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Original Article

A comparative, in-vivo anti-diabetic study of persimmon peel powder in alloxan induced rabbits

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Abstract

Diabetes is a condition when body fails to maintain the glucose levels in the blood properly and the level cross the normal ranges. Natural treatments particularly through bioactive components from fruit and vegetable sources are becoming popular worldwide and are broadly accepted because of no side effects and cost effectiveness. In current study, the antidiabetic potential of persimmon peel powder was investigated by using alloxan induced diabetic rabbits as an animal model. The rabbits were divided into five groups. Persimmon peel powder supplemented diets (0%, 10% and 20%) were given to the diabetic rabbits for the duration of 21 days. The blood samples of rabbits were examined for glucose, serum creatinine and urea levels on weekly basis. There was a significant decline in the levels of blood glucose in the alloxan induced diabetic rabbits. The reduction of blood glucose level was from 357.66 mg/dL to 256.45 mg/dL when the animals were fed on diet supplemented with 20% persimmon peel powder. Similarly, serum creatinine and urea levels were also significantly reduced because of supplementation of persimmon peel powder. It is concluded from the results that persimmon peel powder might be a potential natural best possible therapeutic option for the management and treatment of *Diabetes mellitus*.

Keywords: Diabetes, Antidiabetic potential, Persimmon peel, Bioactive components

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Introduction

A functional food constructively influence specific one or more functions in human beings with its basic nutrition for the reduction of chronic diseases because of containing certain compounds or components (functional components) with biologically active nature (Mack et al., 2003). In other sense, the functional food may be used for the control, management or treatment of human health disorders and diseases including diabetes (Shafi et al., 2014). These biologically active components are frequently present in various parts of foods derived from plant sources especially fruits and vegetables (Tanveer et

al., 2016; Malik et al., 2017). The peels of fruit being processed in agro based industries are the leftovers which are source of environmental pollution (Batool et al., 2015). Although, now it has been well recognized that the fruit peels are not only the waste materials instead these are full of many useful compounds which can be used for antioxidative, antibacterial, and anticancer activities (Hakim et al., 2000; Higashi-Okai et al., 2002), however, there is still lack of research activities to find out the hidden potentials of these wastes against diseases like diabetes (Leontowicz et al., 2003).

Diabetes, a condition when body fails to maintain the glucose levels in the blood properly and the level cross the normal ranges is one of the major health threats all over the world and according to World Health Organization, the diabetes will be among the top 10 leading causes of death on the globe till 2030. This disease is responsible for high number of morbidities and mortalities all over the world and its prevelance is increasing day by day with different rates in different countries (Tanveer et al., 2017). Currently, various therapeutic options like anti-diabetic agents, insulin administration, surgical procedures and pharmacologic interventions are being used for its control but have their own limitations. In such situations, the use of plants or plant based materials are fetching interest of scientist to be explored for effective utilization for possible remedies of chronic diseases (including diabetes) all over the world due to presence of natural bioactive compounds like antioxidants, dietary fibres and many other bioactive compounds (Kazmi et al., 2014). Among fruits, persimmon is a neglected fruit because of its very short shelf life and limited duration of its availability. However, it contains a number of functional compounds like fiber, terpenoids, carotenoids, flavonoids, tannins, naphthoquinones, steroids, amino acids and minerals (Yaqoob et al., 2016) which can be effectively used for the management of various diseases and health disorders.

Keeping in view, the presence of dietary fibres and other bioactive compounds including antioxidants in the peel of persimmon which may be helpful for the management for diabetes, the current activity was designed to evaluate the antidiabetic potential of persimmon peel powder through a biological study using rabbits as animal model.

Material and Methods

Sample preparation

The persimmon purchased from local market of Sargodha, Pakistan was dried after washing and peel was separated from pulp. The peels were dried by using hot air oven at a temperature not more than 50ºC and in freeze drier (Christ alpha 1-4 LD plus, type: 101541 lyophilizer, at -50ºC for 7 days) (Lee et al., 2006). After drying both type of samples (Oven dried and Freeze dried) were grounded into powder form and stored into airtight plastic jars.

Biological study

The biological studies were conducted on white rabbits of both male and female gender (New Zealand strain) with almost uniform initial body weight ranged from 1.36 – 1.67kg. The age of rabbits was ranged from 10-12 weeks. The animals were acclimatized for a period of 2 weeks before start of experiment. For hosting of rabbits, the animal house of University of Sargodha, Sargodha (Pakistan) was used.

Induction of diabetes

Alloxan at a dose of 110 mg/ kg of body weight was used to induce diabetes in rabbits. After 72 hours of alloxan injection, the blood glucose analysis was carried to confirm the induction of diabetes. The blood glucose assay was carried out by using glucometer (ACON Laboratories, Inc. USA).

Treatment diets

The diabetic rabbits were equally distributed into five groups with 3 rabbits in each group. Five experimental diets supplemented by persimmon peel powder at the concentrations of 0 %, 10 % and 20 % were prepared according to the treatment plan (Table 1). Each group was fed on treatment diets having different concentration of persimmon peel powder obtained from both methods of drying (oven dried and freeze dried) for a period of 21 days. The diet was given twice a day and blood analyses were carried out on weekly basis by following the method as given by Shakirin et al. (2010) with some modifications.

Blood analysis

Experimental rabbit's blood was analyzed on weekly basis. Estimation of blood glucose level, serum creatinine and uric acid was done.

Table 1: Treatment Plan for the assessment of OD and FD persimmon pulp powder

Diet Components	Control Diet T_0 (g)	T_1 (10%) (g)	T_2 (20%) (g)	T_3 (10%) (g)	T_4 (20%) (g)
Corn starch	380	350	310	350	310
Barley	370	320	260	320	260
Meat extract	70	50	50	50	50
Mineral mixture	40	40	40	40	40
Vitamin mixture	15	15	15	15	15
Salt	5	5	5	5	5
Sugar	100	100	100	100	100
Corn oil	20	20	20	20	20
Persimmon peel powder OD	0	100	200	0	0
FD				100	200
Total	**1000g**	**1000g**	**1000g**	**1000g**	**1000g**

Mineral supplied: CaCO3, 20g; NaHPO4, 20g. Vitamins supplied (per g of diet): Thiamin, 1mg; Riboflavin, 1mg; Nicotinamide, 10mg; Vitamin C, 10mg; Vitamin D, 1000 IU. T_1 = Normal diet, T_2 = Normal diet + 10 % persimmon peel powder (freeze dried), T_3 = Normal diet + 20 % persimmon peel powder (freeze dried), T_4 = Normal diet + 10% persimmon peel powder (oven dried), T_5 = Normal diet + 20 % persimmon peel powder (oven dried)

Statistical analysis

The data obtained during entire research work was statistically analyzed for analysis of variance (ANOVA) by using STATISTIX 8.1 version software and the coparison of means was assessed through least significant differences as described by Steel et al. (1997).

Results

Blood Analysis

Blood glucose level

Results showed that the persimmon peel powder reduced the blood glucose level. The results of persimmon peel powder concentrations on blood glucose level in rabbits for overnight-fasted diabetic rabbits treated with or without the dietary persimmon peel powder for 21 days. Significantly the highest decline in levels of glucose were found in the blood of rabbits which were treated with diet containing 20% of persimmon peel powder whereas significantly the least change/decline in the glucose levels in the blood of rabbits was observed in case of control group which were fed on diet without supplementation of peel powder. The blood glucose contents were ranged from 249.12 mg/dL to 342.83 mg/dL

The results regarding duration of treatment period depicted that the blood glucose levels were decreased with the passage of time and obviously significantly highest levels of glucose were observed in the blood of rabbits at the start of experiment and significantly the lowest mean blood glucose contents were found at 21st day of treatment period with mean blood glucose contents of 347.44 mg/dL and 225.22 mg/dL, respectively (Fig. 1).

The results for effect of peel powder type (freeze dried and oven dried) on levels of glucose in the blood of animals showed that maximum decline in levels of blood glucose were observed while feeding of animals by freeze dried persimmon peel powder whereas the minimum decrease in glucose level was found in the blood of rabbits when oven dried peel was used in the diet of animals with mean values of 286.08 mg/dL and 291.28 mg/dL, respectively. This difference showed that freeze dried peel powder was better than oven dried.

The combined effect of treatment period and peel powder supplementation levels on blood glucose levels of animals predicted that there was a gradual decline in levels of glucose due to increment in peel powder concentration and with the passage of treatment period (Table 2). In case of control treatment, there was no significant change in blood glucose levels in the rabbits during the entire period of 21 days.

With supplementation of diet by peel powder at a level of 10%, the mean decline in blood glucose levels was from 347.8 mg/dL to 194.1 mg/dL at 21st day of treatment period.

Table 2: Effect of persimmon powder (freeze dried and oven dried) and treatment duration on blood glucose, serum creatinine and urea

Powder Type	Concentration (%)	Weeks			
		0	1	2	3
Blood Glucose					
Freeze Dried	0	337.0±10.13	343.3±8.70	347.0±7.82	344.0±8.09
	10	344.0±7.24	297.7±7.54	249.0±7.95	191.3±8.77
	20	361.7±4.10	284.3±3.48	208.0±3.06	125.7±3.76
Oven Dried	0	337.0±10.13	343.3±8.70	347.0±7.82	344.0±8.09
	10	351.7±2.34	304.3±2.41	257.7±1.77	197.0±2.31
	20	353.3±4.34	288.0±4.05	222.7±3.76	149.3±0.88
Serum Creatinine					
Freeze Dried	0	1.35±0.03	1.42±0.06	1.48±0.04	1.52±0.04
	10	1.41±0.06	1.30±0.06	1.22±0.06	1.12±0.02
	20	1.56±0.03	1.32±0.06	1.18±0.03	0.87±0.09
Oven Dried	0	1.35±0.03	1.42±0.06	1.48±0.04	1.52±0.04
	10	1.48±0.05	1.35±0.04	1.22±0.01	1.10±0.01
	20	1.48±0.05	1.34±0.04	1.21±0.01	0.93±0.09
Urea					
Freeze Dried	0	41.33±2.73	36.67±2.85	32.00±2.52	30.67±2.34
	10	53.00±4.36	35.00±4.59	28.00±4.36	20.67±1.77
	20	46.33±3.48	36.67±2.85	27.00±1.53	18.33±1.20
Oven Dried	0	41.33±2.73	36.67±2.85	32.00±2.52	30.67±2.34
	10	46.67±4.41	41.67±3.29	36.00±0.58	25.67±2.61
	20	51.67±2.34	39.67±3.48	28.00±1.73	18.33±0.61

Significantly the maximum mean reduction in levels of blood glucose (from 357.5 mg/dL to 137.5 mg/dL) was observed in case of animals (rabbits) fed on diet containing 20% of persimmon peel powder at the end of treatment period (3rd Week) as shown in Fig. 1.

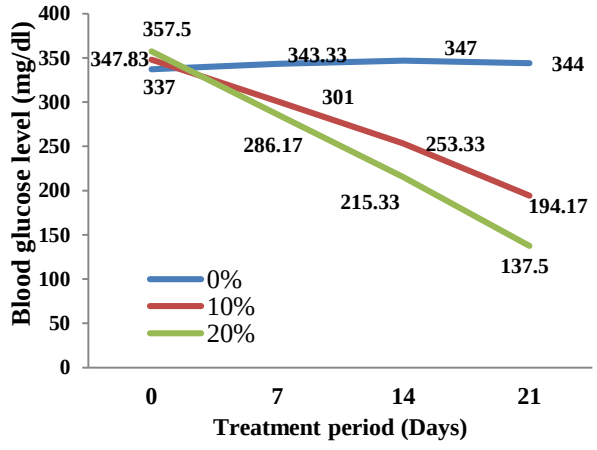

Fig.1: Effect of powder concentrations and treatment period on Blood glucose level

Serum Creatinine

The results regarding effect of various levels of peel powder in the diet of rabbits revealed that there was significant decline in levels of blood serum creatinine with increasing supplementation levels of powder. Significantly the highest mean decline in levels of creatinine level was found in the blood of animals (rabbits) which were treated with the diet containing 20% of persimmon peel powder whereas significantly the least decline in blood serum creatinine was observed in case of control group (treated with diet not containing peel powder). The levels of blood serum creatinine were ranged from 1.24 mg/dL to 1.44 mg/dL due to variation in concentrations of persimmon peel powder in the diet of rabbits.

Due to variation in treatment period, there was gradual decline in blood serum creatinine levels with the passage of time. The results further revealed that significantly the highest levels of blood serum creatinine were observed at initial stage of experiments and significantly the lowest levels were observed after 3 weeks of treatments with mean blood

serum creatinine contents of 1.44 mg/dL and 1.18 mg/dL, respectively.

The results for interactive effect of concentrations of persimmon peel powder and the duration of treatment period on levels of blood serum creatinine indicated that the blood serum creatinine contents were decreases with the increment in treatment period as well as levels of supplementation of diet with peel powder (Table 2). There was no significant change in blood serum creatinine contents in case of control group throughout the treatment period of 3 weeks. With supplementation of diet at a rate of 10% persimmon peel powder the mean decline in levels of serum creatinine in the blood of animals was from 1.44 mg/dL to 1.11 mg/dL after a treatment period of 21 days. However, significantly the highest decline in creatinine levels (0.52 mg/dL to 0.90 mg/dL) was found in the rabbits treated with diet containing 20% persimmon peel powder and after a period of 21 days (Fig. 2).

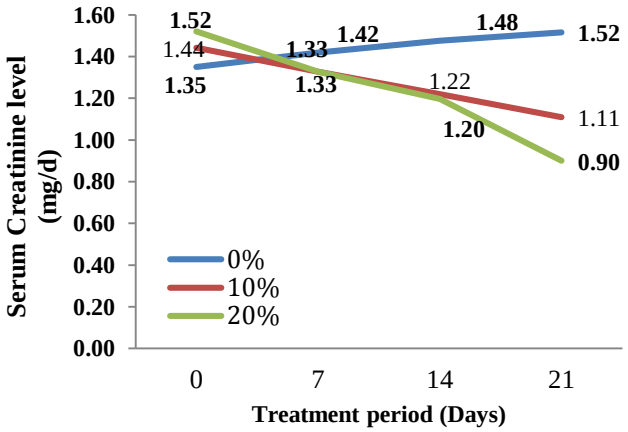

Fig.2: Effect of powder concentrations and treatment period on Serum Creatinine level

Urea

The results regarding urea levels predicted that there was also a gradual decline in urea contents with the passage of treatment period. Significantly, the highest levels of urea were observed at the initial stage of the experiment whereas significantly the lowest levels of urea were assessed after 3 weeks of treatment period with mean urea levels of 46.72 mg/dL and 24.06 mg/dL, respectively.

The cumulative effect of treatment periods and levels of supplementation of diet with persimmon peel powder on urea levels revealed that there was a gradual decline in urea levels with increment in levels of supplementation of diet with persimmon peel powder and with the passage of treatment duration (Table 2). The change in urea level was observed from 49.83 mg/dL to 23.17 mg/dL after three weeks of treatment when rabbits were given a diet containing 10% of persimmon peel powder. However, significantly the highest decline in urea levels was observed in case of rabbits treated with diet containing 20% persimmon peel powder and at 21th day of treatment with mean 49.00 mg/dL to 18.33 mg/dL decline in urea levels. Moreover, there was non-significant change in urea levels in case of control treatment during the entire period of biological study (Fig. 3).

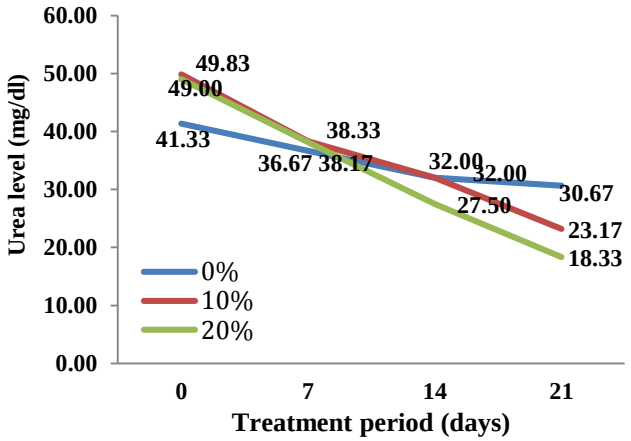

Fig.3: Effect of powder concentrations and treatment period on Urea level

Discussion

The results obtained during current study indicated that due to feeding of rabbits on diet containing different levels of persimmon peel powder gave significantly positive results regarding management of diabetes. This ant-diabetic potential of persimmon peel powder might be due to the presence of fiber and bioactive compounds (having antioxidant activity) in the peel of the fruit. Results further revealed that the effective reduction in hyperglycemia was concentration dependent. Moreover, results regarding serum creatinine and urea level showed significant reduction in serum creatinine and urea level of alloxan induced diabetic rabbits after administration of persimmon peel powder in their diet against control group. These findings were comparable with the observations of Lee et al. (2006) who also found positive impact of persimmon peel on diabetes while working on assessment of antidiabetic potential of

persimmon peel. They used rats as biological model and streptozotocin was used for induction of diabetes. They found high concentration of dietary fiber (40.35% w/w) in persimmon peel and this peel powder showed significant restoration of liver enzyme; Aspartate Amino Transferase (AST) and serum creatinine level which serve as indicator of liver and renal dysfunction. These results concluded that persimmon peel possessed strong antidiabetic efficacy because of the presence of high concentration of dietary fiber and antioxidants.

Azadbakhta et al. (2010) also reported similar outcomes while working on extracts of persimmon fruit. They also used rats in biological study and the diabetes was induced by streptozotocin and after diabetes confirmation, aqueous fruit extract was used to treat the diabetic condition of rats. The results indicated that fruit extract recovered the glycemic level of diabetic rats as well as weight of rats towards control because of the administration of fruit aqueous extract.

Hyperglycemia might be due to the increase in the concentration of free radicals (reactive oxygen species) produced during oxidation reactions. Whereas Yokozawa et al. (2007) reported that persimmon peel possessed strong antihyperglycemic potential and suggested that this anti-diabetic potential may be linked with radical scavenging activity of bioactive compounds present in persimmon peel like proanthocyanidins.

Conclusion

Current study was performed to assess the antidiabetic potential of persimmon peel powder by using alloxan induced diabetic rabbits. Different concentrations (0%, 10% and 20%) and type (freeze dried and oven dried) of persimmon peel was used against alloxan induced diabetes for the duration of 21 days. Study results indicated significant reduction on blood glucose level, serum creatinine and blood urea level of alloxan induced diabetic rabbits. It is concluded from the results that persimmon peel powder might be a potential natural best possible therapeutic option for the management and treatment of *Diabetes mellitus*. However there is need of further pre-clinical s well as clinical investigations against toxicological effect of persimmon peel powder.

Contribution of Authors

Sindu M: Conducted the research
Farooq U: Main Supervisor
Shafi A: write-up and proof reading
Akram K: Co-Supervisor
Hayat Z: Co-supervisor
Riaz M: Write-up and proof reading
Shahbaz M: Write-up and proof reading

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