dose of extracts (400mg/500mL). Animals were grouped into three studies including study-I fed on regular diet, study-II fed on sucrose enrich diet and study-III fed on diet enriched with cholesterol and monitored to evaluate the results. Various parameters like feed consumption, liquid intake of animals measured regularly whereas body weight recorded at the end of each week of study. At the end of the study animals were analyzed for different blood indicators like blood lipid indices (cholesterol, LDL, HDL concentration and triglyceride contents), glucose concentration and insulin contents as well. The maximum feed and drink intake were examined in animals, fed with control diet whereas a non substantial mode of intake was recorded in rest of two groups of animals. The consumption of honeybee propolis based drink reduced cholesterol (6.63% to 10.25%) and LDL (9.96% to 11.23%), whilst a sharp increase in HDL level was ranged as 4.12 to 4.49% among animal groups fed with high cholesterol and high sucrose diet. Blood glucose level was decreased by 10.25% and 6.98% however 6.99% and 4.51% increase were observed in plasma insulin level in both studies, study-II and study-III correspondingly. The overall findings of the study showed that drinks prepared using propolis of propolis found effective for management of hyperglycemia and hypercholesterolemia in present animal modelling system.

Key words: Propolis extract; Low density lipoprotein; Drinks prepared; Hyperglycemia; High density lipoprotein.

Introduction

The sedentary lifestyle and inadequate supply of nutrients are leading factors to promote atherogenic disorders as diabetes mellitus, atherosclerosis, cardiovascular ailments, obesity and even cancer (1). Due to excessive intake of carbohydrate rich diet with significant amount of lipids responsible for development of

degenerative problems like atheroma formation which result in the progression of numerous health issues including hyperglycemia and hyperlipidemia (2). Diabetes mellitus is condition that develops due to abnormal glucose metabolism and ranked as one of the major fatal disease in the world with heavy mortality rate after cancer and heart problems (3). Pakistan falls in categories of some major affected countries as far as diabetes

and other atherogenic diseases are concerned. Recently, our country stands on top sixth position in diabetes with millions of patients and it is being expected that this number of patients will be doubled in coming years (4).

Dietary management of diabetes mellitus and abnormal lipid metabolism is becoming a therapy and has gained a great concern of researchers and nutritionists to fight this menace through modulation of dietary habits (5). The use of bioactive compounds of plants and animals as food ingredients used to strengthen the human body to cope various age-related diseases hence considered as antiaging moieties (6). Nowadays, this dietary approach for prevention of diet related disorder has become popular across the globe to combat life threatening risks such high blood pressure, abnormal cholesterol and glucose concentration in human body (7). Numerous studies showed the propolis possessed numerous bioactive compounds which showed a great role in management of blood glucose and lipid profile in animal modelling systems. It was proved that propolis and its constituents maintains the structural integrity and functionality of pancreatic cells for proper production and utilization of insulin to modulate the hyperglycemia (8).

Bee propolis, a resinous and adhesive produce of honeybees (*Apis mellifera* L.) which is produced from leaves extracts, buds seep and plant secretions. Honeybees gathers propolis from plants and mixed their innate enzyme, pollens, metabolites and waxes to make this valuable product for end usage. The word propolis comes from Greek origins as “pro” and “polis” where Pro stands for “in front” and polis “city”, which showed that propolis is a substance that is used for the protection and defense of beehives (9).

Propolis is a natural moiety having dual nature with characteristics of plant and animal origin as honeybees found secretions of plants as raw material, by mixing with their animal wax and enzymes convert plants exudates into finished propolis through their biological system. Bees use this produce to fill small holes in their hives, moisten the inner structure of honeycomb, to inhibit entry of intruders and protection of hives from diseases (10).

Propolis has been used in natural medicine for treatment of various diseases across the world due to its rich chemistry and potential biological properties. Many of the western civilizations, use propolis in various diseases due to its therapeutic usage with strong remedial properties. It has gained popularity as source of alternative medicine in the world and has average production of 700–800 tons per year for human consumption (11). In recent studies, it has been investigated that intake of propolis and its bioactive ingredients as dietary ingredients to cater different physiological ailments including hyperlipidemia, hyperglycemia and other health problems. Hence propolis and its constituent could be a way forward for management human health and disease prevention (12). Chemically bee propolis consist on 180 or more chemicals of different origin that vary from region to region, season to season hence more that 300 compounds recognized as chemical substance of propolis (13). Functional foods containing potential bioactive compounds and nutraceuticals has potential to cope various problems and could be used as conventional foods system (14). Honeybee propolis found suitable

to promote general health hence can be used in different aspect. Recently, numerous innovations showed that it has biological properties including antimicrobial, anti-inflammatory, antioxidant, antihypertensive, antihyperglycemic and anti-hepatotoxic activities (15). The biological properties of propolis is mainly attributed due to its phenolic components which transfer hydrogen ions to free radicals for protection of cells from oxidative stress. Propolis has the capacity to scavenge free radicals those are responsible for oxidative degeneration of biomolecules including nucleic acid, proteins and lipids (16). By exploring the nature of propolis and its ingredients, the current study designed to elucidate its preventive role by developing functional drink with potential ingredients of propolis against hyperglycemia and hyperlipidemia using rodent modelling system.

Materials and Methods

Laboratory animals

The infectious free animals were breed and made available at National institute of Food Science and Technology and were divided into three groups on the basis of study trials. The research animals were managed as per standards of Animal Institute of Nutrition (AIN) America (17).

Raw material collection and hydroalcoholic extract preparation

Honeybee propolis was obtained from local apiaries from surroundings of Faisalabad, Pakistan. The weighed sample was mixed 65% of ethanol and methanol for preparation of hydroalcoholic extract for further use in preparation of functional drink. For preparation of propolis extracts, weighed amount agitated with alcohol and water under dark conditions for 24 hours adjusting at constant room temperature. The extract filtered through Whatman paper # 2, the resultant material centrifuged at 3000g for 20 mints and supernatant containing bioactive moieties was collected and concentrate by through vacuum distillation in rotary evaporator under specific conditions of reduced pressure at 40°C temperature and stored in dark conditions at 4°C for future use (18, 19).

Development of functional drink

Functional drinks with propolis extracts (T₂ and T₃) developed following standard recipe with addition of hydroalcoholic extracts of propolis and T₁ prepared as control without addition of propolis extract. The addition of propolis extract was made by dose of 400 mg/500 mL in individual drinks for efficacy study (20).

Efficacy study trials and animal housing

Ninety disease free rats kept in designated facility at parent institute and designed three studies varying in diet type including normal diet, sucrose enriched and cholesterol enriched diet. The animals grouped into three groups having thirty animals in each group, in each study, animals divided in three subgroups having ten animals in standard polypropylene cages with stainless steel grills. The environmental conditions were managed at 23 ± 2°C, 55 ± 5% humidity with proper 12 hrs light and dark period. Initially animals were

Table 1. Experiment plan for Diet and functional drink intake.

Groups	Normal			Sucrose rich diet			Cholesterol rich diet		
	1	2	3	1	2	3	1	2	3
Drinks	T ₁	T ₂	T ₃	T ₁	T ₂	T ₃	T ₁	T ₂	T ₃

T₁: Control without propolis extract, T₂: Propolis drink developed by ethanol extract, T₃: Propolis drink developed by methanol extract

fed on basal diet (AIN-76A) to acclimatize according to environment and some of them were sacrificed for initial values as baseline (17). The daily intake of feed and drink measured on regular basis whereas their weight gain monitored weekly and at the end of the study, all the animals were sacrificed, blood sample was used for hematology analysis (Table.1).

Normal diet group (Study-I)

For study-I, animals were supplied with normal diet which composed on 66% starch, 10% protein, 10% cellulose, 10% oil, 3% mineral elements and 1% vitamins along with prepared drinks (T₁, T₂, T₃). By following same protocols other studies also carried out to monitor functional drinks and its effects in respective diets (Table.1).

High sucrose diet group (Study-II)

In study-II, sucrose enriched diet having 40% sucrose mixed with 10% oil, 26% starch, 10% protein, 10% cellulose source with mineral salts and mixture of vitamins in 3 and 1% on weight basis. The supply of functional drinks made available to animal groups simultaneously to observe the effects of propolis bioactive compounds.

High cholesterol group (Study-III)

During study-III, diet with added 1% cholesterol supplied to animal groups with functional drinks to elucidate the effect on subject under studied.

Daily feed and liquid intake

During the study period, feed and drink intake of all groups monitored regularly and functional drink intake in all groups examined through use of standardized bottles. However, weight of animals recorded weekly during study period to observe the effect of drinks on various physical attributes of animals (21).

Blood glucose level and insulin level estimation

At the end of the study, blood sample was collected and evaluated blood glucose level using standard lab protocols according to GOD-PAP procedure as mentioned by Thomas and Labor 1998 (22), whilst the insulin concentration recorded according to guidelines as adopted by Besch, 1987 (23).

Lipid profiling of experimental organisms

The estimation of total cholesterol was done by using the guidelines of CHOD-PAP method, whereas HDL concentration was estimated through HDL Cholesterol Precipitant procedure and LDL level was measured using the protocol as explained by McNamara *et al.*, 1990 (24). Similarly, the triglyceride contents recorded through GPO-PAP, liquid triglycerides protocols explained by Sultan *et al.*, 2014 (25).

Statistical analysis of results

The results of parameters studied obtained in triplicates and imperiled for statistical estimation by using Cohort version 6.1. under technique of completely randomized Design (CRD). Analysis of variance technique applied for estimation of level of significance, means of results compared by using Duncan Multiple Range (25).

Results and Discussion

Net feed and drink intake of animals

The results of means for feed intake in different study groups showed a gradual increase in intake with time (Figure 1) whereas highest values of feed intake recorded in control group. However, a sharp decrease noticed in study groups those supplied with functional drinks (T₂ & T₃). Likewise, mean values regarding daily drink intake exhibited gradual increase with non-substantial variations which showed positive effect for suitability of prepared drinks. The findings of net feed intake in present study supported through earlier experimentation of Kwon *et al.* 2001 (27) they determined the relationship of propolis consumption and feed intake of animals and found that a sharp increase in feed intake by the laboratory animal with passage of time (28). Previously, numerous studies provoked an increase response in feed intake during study period by experimental organisms due to propolis in comparison to control

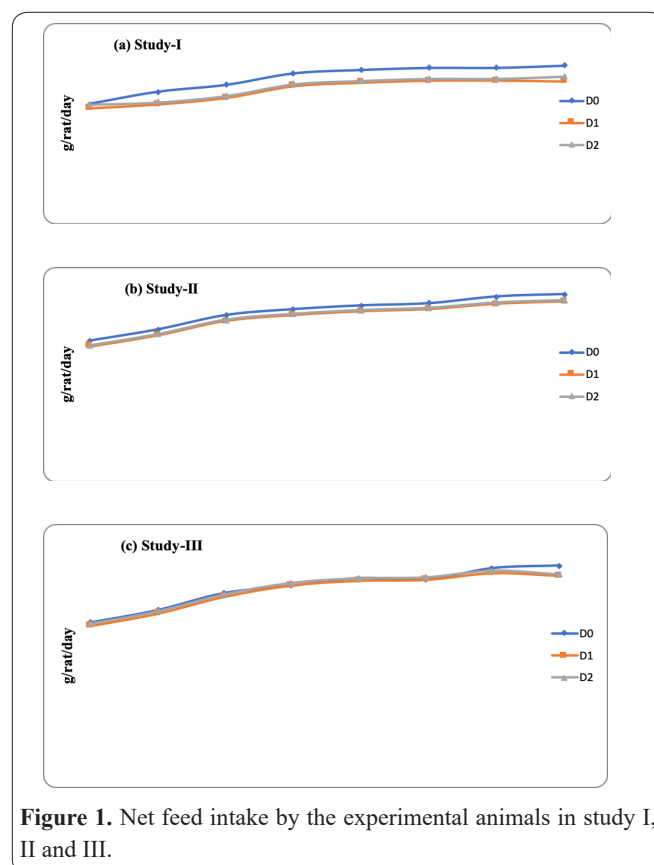


Figure 1. Net feed intake by the experimental animals in study I, II and III.

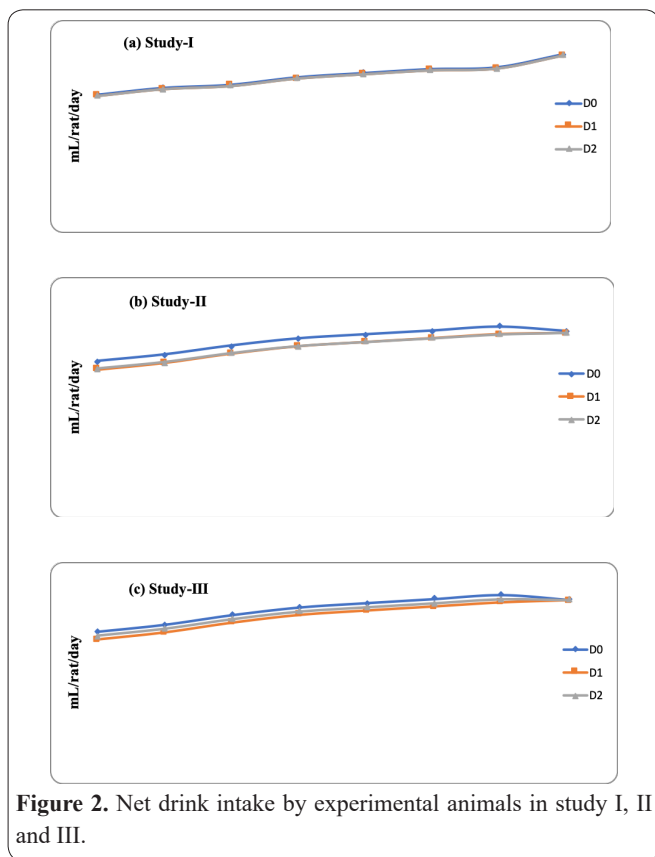


Figure 2. Net drink intake by experimental animals in study I, II and III.

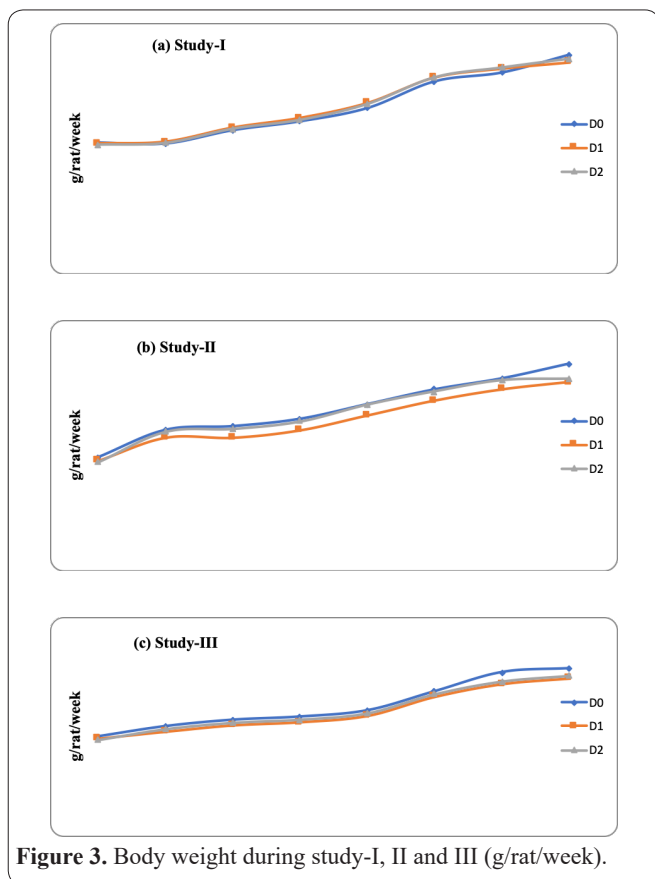


Figure 3. Body weight during study-I, II and III (g/rat/week).

which was not provided by propolis (29). The outcomes regarding drink intake by the animals harmonized with results of researchers working on estimation of feed efficiency ratio and consumption of functional foods for studying their effect on different blood indicators as well (30, 31, 32). Our findings for drink intake showed a non substantial change during study period and in line with investigations of Kim *et al.*, 2012, who determined

a non-momentous change in consumption of drink enriched with polyphenol during animal modelling system (33). In a study conducted by Edward *et al.*, 2008, found a relationship between drinking behavior of animals with fluid homeostatic concentration that promote drink intake as a part of time and phytochemicals intake (34). Similarly, earlier study designed by Jian *et al.*, 2008 showed similar results as observed during present study found during biological study for said attribute (35).

Body weight gain of experimental organisms

The results regarding weight gain by animals showed gradual increase in body weight with time duration throughout the study which is represented in Figure. 2. The overall values depicted that animals those fed on standard diet and drink (T_1) gained more weight in comparison to groups of animals those provided by supply of T_2 and T_3 . It was observed that a substantial body weight gain and increase in values was observed during initial four weeks while clear difference in weight was recorded in last duration of study. The control of animals in this regard exhibited more weight gain in comparison to study groups these supplied with propolis base functional drink on regular basis. The findings of body weight gain during current study are in line and addressed by the earlier investigation of Haro *et al.*, 2000, they demonstrated that propolis with its functional ingredients and pollens consumption in animals with diet enhanced weight gain due to dietary modifications as described in present study as well (36). Similarly, numerous studies conducted by Denli *et al.*, 2005, in which they designed treatment plans for animals those supplied propolis with diet and noticed significant increase in weight of animals due to growth performance and feed consumption as explained in present investigation (37).

Evaluation of changes in lipid profile of animals

The intake of functional drink prepared with propolis extracts exhibited substantial decrease in cholesterol level among all groups of animals and all studies. It was noticed that T_2 (functional drink developed with addition of ethanol extract) imparted more reduction in comparison to T_3 (functional drink developed with addition of methanol extract) whereas their interaction and comparison showed non-significant behavior. Similarly, the results regarding HDL and LDL concentration showed a non-significant increase in HDL whereas substantial reduction in LDL values observed in all three groups which was marked due to functional drink intakes. The results regarding triglycerides concentration showed significant reduction in triglyceride values that noticed during study III, however, rest of groups of animals (study-I and study-II) exhibited non-substantial effect depicting no variation in triglycerides of animals (Table.2). The means concerning cholesterol level observed in ranges from 82.20 ± 5.57 to 83.61 ± 5.42 mg/dL among animals of study group having high cholesterol values due to high cholesterol diet found higher with respect to other studies. In a same manner, functional drink of ethanol extract of propolis showed more response towards cholesterol reduction in all study groups in comparison to other treatments and this effect shown more in animal

groups fed on diet containing cholesterol in excess. The means of LDL ranged as 28.16±2.14 to 29.33±2.32 mg/dL in group of animals those fed on control die whereas these values found higher in rest of study groups and ethanol extract of propolis decreased the values substantially as part of functional drink intake. Likewise, the mean values of HDL showed contrast nature in comparison to cholesterol whereas the trend for increase in HDL concentration found non-significant as a part of drink intake among all study groups.

As far as values of HDL concerns, recorded between 28.16±2.14 to 29.33±2.32 mg/dL among animals of control group and higher values were observed in other study groups during the study period. At the end of study, a pattern of decrease in LDL concentration was observed which was significantly reduced in groups due to drink intake as a function of ethanol extract base functional drink. The values for low density lipoproteins (LDL) ranged from 28.16±2.14 to 29.33±2.32mg/dL in animals having fed on regular diet, whereas higher in other study groups. The propolis based functional drink containing ethanol extract decreased the trait significantly. The decrease in cholesterol found high in animals with cholesterol enriched diet as 10.25% and 5.59% for D₁ and D₂ respectively. In comparison to drink prepared with methanol extract of propolis, drink prepared with ethanol extract of propolis reduced LDL more effectively. The mean values for triglyceride showed lowest values in group of animals supplied with control diet but highest values were noticed in group of animals fed on diet with high cholesterol followed by group of animals with high sucrose diet. The same trait was reduced by 8.96% and 3.50% correspondingly among animals of group fed on diet with added cholesterol and 6.32% and 3.21% reduction was noticed in other group with high sucrose in their diet due to consumption of functional drinks with propolis extracts.

Cholesterol is an important type of lipid associated with numerous cellular parts of animal tissues. The source of cholesterol in animal bodies is mainly from *de novo* synthesis in the liver cells as well as from the endocytic uptake of plasma low density lipoprotein (LDL) through receptor mediated process (38). Cardiovascu-

lar complications are the leading cause of mortality in the world due to poor eating habits and sedentary life pattern. In particular, high cholesterol level is the major factor in the progression of atherosclerosis and other cardiovascular problems (39).

The findings of decrease in cholesterol level were supported by previous studies in which orally supply of propolis extract significantly reduced cholesterol in rats (40). Propolis has bioactive compounds of varied nature those involved in regulation of blood lipid chemistry through altering the response of certain genes and physiological actions of certain enzymes (41). Propolis inhibit the onset of atherosclerosis through lipid profile interventions due to inhibition of pro-inflammatory cytokines, chemokines and altering the influence of mRNA expressing genes including MCP-1 (42). Propolis and its constituents when consumed with diet reduced oxidation of LDL and help to enhance the level of HDL concentration in blood of laboratory rats. This mechanistic approach may attribute towards management of lipid abnormalities and in harmonization to present study (43, 44). In diabetic induced rats, intake of propolis significantly increase HDL concentration and inhibit LDL concentration as a result of functions of bioactive moieties associated with propolis and similar effect was observed during present study. Propolis could be a source of alternative medicine for addressing dyslipidemia due to rich phytochemistry and spectra of ingredients. Regular intake improves hyperlipidemia due to antioxidant properties found in propolis ingredients through interfering metabolic pathways of lipid and inhibition of free radical oxidation process (45).

Effect of functional drink on blood glucose and insulin

During the present study consumption of propolis functional drink significantly lowers the blood glucose among the animals in which hyperglycemia induced through diet whereas in rest of groups nonsignificant reduction was monitored. The means for blood glucose level recorded in control group was ranged from 86.78±7.18 to 89.63±6.24mg/dL whereas in group of animals supplied diet with high sucrose contents values

Table 2. Propolis based drink and lipid profile of experimental animals.

Attributes	Studies	Propolis base functional drinks		
		T ₁	T ₂	T ₃
Total Cholesterol value (mg/dL)	Study I	83.61±5.42	81.11±6.14	82.20±5.57
	Study II	98.68±8.51 ^a	91.34±7.04 ^b	92.75±7.12 ^{ab}
	Study III	152.1±8.69 ^a	136.51±9.21 ^c	147.88±6.23 ^b
LDL concentration (mg/dL)	Study I	29.33±2.32	28.16±2.14	28.68±2.58
	Study II	45.96±3.58 ^a	41.38±3.43 ^c	43.67±3.21 ^b
	Study III	63.20±5.29 ^a	56.10 ±4.21 ^c	58.06±5.48 ^b
HDL concentration (mg/dL)	Study-I	33.63±2.34	34.31±2.54	34.27±2.81
	Study-II	41.89±3.63 ^b	43.62±3.41 ^a	42.66±4.24 ^{ab}
	Study-III	58.69±4.29 ^b	61.33 ±4.79 ^a	60.46±5.41 ^a
Triglycerides level (mg/dL)	Study-I	67.25±4.42	65.69±5.14	66.57±5.58
	Study II	75.96±5.41 ^a	71.76±6.43 ^b	73.52±6.21 ^{ab}
	Study III	99.36±7.69 ^a	90.46±8.21 ^c	95.88±7.48 ^b

Values represented by means ± SD. Values with varied lettering differed significantly (P < 0.05). Study I: Diet with standard composition, Study II: Diet with sucrose, Study III: Diet with cholesterol, T₁: Drink without propolis extract, T₂: Propolis drink developed by ethanol extract, T₃: Propolis drink developed by methanol extract

Table 3. Effect of functional drink intake on blood glucose and insulin in sprague dawely rats.

Parameters	Studies	Propolis base functional drinks		
		D1	D2	D3
Blood Glucose (mg/dL)	Study I	89.63±6.24	85.94±6.39	86.78±7.18
	Study II	140.25±12.19 ^a	125.87±17.58 ^c	130.26±18.14 ^b
	Study III	101.23±9.53 ^a	94.16 ±8.76 ^b	94.76±8.41 ^b
Plasma Insulin (µm/mL)	Study I	7.21±0.34	7.38±0.27	7.35±0.81
	Study II	9.12±0.24 ^b	11.80 ±0.73 ^a	11.03±1.13 ^a
	Study III	8.88±0.29 ^c	11.49±1.41 ^a	9.28±0.79 ^b

Values represented by means ± SD. Values with varied lettering differed significantly (P < 0.05).

were found as 130.26±18.14 to 140.25±12.19 mg/dL during the period of study. The provision of functional drink (T₁, T₂) marked minimum 7.12% and maximum 10.25% reduction in among animals provided with high sucrose diet. The results for insulin level was examined and ranged between minimum (7.21±0.34µm/mL) to maximum (9.12±0.24 µm/mL) among animals fed on normal and high sucrose diet correspondingly. It was noticed that insulin level increased by 6.99% and 4.21% in animals with induced hyperglycemia which clearly depict the role of propolis drink i.e T₁ and T₂, whereas, drink prepared ethanol extract showed more positive response than other treatments (Table 2).

In diabetes, metabolism of carbohydrates and lipids is improperly regulated due to abnormalities in insulin production and sensitivity which leads to increased fasting and postprandial blood glucose level and the condition over a long period of time caused hyperglycemia which ultimately is converted to diabetes mellitus (46). The intake of encapsulated propolis contents significantly lowers blood glucose concentration through modulating the insulin activity among animals those were induced hyperglycemia by streptozotocin injections (47). High glucose concentration managed through propolis consumption by different mechanism including more uptake by liver and tissue utilization which indicates insulin sensitivity increased by propolis ingredients and findings of present study showed similar with the study conducted by Al-Hariri *et al.*, 2011 (48). In another study, it was confirmed that pancreatic regeneration may promote production of insulin which ultimately lowers blood glucose. The folk medicines could be a source of adjuvant therapy for management of insulin and diabetic syndrome as well (49). It has been shown that caffeic acid phenyl amide showed hypoglycemic role in diabetic induced animals. This compound can be derived from propolis extract being major ingredient in propolis composition and similar result observed in present study (50). Propolis collected from Brazilian region in dose of 100-300mg/kg imparted synergistic role in lowering blood sugar as a function of improvement in insulin sensitivity and glycemic metabolic modification (51).

In an investigation by Li *et al.* (47) on male rats to observe role of encapsulated propolis on glycemic indices, lipid regulations and insulin sensitivity induced diabetic. They concluded that propolis encapsulated granules improve glycemic indices and promotes insulin activity through increase cellular-insulin interaction. Polyphenols found in propolis involved management of blood glucose through malondialdehyde inhibition and superoxide dismutase production as well. CAPE of pro-

polis, lowers oxidative damage induced by diabetes mellitus by lowering the production of ROS and protection of β-cells (52,53,54).

Bee Propolis is resinous product with plenty of bioactive compounds with potential biological activity. The extraction and composition of bioactive compounds depends on the seasonal variation and solvent concentration used for extraction. The ethanol extract showed spectra of bioactive compounds and found suitable to manage hyperlipidemia and blood glucose concentration. Among different treatments the functional drink prepared with ethanol extract showed good results in comparison to other treatments for uplifting plasma insulin and glucose management. Furthermore, new clinical trials should be planned to explain the metabolic role of propolis in disease management and toxic effect caused by propolis residues in the biological system.

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