

Research Article

ANTIDIABETIC POTENTIAL OF PERSIMMON PULP POWDER ON
ALLOXAN INDUCED DIABETIC RABBITSFiaqa Choudhry^{1*}, Umar Farooq², Kashif Akram³ and Afshan Shafi²¹ Department of Allied Health Sciences, Sargodha Medical College, University of Sargodha² Department of Food Science & Technology, MNS-University of Agriculture Multan, Pakistan³ Department of Food Sciences, Cholistan University of Veterinary and Animal Sciences, Bahawalpur, Pakistan

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Abstract

Diabetes, a condition when blood glucose level goes beyond the normal range. 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. The present study was planned to investigate the antidiabetic potential of persimmon pulp powder by using alloxan induced diabetic rabbits as an animal model. For this purpose, fifteen rabbits were induced Diabetes mellitus by alloxan and divided into three groups. Persimmon pulp 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. The results indicated that there was a significant decline in glucose as well as creatinine and urea levels in blood of diabetic rabbits. The reduction of blood glucose level was ranged from 357.66 mg/dL to 256.45 mg/dL on administration of 20% persimmon peel powder in the feed of rabbits. Similarly, serum creatinine and urea levels were also significantly reduced 1.42 mg/dl and 1.22 mg/dl and 47.50 mg/dl and 24.22 mg/dl, respectively because of supplementation of 20% persimmon pulp powder. It is concluded from the results that persimmon pulp powder might be a potential natural antidiabetic treatment of diabetic complications.

1. Introduction

Diabetes mellitus, mother of diseases and an ancient metabolic disorder of human being, is affecting millions of people all over the sphere. It has become a main cause of death in present days. Basically this disease condition is a metabolic disorder, in which level of blood sugar is raised. Food digested through metabolic reactions and then this digested food used by the body for its growth and development as well as energy production. Any type of food that we eat is a large quantity of it is broken down into glucose. Glucose is a form of sugar that is present in blood and it is one of the principal source of energy/fuel for human beings. Digested food that is glucose move towards bloodstream. Living cells utilize this glucose for energy and development. But glucose cannot be entered into body cells without the presence of insulin. Insulin is helpful for cells to take glucose (Alemzadeh, & Ali, 2011). Diabetes mellitus has multifaceted and different levels of heterogeneity by its etiology. Exact mechanism of the development and expansion of diabetes is still undefined. It is assumed that diabetes occurs when the firmly measured mechanisms have been disturbed because of any reason (Tanveer, Farooq, Akram, Hayat, & Shafi, 2018). Diabetes is lifetime ailment and its cure is not found yet. Though,

numerous pharmacological and non-pharmacological therapeutic actions have been applied to improve the life worth of persons that are suffering from diabetes mellitus and its micro and macro-vascular difficulties (ADA, 2011). Therapeutic anti-diabetes agents that are recently used are sulfonylureas, glinides, biguanides, and α -glucosidase inhibitors that are employed individually or in amalgamation (Kim, & Egan, 2008). Unfortunately, the diabetic treatment through antidiabetic agents has defects that involves the increased resistance and absence of responsiveness in a large part of the patient population. Moreover, no any antihyperglycemic agent suitably tackles the raised lipid level (Chitra et al., 2010). Maximum number of these oral antidiabetic agents cause severe side effects, so it is a challenge to manage diabetes without any bad outcomes (Liu et al., 2010). Consequently complete examination of more active and safer therapeutic agents in abolishing diabetic syndromes is a significant area of study. Therapeutic options that are presently used for the control of diabetes, like dietary amendments, oral hypoglycemic medicines, and insulin have their own restrictions (Kao et al., 2000).

In current years, a number of studies have been done to recognize the precise plant components which impart beneficial impact on human health. Different

studies are verifying that by including plant based diet in our daily routine can reduce the threats of numerous ailments to a significant level due to the occurrence of biologically active constituents (Dauchet et al., 2006). The current research work was scheduled to measure the anti-diabetic potentials of persimmon through biological studies on rabbits with following objectives: Compositional assessment of persimmon pulp powder. To assess anti-diabetic potential of persimmon pulp powder using rabbits as biological model.

2. Material and Methods

The current work was performed to prepare persimmon pulp powder by two different drying techniques (oven dried and freeze dried) in order to examine their therapeutic potential (anti-diabetic potential). The detail of work is as under:

2.1. Sample preparation

Persimmon fruit was obtained from fruit market of Sargodha. It was properly washed, pulp removed through fine pulper and dried by two different methods; 1: drying by hot air oven (Binder Company) at 40-50°C till constant weight (Lee et al., 2006) and 2: drying by lyophilization in a lyophilizer (Christ alpha 1- 4 LD plus, type: 101541) at -50° C (Jang et al., 2010).

Then dried pulp was grinded and sieved by using uniform mesh size (40-60 mesh sieves) to convert it into fine powder. Then this pulp powder was kept in air tight plastic containers at room temperature for further use (Lee et al., 2006).

2.2. Proximate analysis of persimmon pulp powder

The proximate composition of both types of persimmon pulp powder for the parameters of moisture, crude fiber, neutral detergent fiber, acid detergent fiber, hemicellulose, cellulose, lignin, crude protein, crude fat, and ash were analyzed according to the AOAC methods (AOAC 2000).

2.3. Biological study

Fifteen white rabbits (Male and female) of the New Zealand strain, weighing between 1.36 – 1.67 kg at age of 10-12 weeks were kept under controlled conditions at the university animal house and housed in stainless steel cages. These rabbits were acclimatized for two weeks before the onset of biological studies.

2.3.1. Diets

Diets of rabbits were prepared at Food Microbiology Laboratory at Institute of Food Science and Nutrition, University of Sargodha, Sargodha. Diet was prepared to control blood glucose level of rabbits.

2.3.2. Procurement of diet ingredients

Diet of experimental animals consists of barley powder, corn starch, common salt (NaCl), sucrose, corn oil, calcium carbonate, sodium hydrogen phosphate and meat extract. Which were purchased from the local market.

2.3.3. Diet preparation for treatment of rabbits

All diets contained basic components like barley powder, corn starch, common salt, sucrose powder, vitamin and minerals. Each diet was kneaded well by addition of some distilled water to prepare the pellets used in feed.

Five experimental diets differing by percentage of persimmon pulp powder were prepared as given in Table 1 (Shakirin et al., 2012)

2.3.4. Induction of diabetes

Alloxan (as per body weight) was dissolved in 1mL saline solution. The 12 hours fasted rabbits (diabetic control and test rabbits) were injected with portions of this solution at a dose of 110mg/ kg body weight. After 3days (72 hours) of alloxan injection, the rabbits were fasted for 12 hours and diabetes was confirmed by blood glucose estimation by using glucometer of ACON Laboratories, Inc. USA.

2.3.5. Feeding of rabbits

On the basis of five treatments all the fifteen diabetic rabbits were divided into five groups on the basis of diets. Each group contained 3 rabbits and was fed on separate diet as per treatments.

2.3.6. Blood analysis

All rabbits were trap in rabbits stand and the blood was withdrawn from jugular vein of all rabbits with help of 3 cc BD syringe and put in different blood collection tubes of 5ml containing Ethylene diamine tetraacetic acid (EDTA) to separate serum. Serum was separated in a table top centrifuge at 14000 rpm for 5 minutes.

Serum was sent to diagnostic laboratory for estimation serum creatinine and uric acid at start of the study and later on after every week for a period of three weeks. The blood glucose under fasting condition was estimated at the spot by using glucometer.

2.4. Statistical analysis

The final data obtained was subjected to statistical analysis using analysis of variance technique (ANOVA) under multi factor factorial completely randomized designs (CRD). The P values of <0.05 were adopted as statistically significant. The mean of all treatments were also compared by using LSD test adopting the method as described by Steel et al.

(1997). The data was analyzed by using SPSS version 17.00 (Statistical Package for Social Sciences)

3. Results and discussion

3.1. Proximate analysis of persimmon pulp powder

Six samples of persimmon pulp powder obtained after drying by hot air oven and lyophilizer were separately analyzed for proximate analysis. Their average value was calculated and results are given in Table 3.

In hot air oven dried persimmon pulp powder moisture contents 7.08%, 3.70 3.61%, fat contents 1.98%, minerals 3.80%, fiber contents 36.8.5% including cellulose, hemicellulose and lignin contents with values 17.08 %, 1.08 % and 16.50% were noted. In freeze dried persimmon pulp powder moisture contents were 7.18%, protein 3.68%, fat contents 2.13%, minerals 3.76 %, fiber contents 37.1% including cellulose, hemicellulose and lignin contents with values 18.50%, 0.53 % and 14.20%.

Salama, (2008) found similar results of proximate composition of persimmon pulp. According to him protein content was in range of 2.8 ± 1.42 , fat content 1.77 ± 1.059 , ash content was in range 2.12 ± 0.926 , crude fibre content range from 2.65 ± 0.494 .

3.2. Biological studies

The control of diabetes using persimmon pulp was done on rabbits and following final results were found. In these results effect on weight, blood glucose level, serum creatinine level and urea level was observed.

3.2.1. Blood glucose level

The statistical results (ANOVA) about diabetes discovered that the effect of concentration of persimmon pulp powder, duration of treatment and pulp powder (oven dried and freeze dried) was found to be highly significant (Table 3). The interactive effect of powder concentration and duration of treatment and powder concentration and pulp powder type was also found significant.

The interactive effect of powder treatment time and powder type was found non-significant and other interactions (powder concentration x duration of treatment, treatment duration x powder type, powder concentration x powder type x treatment duration) showed non-significant results.

Effects of various concentrations of persimmon pulp powder on blood glucose level in rabbits significantly showed decreasing (from 357.66mg/dl to 256.45 mg/dl) trend. The lowest blood glucose level was observed in rabbits fed on diet supplemented with 20% pulp powder whereas the highest blood glucose level was found in rabbits fed on diet not supplemented with persimmon pulp powder. The effect of treatment

period on total blood glucose level indicated that the maximum blood glucose level was observed at start of experimentation and minimum blood glucose level was found at end of experimentation with mean value 348.39 mg/dl and 241.28 mg/dl, respectively. A significant decreasing trend in blood glucose level was observed with passage of treatment time in duration of three weeks. The effect of type of persimmon pulp powder (freeze dried and oven dried) on glucose level showed that average minimum glucose level was observed in rabbits fed on diet supplemented with freeze dried pulp powder whereas average maximum glucose level was found on rabbit fed on diet supplemented with oven dried pulp powder. The interactive effect between treatment, time periods and various concentrations of persimmon pulp powder on blood glucose level indicated that the lowest glucose contents were found in blood of rabbits fed on diet having 20% persimmon pulp powder, after 3 weeks of treatment followed by glucose contents in the blood of rabbits fed on diet supplemented with 10% persimmon pulp. The maximum reduction in blood glucose level was observed after 3 weeks in the rabbits fed on food supplemented with 20% pulp powder with mean values of 353.17 and 156.83(mg/dl), respectively followed by 10% persimmon pulp with mean value of 341.67 mg/dl and 208.00 mg/dl.

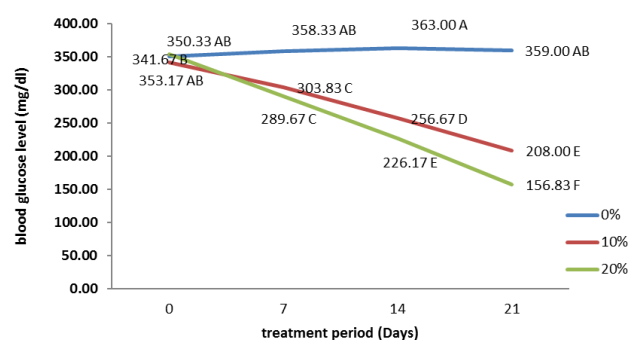


Fig 1: Effect of powder concentrations and treatment period on blood glucose level

However the significant highest glucose level at the start of experiment was found in 20% pulp powder with mean value of 353.17 mg/dl.

The interactive effect of persimmon pulp powder concentration and type of pulp powder indicated that glucose level slightly decreases. Maximum decrease in blood glucose level was observed in the blood of rabbits fed on the diet with 20% freeze dried pulp followed by 20% hot air oven persimmon pulp with mean value of 268 and 245. However the highest blood glucose contents were observed at the start of experiments in rabbits which are fed with the diet

supplemented with 0% persimmon pulp powder with mean value of 358 mg/dl Fig.1.

These results showed that persimmon pulp significantly decreased glucose levels in diabetic rabbits and this reduction might be due to the combined effects of dietary fiber and antioxidants present in persimmon Pulp. Perpetuo, and Salgado, (2003) also found similar results while working on mango pulp. Results also concluded that oral administration of persimmon pulp powder for 21 days effectively controlled hyperglycemia and it was concentration dependent. These findings are related to the findings of Azadbakhta et al. (2010) by working on anti-diabetic effects of aqueous fruits extract of persimmon on streptozotocin-induced diabetic rats.

Table 1: Composition of the experimental diets (g/kg)

Components	Normal Diet	F.D Diet			H.A.O Diet	
	T1	T2	T3	T4	T5	
	0%	10%	20%	10%	20%	
Corn starch	380	360	315	360	315	
Barley	370	355	300	355	300	
Meat extract	70	50	50	50	50	
Mineral mixture 1	40	40	40	40	40	
Vitamin mixture2	15	10	10	10	10	
Salt	5	5	5	5	5	
Sugar	100	60	60	60	60	
Corn Oil	20	20	20	20	20	
Persimmon pulp powder						
F.D pulp	-	100	200	-	-	
H.A.O pulp				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.

T₁ = Normal diet

T₂ = Normal diet + 10 % persimmon pulp powders (freeze dried)

T₃ = Normal diet + 20 % persimmon pulp powders (freeze dried)

T₄ = Normal diet + 10% persimmon pulp powders (oven dried)

T₅ = Normal diet + 20 % persimmon pulp powders (oven dried)

3.2.2. Serum creatinine level

The statistical results (ANOVA) about diabetes revealed that the effect of concentration of persimmon pulp powder and duration of treatment was found to be highly significant. While the effect of pulp powder type was non-significant. The interactive effect of powder concentration and duration of treatment was also found significant. The interactive effect of powder concentration and pulp powder type was found non-significant. While other relations (duration of treatment x powder type and powder concentration x

treatment duration x powder type) was also non-significant (Table 3).

Table 2: Proximate composition of Persimmon Pulp powder (g/100)

Parameters (%)	Freeze Dried	Oven Dried
Moisture	7.18±0.12 ^a	7.08±0.14 ^a
Protein	3.68±0.87 ^a	3.70±0.18 ^a
Fat	2.13±0.36 ^a	1.98±0.12 ^b
Fibre	37.10±0.56 ^a	36.80±0.56 ^a
Fibre (Cellulose)	18.5±0.43 ^a	17.08±0.39 ^a
Fibre (Hemicellulose)	0.53±0.23 ^b	1.08±0.54 ^a
Fibre (Lignin)	14.2±0.49 ^b	16.5±0.31 ^a
Ash	3.76±0.23 ^a	3.80±0.33 ^a

Effects of various concentrations of persimmon pulp powder on serum creatinine level in rabbits significantly showed decreasing trend. The lowest serum creatinine level was observed in rabbits fed on diet supplemented with 20% pulp powder whereas the highest serum creatinine level was found in rabbits fed on diet not supplemented with persimmon pulp powder. The serum creatinine contents ranged from 1.49mg/dl to 1.24 mg/dl.

Table 3: Mean Sum of squares of blood glucose, serum creatinine and urea

Source	DF	Blood Glucose	Serum Creatinine	Urea
Concentration (A)	2	69582.9**	0.42588**	183.01**
Day (B)	3	38596.7**	0.14211**	1840**
Pulp Power Type(C)	1	1682.0**	0.1967 ^{NS}	45.12 ^{NS}
AXB	6	12458.2 ^{NS}	0.11259**	172.77**
AXC	2	707.5*	0.01513 ^{NS}	24.04 ^{NS}
BXC	3	144.9 ^{NS}	0.00467 ^{NS}	8.64 ^{NS}
AXBXC	6	168.7 ^{NS}	0.00133 ^{NS}	3.84 ^{NS}
Error	48	88.4	0.1101	27.75 ^{NS}
Total	71			

NS= Non Significant

**=significant

The effect of treatment period on total serum creatinine level indicated that the maximum serum creatinine level was observed at start of experimentation and minimum serum creatinine level was found at end of experimentation with mean value 1.42 mg/dl and 1.22 mg/dl, respectively. A significant decreasing trend in serum creatinine level was observed with passage of treatment time in duration of three weeks. The interactive effect between treatment periods and various concentrations of persimmon pulp powder on serum creatinine level indicated that the lowest serum creatinine contents were found in blood of rabbits fed on diet having 20% persimmon pulp powder after 3 weeks of treatment followed by creatinine in the blood of rabbits fed on diet supplemented with 10% persimmon pulp after 3 weeks of treatment. The maximum reduction in blood serum creatinine level was observed after 3 weeks in the

rabbits fed on food supplemented with 20% pulp powder with mean values of 0.94 mg/dl and 1.37 mg/dl, respectively followed by 10% persimmon pulp with mean value of 1.45 mg/dl to 1.37 mg/dl. However the significant highest serum creatinine level at the start of experiment was found in 20% pulp powder with mean value of 1.45mg/dl as shown in Fig. 2.

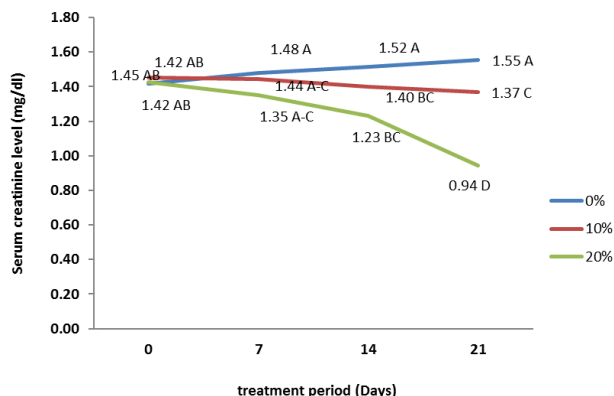


Fig. 2: Effect of powder concentrations and treatment period on serum creatinine level

These results concluded that serum creatinine level was significantly decreased in the persimmon pulp supplemented diabetic group of rabbits. Diabetic induces an elevation in urea and creatinine levels and it is concluded to be significant marker of renal dysfunction also found by Pandya, Nagrajappa, & Ravi (2016). As persimmon pulp decreases the glucose level this might be linked with decrease in creatinine level as we founded by Perpetuo and Salgado, (2003) also found similar results while working on mango pulp.

3.2.3 Urea level

The statistical results (ANOVA) about diabetes discovered that the effect of concentration of persimmon pulp powder and duration of treatment was found to be highly significant. While the effect of pulp powder type (oven dried and freeze dried) was found non-significant. The interactive effect of powder concentration and duration of treatment was also found significant.

The interactive effect of powder treatment time and powder type was found non-significant and other interactions (concentration pulp powder type, treatment duration x powder type, powder concentration x treatment duration x powder type) showed non-significant results (Table 3).

Effects of various concentrations of persimmon pulp powder on urea level in rabbits significantly showed decreasing trend. The lowest urea level was observed in rabbits fed on diet supplemented with 20% persimmon pulp powder. While highest urea level was

observed in rabbits fed on diet supplemented with 0% pulp powder. The urea level ranged from 16.58 mg/dl to 19.08mg/dl respectively. The effect of treatment period on total urea level indicated that the maximum urea level was observed at start of experimentation and minimum urea level was found at end of experimentation with mean value 47.50 mg/dl and 24.22 mg/dl, respectively. A significant decreasing trend in urea level was observed with passage of treatment time in duration of three weeks. The interactive effect between treatment time periods and various concentrations of persimmon pulp powder on urea level indicated that the lowest urea contents were found in blood of rabbits fed on diet having 10% persimmon pulp powder after 3 weeks of treatment followed by urea in the blood of rabbits fed on diet supplemented with 20% persimmon pulp after 3 weeks of treatment. The maximum reduction in urea level was observed after 3 weeks in the rabbits fed on food supplemented with 10% pulp powder with mean values of 53.50 mg/dl and 8.83 mg/dl, respectively followed by 20% persimmon pulp with mean value of 45.33 mg/dl and 10.83 mg/dl.

However the significant highest urea level at the start of experiment was found in 10% pulp powder with mean value of 53.50 mg/dl as shown in Fig.3.

These results concluded that Urea level was significantly decreased in the persimmon pulp supplemented diabetic group of rabbits. Diabetic induces an elevation in urea and creatinine levels and it is concluded to be significant marker of renal dysfunction also found by Pandya et al. (2016). As persimmon pulp decreases the glucose level this might be linked with decrease in urea level as we founded by Perpetuo frand Salgado, (2003) also found similar results while working on mango pulp.

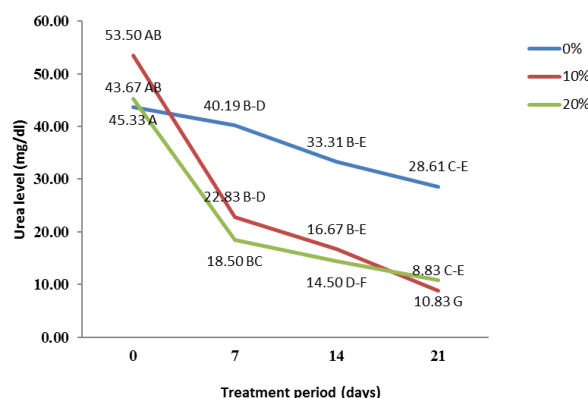


Fig 3: Effect of powder concentration and treatment period on urea level

4. Conclusion

At the end, by viewing on the result of all factors such as weight, blood glucose level, serum creatinine level and urea level it is concluded that persimmon pulp powder has the potential to treat diabetes especially at 20% pulp concentration. It is further revealed that there is no significant effect of drying methodology on its effectiveness but on comparison, freeze dried pulp proves to be more beneficial to reduce diabetic complications. Although this study results has proved the effectiveness of persimmon but still there is need to perform further pre-clinical as well as clinical studies to explore its more benefits for the betterment of human life.

5. Conflict of Interest

Authors declared no conflict of interest

References

- Alemzadeh, R., & Ali, O. (2011). Diabetes Mellitus. In: Kliegman, R.M., ed. *Kliegman: Nelson Textbook of Pediatrics*. 19th ed. Philadelphia, Pa: Saunders:chap 583
- AOAC. (2000). Official Methods of Analysis. The Association of Analytical Chemists. 17th Ed. Arlington, USA.
- Azadbakhta, M., Safapour, S., Ahmadi, A., Ghasemi, M. & Shokrzadeh, M. (2010). Anti-diabetic effects of aqueous fruits extract of *Diospyros lotus* L. on streptozotocin-induced diabetic rats and the possible morphologic changes in the liver, kidney and heart. *Journal of Pharmacognosy and Phytotherapy*, 2(2):10-16.
- Chitra, V., Venkata, K. R., Raju, M. V. R. K. K., & Prakash, J. (2010). Study of antidiabetic and free radical scavenging activity of the seed extract of *strychnosnux-vomica*. *International Journal of Pharmacy and Pharmaceutical Sciences*, 2: 106–110.
- Dauchet, L., Amouyel, P., Hercberg, S., & Dallongeville, J. (2006). Fruit and vegetable consumption and risk of coronary heart disease: a meta-analysis of cohort studies. *The Journal of Nutrition J*, 136: 2588-2593.
- Jang, I. C., Jo, E. K., Bae, M. S., Lee, H. J., Jeon, G. I., Park, E., & Lee, S. C. (2010). Antioxidant and antigenotoxic activities of different parts of persimmon (*Diospyros kaki* cv. *Fuyu*) fruit. *Journal of Medicinal Plants Research*, 2: 155-160.
- Kao, Y. H., Hiipakka, R. A., & Liao, S. (2000). Modulation of endocrine systems and food intake by green tea epigallocatechin gallate. *Endocrinology*, 141: 980–987.
- Kim, W. and Egan, J. M. (2008). The role of incretins in glucose homeostasis and diabetes treatment. *Pharmacological Reviews*, 60:470–512.
- Lee, S.O., Chung, S.K., & Lee, I.S. (2006). The antidiabetic effect of dietary persimmon (*Diospyros Kaki* L.cv. Sangjudungsi) peel instreptozotocin-induced Diabetic Rats. *Journal of Food Science*, 71: 293–298.
- Liu, Q., Chen, L., Hu, L., Guo, Y., & Shen, X. (2010). Small molecules from natural sources, targeting signaling pathways in diabetes. *Biochimica et Biophysica Acta (BBA)-Gene Regulatory Mechanisms*, 1799: 854–865.
- Pandya, D., Nagrajappa, A. K., and Ravi, K. S. (2016). Assessment and correlation of urea and creatinine levels in saliva and serum of patients with chronic kidney disease, diabetes and hypertension– a research study. *Journal of Clinical and Diagnostic Research*, 10(10):ZC58-ZC62.
- Perpetuo, G.F., & Salgado, J.M. (2003). Effect of mango (*Mangifera indica*,L.) ingestion on blood glucose level of normal diabetic rats. *Plant Foods for Human Nutrition*, 58: 1-12.
- Roberta, Larson. Duyff. (2011). ADA (American Dietetic Association). 688 pages - Preview
- Salama, M.F. (2008). Effects of pretreatments on quality of dried persimmon (*Diospyros kaki*) fruit sheets. *Journal of Food Science & Technology*, 5(1):13-20.
- Shakirin, F.H., Azlan, A., Ismail, A., Amon, Z., & Yuon, L.C. (2012). Protective Effect of Pulp oil extracted from *Caranium odontophyllum* Miq.Fruit on Blood Lipids, Lipid Peroxidation, and Antioxidant Status in Healthy Rabbits. *Oxidative Medicine and Cellular Longevity*, 2012: Article ID 840973
- Tanveer, A., Farooq, U., Akram, K., Hayat, Z., and Shafi A. (2018). Management of diabetic complications through fruit flavonoids as a natural therapy. *Critical Reviews in Food Science and Nutrition*, 57: 1411-1422.