Histological Study On the Effect of *Punica Granatum* On the Liver of STZ-Induced Diabetic Male Rats

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ABSTRACT

Diabetes mellitus (DM) is one of the oldest known human diseases and one of the biggest health problems of researchers. Many studies have shown diabetes complications in different body organs. In the present study, diabetes mellitus (DM) was induced via administration of 40 mg/kg Streptozotocin (STZ), intraperitoneally. Rats were divided into three groups, control group (n=5), diabetes group (n=10), and diabetes treated with *Punica granatum* peel (n=10) (3 ml/animal). The study continued for 4 weeks. The liver was processed for examination under a light microscope. The results showed many pathologic changes in the liver tissue in diabetic rat, which were seen by different changes appearing in the liver tissue such as several necrotic cells, atrophic nucleus, and acute tissue deformities. While the tissues treated with *Punica granatum* showed a significant improvement in liver tissue of the diabetic rats treated with *Punica granatum* compared to the diabetic control rats group.

Key Words: *Punica granatum, Diabetes mellitus, Liver, Hepatocytes, Portal Area, Blood Sinusoids, Kupffer cells.*

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INTRODUCTION

Diabetes mellitus (DM) is one of the oldest known human diseases and one of the biggest health problems of researchers, especially after the spread among children largely due to unhealthy nutrition and unhealthy lifestyle with less physical activity and the riskiness of this is on the health of individuals and society [1-3]. Persians, Indians, and ancient Egyptians described the symptoms of the disease, but the proper understanding of the situation has developed over thelast 100 years. Diabetes has 3 major types: Diabetes mellitus type 1: the result of the destruction of insulin-producing cells. These cells are called beta-cells (β-cells), which are treated with insulin, and diabetes mellitus type 2: which does not depend on insulin and depends on diet and medicines; betacells secrete insulin, but the inability is to identify and benefit from insulin by the cells and is treated with drugs, including Sulfonylurea in addition to diet and physical activity. In addition, diabetesmay appear during pregnancy and is called Gestational Diabetes. The risk of diabetes is represented by itsserious long-term complications, dysfunction, and malfunction in various organs such as the eyes, kidneys, heart, nerves, and blood vessels. Diabetes quickly turns into a global health problem due to its complications, especially with the high population rate, aging, urbanization, urbanization, increased physical inactivity, and obesity [4]. *Punica granatum*, also called (Pomegranate), has been described as a treatment for diabetes in traditional Greek medicine Unani system of medicinesinceancienttimes. As *Punica granatum* contains high levels of a variety of phytochemicals including polyphenols, sugars, fatty acids (conjugated and non-conjugated), aromatic compounds, amino acids, tocopherols, sterols, terpenoids, and alkaloids, it has therapeutic benefits including anti- inflammatory, antioxidant, antimicrobial, and antineoplastic effects. Moreover, it plays a therapeutic role in hypoglycemia and hypolipidemia [5]. *Punica granatum* has a protective role against type 2 diabetes. The pomegranate peel contains higher amounts of components with active and biological nutritional values compared to its edible part (pulp). The pomegranate peel also contains phenols that show strong resistance to mutations, antioxidants, and antimicrobials [6].

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MATERIALS AND METHODS

Twenty-five (n=25) male adult Wister rats with an average weight of 200-250g were used in the experiment conductedin King Fahad Medical Research Center (KFMRC). Diabetes was induced using 40 mg/kg Streptozotocin (STZ) [7], purchased from Sigma Chemicals (Germany), and freshly prepared in citrate buffer (0.1 M, pH 4.5). Rats with a blood glucose level of 200 mg/dl or higher were considered diabetic. The *Punica granatum* was obtained from the local herb store in Jeddah, Saudi Arabia. The *Punica granatum* peels (PGP) were extracted as describedby (Berto BP *et al., a*nd Khalil, Enas A.M) [8, 9] with some modifications. 5g of *Punica granatum* peels (PGP) powder was dissolved in 200 ml Distilled Water. The rats received normal rat chow and tap water ad libitum in a constant environment (room temperature 28± 2°C, room humidity 60±5%) with a 12 h light/dark cycle. The rats were divided into 3 groups; group 1: normal control group containing5rats;group 2:control diabetic group containing 10 rats; group 3: containing 10 diabetic rats treated with 3ml/rat of PGP for 28 days. The liver was removed, processed, and stained with hematoxylin & eosin (H& E) for examining under a light microscope.

RESULT AND DISCUSSION

Group 1: Normal Control Group:

In a histological study with a light microscopic examination, the liver tissue was seen consisting of lobed hepatic lobules with no barriers separated. It consists of hepatocytes with athickness of one cell, organized in the form of radially shaped ribbons around the central vein located at the center of the hepatic lobule and padded with a Simple Squamous Epithelial Cells. Hepatocytes appear as the structural unit and the main function of the liver tissue in the natural polygonal state and have one central nucleus or two round nuclei containing one or morenucleiinside them, and they are highly pigmented to the base dyes. Kupffer Cells are characterized by their emergence within a cavity the blood sinus and the endothelial cells that line the blood sinuses, which form a barrier against pathogens, are a sieve to purify substances that pass from the blood to the hepatocytes (Figures 1 and 2). Swian *et al.,*also agreed, where microscopic examination images of the liver from the normal rat group of liver cells showed regular hepatocytes in normal structure, central venous, and portal area integrity and blood sinuses [10].

Group 2: control diabetic group:

The photomicrograph of the liver ofthe diabetic control rat group showed acute inflammation in the liver tissues as necrosis cellular of hepatocytes, damage of central vein, dilation of blood sinuses, Kupffercellsproliferation, cytoplasmic degeneration, and congestion of blood in liver tissue and damage of the portal area, hepatic artery fibrosis, bile duct proliferation, karyolysis cells, and separation of endothelial cells, and increase in lipid droplets in hepatocytes (Figures 3 - 7). Diabetic rats showed loss of normal architecture of liver like hepatocellular injury, dilation of central vain, and fat droplets [10]. The hepatopathy of the liver in diabetic rats was observed like, vacuolization of hepatocytes, variation of nuclear size, dilated sinusoidal, and lipid droplets [11]. In addition, histological changes of liver were shown in diabetic rats, such as inflammatory infiltration, disorganized hepatocytes, and vacuolar degradation [12]. Moreover, the hemorrhage, fatty and vacuolization changes, necrosis, and loss of parenchymal cells were evident in the liver tissues [13]. The histologicaldamagesof the liverwere also seen indiabetic rats like cellular necrosis, vacuolated nuclei, and some fatty droplets [14].

Group 3: diabetic treatment with *Punica granatum* peel (PGP) group:

The photomicrograph of liver tissues of diabetic rats treated with *Punica granatum* peel (PGP) showed significant improvement as hepatic cells appeared as hepatic bands, central veins are normal, presence of two-nucleus (2N) cells, mild inflammation, and reduction of congestion of blood (Figures 8-11). Many studiesshowed a strong correlation between antioxidant activity and to talphenolics content (TPC). In addition, *Punica granatum* peel has a higher amount of TPC compared with aril juices or seeds [15, 16]. In a study by Aboonabi*et al.,* the results showed a decrease in histopathological changes in the liver, the liver cells appeared closer to the arrangement and the natural form, and a slight expansion of the blood pockets without any inflammation around the central vein in addition to a decrease in the level of blood glucose and an increase in the level of insulin in the blood serum in diabetic rats treated with *Punica granatum* when compared to the untreated diabetic group [17]. In a recent study, Wang *et al.*, showed the positive effect of pomegranate on the liver, and the study addressed the protective effect of *Punica granatum* peel (PGP) extract on the liver tissue of autoimmune hepatitis (AIH); the results showed a sharp change in the affected liver, while pre-treatment with *Punica granatum* peel (PGP) extract resulted ina significant reductioninjury, acutechangesintheliver, and tissue return closer to normal shape, which proves the positive effect of the *Punicagranatum* in the treatment of hepatic tissue [11]. Also, Xu*et al.*, studied the effect of *Punica granatum* flower PGF extract on hepatic fat levels and the histological results confirmed that pomegranate reduces diabetes and hepatic fat associated with obesity (at least in part) [18]. *Punicagranatum* has potent positive activity in reducing blood glucose levels, serum biomarkers such as aspartate

aminotransferase (AST) and alanine aminotransferase (ALT), serum cholesterol, and triglyceride levels, while showed a significant increase in serum insulin in diabetic rats [19, 20]. A study on different parts of *Punica granatum* showed the positive activity of *Punica granatum*in diabetic rats, the *Punica granatum* juice (PJ) showed significantly repairment in the islets of Langerhans, while the *Punicagranatum* juice (PJ) and *Punicagranatum* seeds (PS) showed significantly decreased levels of plasmainflammatory biomarkers [20]. The *Punica granatum* peel has a positive activity in reducing plasma glucoseand serum levels of triglyceride, cholesterol, and low-density lipoproteins [21].

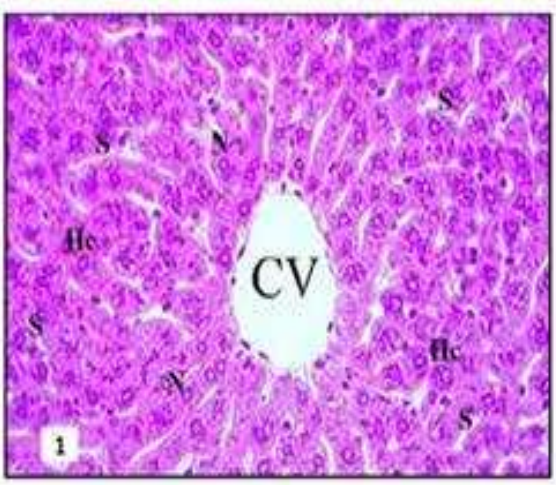

Figure 1: Photomicrograph of liver tissues from normal control rats showing hepatocytes (Hc), nucleus (N), central vein (Cv), and blood sinusoid(S)(H&E stain; X400).

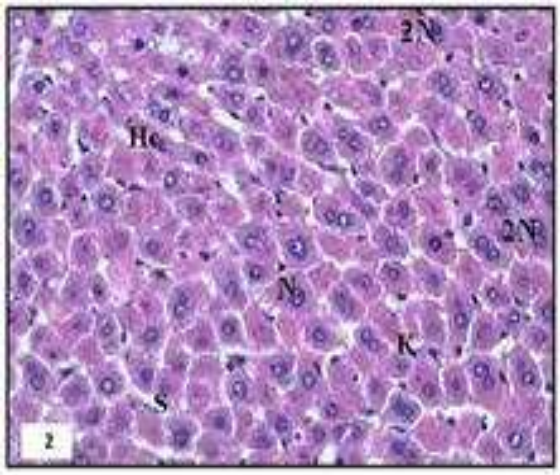

Figure 2: Photomicrograph of liver tissues from normal control rats showing hepatocytes (Hc), nucleus (N), or two-nucleus (2N), blood sinusoid (S), and Kupfer cells(K) (H&E stain; X400).

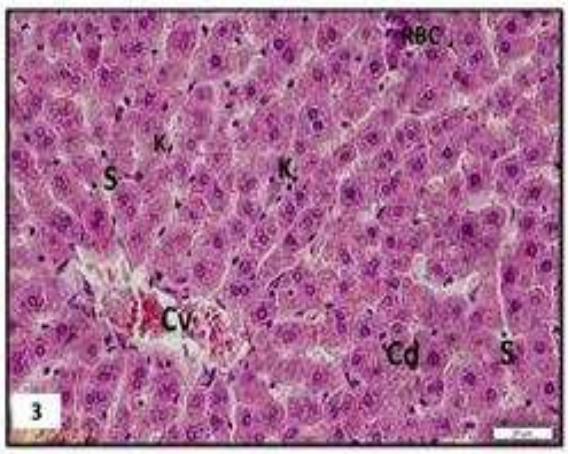

Figure 3: Photomicrograph of liver tissues from diabetic control rats showing damage of central vein (Cv), dilation of blood sinuses (S), Kupffer cells proliferation (K), cytoplasmic degeneration (Cd), and congestion of blood in liver tissue (RBC)(H&E stain; X200).

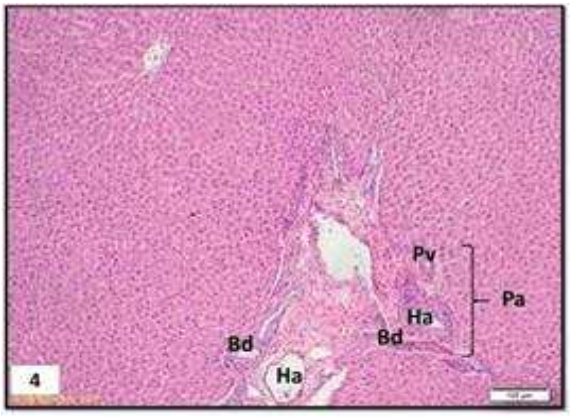

Figure 4: Photomicrograph of liver tissues from diabetic control rats showing damage of the portal area (Pa), portal vein (Pv), hepatic artery (Ha), and bile duct proliferation (Bd)(H&E stain; X100).

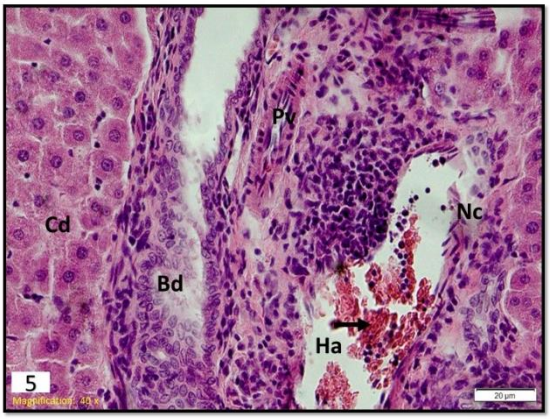

Figure 5: Photomicrograph of liver tissues from diabetic control rats showing damage in the portal area as hepatic artery fibrosis (Ha), cytoplasmic degeneration (Cd), bile duct proliferation (Bd), karyolysis cells and separation of endothelia cell (arrow), necrosis cellular of hepatocytes (Nc) (H&E stain; X400).

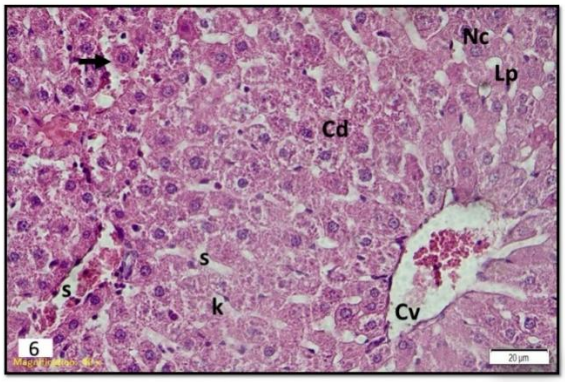

Figure 6: Photomicrograph of liver tissues of diabetic control rats showing damage in the central vein (Cv), dilatation of blood sinuses (S) and Kupffer cells (K)in liver tissues, acute inflammation (arrow), necrosis cellular of hepatocytes (Nc), cytoplasmic degeneration (Cd), and increase in lipid droplets in hepatocytes (Lp) (H&E stain; X400).

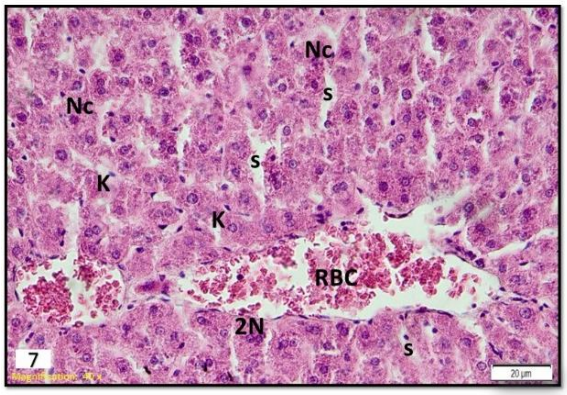

Figure 7: Photomicrograph of liver tissues from diabetic control rats showing damage acute inflammation in central vein as congestion of blood (RBC), dilatation of blood sinuses (S), Kupffer cells (K), increase cytoplasmic degeneration and necrosis cellular of hepatocytes (Nc) (H&E stain; X400).

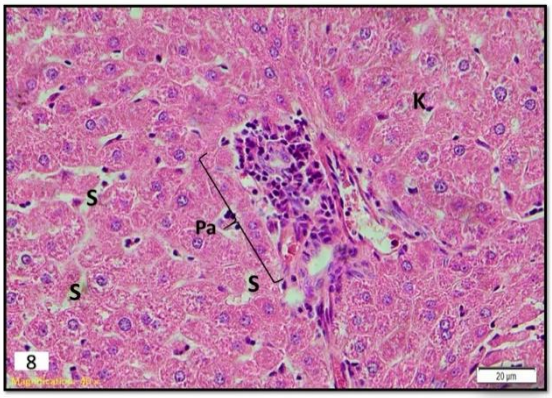

Figure 8: Photomicrograph of liver tissues from diabetic rats treated with *Punica granatum*peel (PGP) showing significant improvement in portal area (Pa) as reduction of damage in bile duct and congestion of blood in liver tissues (H&E stain; X400).

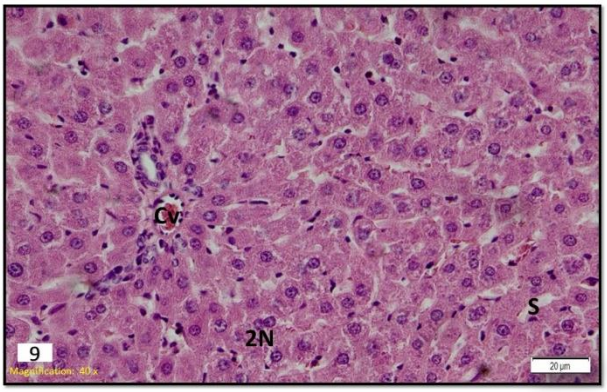

Figure 9: Photomicrograph of liver tissues of diabetic rats treated with *Punica granatum* peel (PGP) showing significant improvement as hepatic cells appear as hepatic bands and central veins (Cv) are normal and two-nucleus (2N) cells are present (H&E stain; X400).

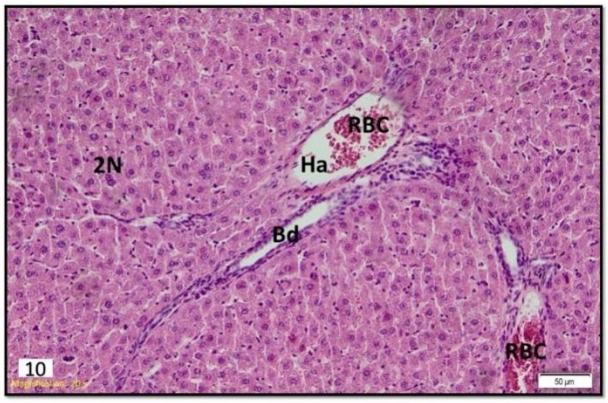

Figure 10: Photomicrograph of liver tissues of diabetic rats treated with *Punica granatum* peel (PGP) showing significant improvement as reduction of congestion of blood (RBC) in the hepatic artery (Ha) with mild damage in the bile duct (Bd) form and presence of two-nucleus (2N) cells (H&E; X200).

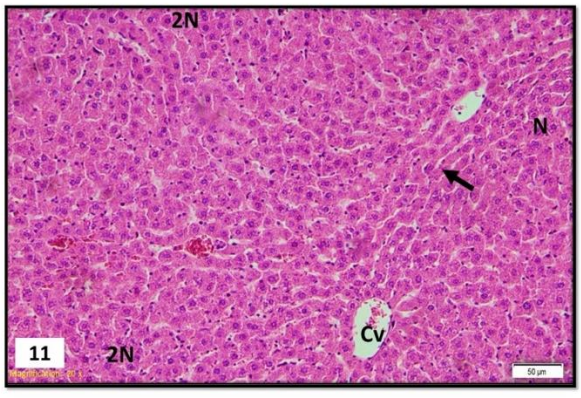

Figure 11: Photomicrograph of the liver tissues of diabetic rats treated with *Punica granatum* peel (PGP) showing significant improvement as hepatic cells appear as hepatic bands and central veins (Cv) are normal, mild damage is observed in liver tissues, and bi-nucleus (2N) cells are present (H&E stain; X200).

CONCLUSION:

Diabetic Mellitus (DM) has a potent effect on the liver, which leads to the development of hepatitis causing acute inflammation in liver tissue as hepatic dysfunction, congestion of blood (RBC), and deformity of hepatocytes. However, the use of *Punicagranatum* peel (PGP) causing improved liver tissue and function of hepatocytes, stop of bleeding blood (RBC), which means *Punicagranatum* peel improvs hepatic function.

Recommendations:

1. As the study is recent, it is recommended to do more histological studies on the positive effect of Punica granatum ontissues.
2. In addition, it is recommended to increase the nutritional health awareness of the community and the importance of eating *Punica granatum* daily by moderate amounts, especially for patients with diabetes mellitus (DM).

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REFERENCES

[1] Alkhoshaiban A, Almeman A. Knowledge of pharmacists about Diabetes Mellitus. Arch. Pharm. Pract. 2018;9(4):4-10.

[2] Ragab M, Rashed LA. Effect of experimentally induced diabetes mellitus on the exocrine part of pancreas of adult male albino rat and the possible protective role of Silymarin: light and electron microscopic study. J. Adv. Pharm. Educ. Res. 2018;8(1):75-81.

[3] Aziz N, Wal A, Wal P, Pal RS. Preparation and Evaluation of the Polyherbal Powder: The Nature's Pharmacy for the Treatment of Diabetes Mellitus and Its Complications. Pharmacophores. 2019;10(1):60-7.

[4] Sen S, Chakraborty R, De B. Complications of DiabetesMellitus. Diabetes Mellitus in 21st Century pp 69-100| Cite as. 2016.

[5] Banihani S, Swedan S, Alguraan Z. Pomegranate and type 2 diabetes. Nutr- Ition Resear CH33, 2013 ; 341–348.

[6] Akhtar, S., Ismail, T. Fratenale, Daniele. Piero, Sestili. (2015). Pomegranate peel and peelextracts: Chemistry and foodfeatures. Food Chemistry, 2015 ; Volume 174, 417-425.

[7] Tella T, Masola B, Mukaratirwa S. The effect of *Psidium guajava* aqueous leaf extract on liverglycogenenzymes, hormone sensitive lipase and serumlipid profile in diabetic rats. Biomedicine &Pharmacotherapy, 2019 ; Volume 109 : 2441-2446.

[8] Berto B.B, Borba H.R, Santos H.L.C.D, Lima V.M, Flausino W, Teixeira-Filho W, Lopes C.W.G. Evaluation of pomegranate (*Punica granatum*) pericarp aqueous extract on Eimeriaspp. From Japanese quails (*Coturnix japonica*).2014.

[9] Khalil, Enas A.M. Antidiabetic effect of an aqueousextract of Pomegranate (P*unica granatum* L.) peels in normal and alloxandiabetic rats.2004.

[10] Swian S K, Dash U C, Kanhar S, Sahoo A K. Ameliorative effects of Hydroleazeylanica in streptozotocin-induced oxidative stress and metabolic changes in diabetic rats. Journal of Ethnopharmacology, 2010 ; 247 : 112257.

[11] Wang, T., Men, R., Hu, M., Fan, X., Yang, X., Huang, X.,Ye, T., Yang, L. Protective effects of *Punica granatum* (pomegranate) peel extract on concanavalin A-induced autoimmune hepatitis in mice. Biomedicine & Pharmacotherapy, 2018 ; 100, 213–220.

[12] Luo L, Fang K, Dan X, Gu M. Crociname- liorates hepaticsteatosis through activation of AMPK signaling in db/dbmice. Luo et al. Lipids in Health and Disease, 2019 ;18:11.

[13] Banu B.B, Kalaivani K, Vijayakumar M. Moringa concanensis Nimmo extracts ameliorates hyperglycemia-mediated oxidative stress and upregulates PPARγ and GLUT4 gene expression in liver and pancreas of streptozotocin-nicotinamide induced diabetic rats. Biomedicine & Pharmacotherapy, 2019 ; 112 : 108688.

[14] Makena W, Hamman W.O, Buraimoh A.A, Dibal N.I, Obaje S.G. Therapeutic effects of balanitoside in streptozotocin-induced diabetic rats. 2018.

[15] Amir R, Borochov-Neori H, Tian L, Holland D. The biodiversity of different traits of pomegranate fruit peelsfrom a broad collection of diverse cultivars. Scientia Horticulturae, 2019 ; 246 : 842–848.

[16] Derakhshan Z, Ferrante M, Tadi F, Ansari F, Heydari A, Hosseini, Conti G.O, Sadrabad E.K. Antioxidantactivity and total phenolic content of ethanolic extract of pomegranate peels, juice and seeds. Food and Chemical Toxicology, 2018 ; 114 : 108–111.

[17] Aboonabi, A., Rahmat, A., Othman, F. Antioxidant effect of pomegranate against streptozotocin-nicotinamid generated oxidative stress induced diabetic rats. Toxicology Reports, 2014 ; Volume 1 : 915-922.

[18] Xu, Kevin Zhe-Yang. Zhu, Chenchen. Kim, Moon Sun. Yamahara, Johji. Li, Yuhao. Pomegranate flower ameliorates fatty liver in an animal model of type 2

diabetes and obesity. Journal of Ethnopharmacology2009 ; 123(2) : 280-287.

[19] Chakraborty M, Ahmedb M.G, Bhattachar jeec A. The potential for interaction of tolbutamide with pomegranate juice against diabetic induced complications in rats. Integrmedres 2017.

[20] Rouhi S Z T, Sarker M R, Rahmat A, Alkahtani S A. The effect of pomegranate fresh juice versus pomegranate seed powder on metabolic indices, lipid profile, inflammatory biomarkers, and the histopathology of pancreaticislets of Langerhans in streptozotocin-nicotinamide induced type 2 diabeticSprague–Dawley rats. Rouhi et al. BMC Complementary and Alternative Medicine, 2017 ;17:156.

[21] Shujaat, A. Hussain M.M. Effect of Pome- granate peel Alone and combination with Rosiglitazone on Hyperglycemia and Dys- lipidemia in type 2 DiabetesMellitus. PakArmed Forces Med J 2016 ; 66(4):469-74.