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Propolis impact on Lipid Profile and Renal Function in diabetic rats

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Abstract

SummeryPropolis is a honeybee product with various biological properties involving modulating lipid profile with attenuate renal lesion by its antioxidant effect. The present study was designed to evaluate hypolipidemic, renoprotective and weight improvement effects of propolisas an add-on therapy to metformin and sitagliptinin alloxan-induced diabetic rats. Single intrapertonial dose of alloxan (150mg/kg) induced diabetes. The animals was divided into seven groups (6 rats per each); group 1 (non-diabetic rats) and group 2 (diabetic rats) both received distilled water. Groups 3, 4, 5, 6 and 7 were diabetic receiving 100mg/kg metformin, 100mg/kg propolis, 50 mg/kg sitagliptin, 100mg/kg metformin + 50mg/kg sitagliptin, and 100mg/kg propolis +100mg/kg metformin + 50mg/kg sitagliptin respectively. After 21 days of alloxan injection, there were significant elevation in the levels of Total cholesterol, Triglyceride, LDL-cholesterol, Cardiac-risk ratio, blood Urea nitrogen and Creatinine, with significant reduction in HDL-cholesterol, Cardioprotective index and animals body weights. On other hand, oral daily supplementation with propolis to diabetic rats improved lipid profile parameters, renal function and body weight.The findings of this study were significant (P-value<0.05). In conclusion, propolis possesses hypolipidemic and renal protective activitieswith good protection effect of body weight, so it was delay the occurrence of diabetic complication.

Key words: propolis, add-on therapy, hypolipidemic, renoprotecive effect.

Introduction

Diabetes is a chronic metabolic syndrome characterized by hyperglycaemia that presents a major around the world wellbeing issue (El-Awady et al., 2014).Hyperglycemia impedes antioxidant defense system and metabolism of carbohydrate, lipid, and protein. Many disorders arerelated to diabetes mellitus, that include cardiovascular disorders, renal impairment and dyslipidemia. Elevatation ofblood glucose levellead to overproduction of ROS and to diabetic complications, in specific, impaired renal function and lipid metabolism ,which can be anticipated with the utilize of natural antioxidants (Oladayo, 2016; Al-Waili et al., 2017).

Analysts are presently inquisitive about the wellbeing benefits of elective therapeutic nourishments with normal antioxidant bioactive components as modern adjunctive pharmaceuticals to diminish occurance of diabetes complications(Sforcin et al., 2011). Natural bioactive items may behave ability to manage glycemic parameters and diminish complicationshazard (Davi et al., 2010).

Propolisis a gum as substance gathered via bees from different plants. It is differes in colour from light yellow to dark brown (Aldahmash et al., 2016).It has different biological and pharmacological effects, involving antioxidant, antiinflammatory, hepatorenal protective and

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antimicrobial activities, with wound healing properties (Teles et al., 2015; El Menyiy et al., 2019).These activities may be associated with propoliscomponents gotten from plants, especially flavonoids. Flavonoids are phenolic compound with potent antioxidant ability asfree radical scavenger and chelating of metal activities (Perron and Brumaghim, 2009). At slightest 38 diverse flavonoids have been detailed in propolis(El-Kott and Owayss, 2008). Some of the propolisconstituents are retained then circulate within the blood and carry on as a hydrophilic free radical scavenger and spare vitamin C (Sun et al., 2000).

Therefore, present study designed to evaluates the impact of propolisorally administered against hyperlipidemia, renal dysfunction and changing in body weight when administered as an add-on therapy to metformin and sitagliptin.

Materials and methods

Chemicals

Chemicals were purchased from different companies. Propolis from Now food chemical company/ USA.Alloxan from sigma chemical company (St. Louis, MO, USA).Metformin was purchased from Merck/France and sitagliptin from pioneer/Iraq.

Animals

In this study,42 adult male albino rats weighing (180-250 g) were used. They gotten from the animal house of the Veterinry College, University of Mosul, Iraq. Rats were housed in rodent plastic cages (6/cage) bedded with wood shavings and kept beneath standard research facility conditions of air circulation and room temperature at around (22 ±2)°C and adapted for one week recently being tested.They were permitted to free get to standard diet and water advertisement libitum.

Induction of diabetes

The animal was fasted overnight with free access drinking water. Diabetes was induced through single intraperitonealalloxan injection that freshly prepared (150 mg/kg) dissolved in0.9% sterile normal saline. To avoid hypoglycemia and death through hypoglycemic phase, oral solution of (5%) glucose in tap water givenimmediately after injection to rats by water bottle for next 24 hours. A pinch of blood collected from the tail was analyzed for glucose level with the aid of a portable glucometer (Joycoo. Hamburg, Germany) at 48 and 72 hours after injection and those with blood glucose levels above 10 mmol/L were considered diabetic.

Experimental protocol

The animals were used in this experiment classified into seven groups (6 rats/ group) and treated for 21 days as follow:

G 1: control (non-diabetic); received DW.
G 2: control (untreated diabetic experimental);received DW.
G 3: diabetic experimental; received 100 mg/kg metformin.
G 4: diabetic experimental; received 100 mg/kg propolis.
G 5:diabetic experimental; received 50 mg/kgsitagliptin.
G 6: diabetic experimental; received (100 mg/kgmetformin + 50mg/kg sitagliptin).
G 7: diabetic experimental; received (100 mg/kgmetformin + 50mg/kg sitagliptin + 100mg/kg propolis).

Blood Sampling and Biochemical Analysis

For serum preparation, blood samples (in plain centrifuge bottles) were permitted to stand for 30 minutes and then centrifuged at 3000 round/minute for 15minute at 19°C to obtain the serum. Obtained serum was analyzed for TC measured by enzymatic colorimetric method using

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Cholesterol CHOD-PAP kit,TG measured by enzymatic colorimetric method using TRIGLYCERIDES GOP kit, HDL-c measured by the precipitation method using the HDL-cholesterol (PTA) and cholesterol CHOD-PAP kits. All kits wasmanufactured by BIOLABO (France).Also, obtained serums were utilized for the estimation serum level of blood urea measured by enzymatic colorimetric method using Urea-kit S supplied by BioMerieux (France) and creatinine measured by Jaffe reaction method using a kit supplied by SYRBIO laboratories under license of EUROBIO laboratories (France).

LDL-cholesterol level was calculated from the Friedewald equation concurring to the manufacturer's informational (Friedewald et al., 1972):

$$(LDL\text{-}c) = (TC) - (HDL\text{-}c) - (TG/5)$$

atherogenic indices:

Cardiac risk ratio {CRR} = (TC/HDL-c) (Oršoli´c et al., 2014a)

Cardio-protective index{CPI}= (HDL-c/LDL-c) (Oršoli´c et al., 2014b)

Statistical Analysis

One way ANOVA-test then Tukey(s) Pair wise test utilized to analyze the statistical differences between results of each group. The results were expressed as means ± SD and when $P<0.05$, the differences were statistically significant.

Results

Table 1and table 2were showed comparison of the post-trial values of lipid profile, renal function and body weight under study between all the groups. Propolis supplementation as monotherapy or as combined therapy was showed statistically significant improvement in lipid profile , BUN, Cr and rats' weight compared to diabetic non-treated grouop ($P<0.05$).

Lipid profile parameters	**Groups**							**P-value**
	G1 Mean ± Standard deviation	**G2 Mean ± Standard deviation**	**G3 Mean ± Standard deviation**	**G4 Mean ± Standard deviation**	**G5 Mean ± Standard deviation**	**G6 Mean ± Standard deviation**	**G7 Mean ±Standard deviation**	
Cholesterol (mg\dL)	108.50 ± 4.79 **B**	128.38 ± 5.70 **A**	101.87 ± 5.94 **B**	107.72 ± 7.16 **B**	109.33 ± 5.42 **B**	108.20 ± 8.84 **B**	107.63 ± 7.07 **B**	**0.001**
TG (mg\dL)	96.45 ± 11.50 **B**	121.97 ± 12.82 **A**	95.27 ± 5.54 **B**	99.80 ± 6.54 **B**	101.33 ± 7.50 **B**	94.47 ± 10.24 **B**	92.23 ± 11.29 **B**	**0.001**
HDL (mg\dL)	45.38 ± 4.04 **AB**	39.45 ± 1.50 **C**	42.66± 2.01 **BC**	48.10 ± 1.71 **A**	46.45 ± 1.56 **AB**	45.87 ± 1.31 **AB**	48.55 ± 1.28 **A**	**0.001**
LDL (mg\dL)	43.79 ± 5.94 **B**	59.35 ± 4.98 **A**	40.20 ± 5.87 **B**	39.62 ± 4.80 **B**	42.45 ± 4.54 **B**	43.44 ± 6.15 **B**	40.64 ± 4.03 **B**	**0.001**
HDL\LDL	1.06 ± 0.22	0.67 ± 0.04	1.08 ± 0.19	1.22 ± 0.14	1.10 ± 0.11	1.07 ± 0.15	1.12 ± 0.11 **A**	**0.001**

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	A	**B**	**A**	**A**	**A**	**A**		
Chol\HDL	2.395 ± 0.262 **B**	3.250 ± 0.241 **A**	2.380 ± 0.180 **B**	2.233 ± 0.107 **B**	2.350 ± 0.115 **B**	2.350 ± 0.138 **B**	2.211 ± 0.111 **B**	**0.001**

Table (1): Comparison in lipid profile among the study sampled groups after 3 wks of managment.

Variables	**Groups**							**P-value**
	G1 Mean ± Standard deviation	**G2 Mean ± Standard deviation**	**G3 Mean ± Standard deviation**	**G4 Mean ± Standard deviation**	**G5 Mean ± Standard deviation**	**G6 Mean ± Standard deviation**	**G7 Mean ±Standard deviation**	
Bl. urea (mg/dl)	25.20 ± 2.72 **B**	60.03 ± 12.04 **A**	31.45 ± 3.17 **B**	27.93 ± 4.00 **B**	28.55 ± 2.90 **B**	30.37 ± 4.99 **B**	25.99 ± 4.41 **B**	**0.001**
S. creatinin (mg/dl)	0.66 ± 0.15 **B**	1.90 ± 0.20 **A**	0.77 ± 0.05 **B**	0.62 ± 0.16 **B**	0.72 ± 0.26 **B**	0.93 ± 0.41 **B**	0.64 ± 0.19 **B**	**0.001**
Rats' weight (gm)	251.2 ± 32.3 **A**	185.3 ± 11.7 **B**	207.3 ± 39.4 **AB**	225.3 ± 48.5 **AB**	180.3 ± 10.3 **B**	196.8 ± 19.2 **B**	205.0 ± 16.5 **AB**	**0.002**

Table (2): Comparison in RFT and rats' weight among the study sampled groups after 3 wks of managment.

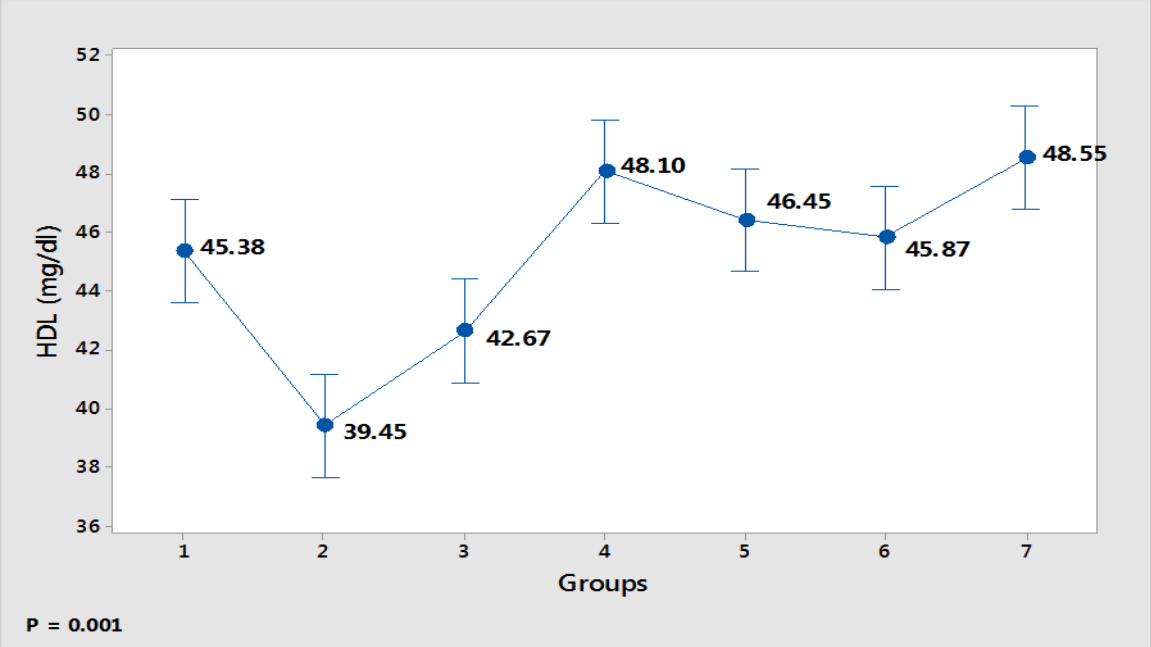

Figure (1): The differences of mean HDL among the study sampled groups after 3 wks of managment.

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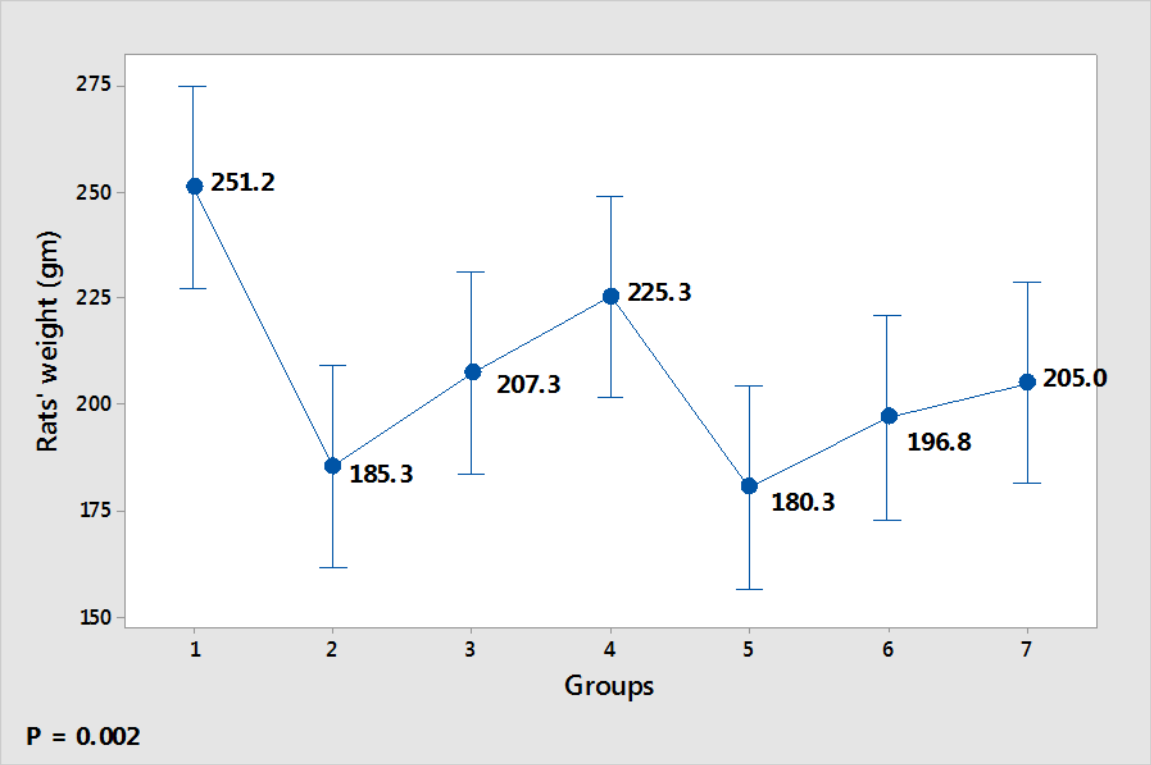

Figure (2): The differences of mean rats' weight among the study sampled groups after 3 wks of managment.

Disscution

The present exprement was conducted to investigate the possible protective effect of propolis supplementation against alloxan-induced diabetes complication in rats. Diabetes mellitus is associated with microvascular/macrovascular complications risk(King et al., 2005). Oxidative stress plays critical role in the pathogenesis of DM and its complications (Maritim et al., 2003).Supplemention with antioxidant products controls serum level of glucose concentration, attenuate oxidative stress, improve lipid profile, also inhibit renal impairment (Zhu et al., 2011; Chen et al., 2018).

In present study, alloxan was caused significant elevation in levels of TC, TG,LDL-c and CRR with significant reduction of HDL-c and CPI in diabetic untreated rats, those are risk factors for the advancementof atherosclerotic lesions and other cardiovascular event. This results was accordance with the results of Oršolić et al. (2019).Administration of propolisto diabetic animals as monotherapy (100 mg/kg) and as combined therapy (propolis + metformin+sitagliptin) significantly lowered TC, TG, also LDL-cholestrol,so prpolis can viablycontrol lipid metabolism of induced diabetic rats. Hence, it is useful for managing patients suffering from dyslipidemia.These results was consistentwith Chen et al., (2018) and El Menyiyet al. (2019) findings.

Propoliswas caused statistically significant elevation in the serum level of HDL-c in diabetic rats.This impact due to flavonoids consitituent of propolis. An important lipoprotein particle HDL-cholesterol gives protection againstmacrovascular complications, prevent oxidation of bad cholesterol (LDL-c), and neutralizes atherogenic impacts in the vascular walls (Zakerkish et al., 2019). This result confirm to that of Mujica et al. (2017), who found that supplementation with propoliscaused significant rise of HDL-C levels.Propolis also was caused significant differences

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on atherogenic Indices, by reduction of CRR and elevation of CPI this lead to reduce a predisposition toatherogenic disorders. Oršolić et al. (2019) was found the administration of propolis to mice had no significant effect on HDL level while there were significant reduction of TC, TG and LDL-cholesterol levels, and there were significant lowering of CRR with significant elevation of CPI.

Agreeing to our findings depened on the free radical scavenger ability ofpropolis, it appears that propoliswith its components arepotential suppresser of bad cholesterol (LDL) oxidation andinhibit inflamed vascular endothelium.This scavenger capacity seemrelated to the defensive impact against chronic diseases contributed to oxidativestress.

Urea and creatinine are markers of optimal renal function. Elevated levels of these metabolites signify impairment in kidney(s) function (Shokeen et al., 2008). In the present experiment, significant rising in the serum urea and ceratinin levels were observed in the untreated group of animals demonstrating kidney damage, while significant lowering in both these parameters was watched in the treated animals groups. Treatment with propolis as monotherapy and as combined therapy was significantly ameloriated the clearance of these metabolites by the kidney, thus restoring the serum level of urea and creatinine to normal ($P < 0.05$). El Menyiy et al. (2019) wasobserved that the administration of propolis extract caused a significant diminished of the elevated serum levels of creatinine and blood urea. Whereas the findings of Zakerkish et al. (2019) andOršolić et al. (2019) studies were showed significantly deceased in the serum level of urea with non-significant effect on Serum Cratinine level. These perceptions demonstrate that propolis supplementation can anticipates disintegration of renal work in diabetic patients.

Alloxan also was caused a significant body weight loss of diabetic rats ($P<5\%$). Significant increase in body weight of treated rats with propolis were observed as compared to other groups after 3 weeks of management ($P<0.05$).This was consistent with the findings of Rivera-Yañez et al. (2018);and Alassaf et al. (2020).So that, oral daily supplementation of diabetic animals with propolis ameliorated alloxan-induced alterations in the animals body weight.

Conclusion

propoliscontrols serum lipid profiles, renal parameters and diminished body weight loss in diabetic rats. It alsohas significant impact on the atherogenic indices by lowering CRR, and enhancing CPI in alloxan-induced diabetic rats. Antioxidative capacity of propolis because of its Flavonoids components.Hence, this gives trust that the utilize of propolismay be manage patients with dyslipidemia and to reduce diabetes complication.

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