

# Antihyperglycemic and antioxidant effect of the total flavones of *Potentilla kleiniana* Wight et Arn. in streptozotocin induced diabetic rats

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**Abstract:** To investigate the antihyperglycemic and antioxidant activity of the total flavones of *Potentilla kleiniana* Wight et Arn. (TFP) in streptozotocin (STZ) induced diabetic rats. STZ-induced diabetic rats were treated with TFP weekly for 4 weeks at three doses (100 mg/kg, 200 mg/kg and 400 mg/kg). Blood glucose levels (BGL), body weight, insulin, total cholesterol (TC), triglyceride (TG), high density lipoprotein (HDL), very low density lipoprotein (VLDL), low density lipoprotein (LDL), malondialdehyde (MDA), glutathione (GSH), super oxide dismutase (SOD) and catalase (CAT) levels of liver and pancreas were measured weekly for 4 weeks. STZ administration resulted in oxidative damage of pancreas, then hyperglycemia proved by higher MDA, lower insulin and higher BGL in comparison to normal rats. TC, TG, LDL and VLDL cholesterol levels were also significantly elevated with decreased GSH, SOD, and CAT levels. A steady decrease in BGL and increase in insulin level were observed 4 weeks after TFP treatment in a dose dependent manner, as well as remarkable improvement in body weight and biochemical parameters. TFP have the effect of inhibiting hyperglycemia and oxidative stress, and the administration may be helpful in the prevention of diabetic complications associated with oxidative stress.

**Keywords:** *Potentilla kleiniana*; total flavones; blood glucose levels; insulin; total cholesterol.

## INTRODUCTION

Diabetes is the most important chronic metabolic disease and considered to be one of the five leading causes of death in the world (Zimmet, 1999). It is increasing rapidly in every part of the world, the prevalence of the disease will grow to 300 million people by 2025 (Pradeepa and Mohan, 2002). It is one of the most challenging disease facing health care professional today, putting great burden on society and public health sector. Diabetes is caused by an inherited or acquired deficiency of insulin secretion that results in an increased blood glucose level, which in turn produces adverse effects on different body systems (Chandra *et al.*, 2002). Plants have been used as an exemplary source of drugs, and many of the currently available drugs have been derived directly or indirectly from plants. Plant materials are considered to be the alternative sources, for discovering new leads for anti-diabetic agents. More than 400 species have been reported to display anti-diabetic effects, but few of them have been investigated scientifically (Bailey and Day, 1989).

*Potentilla kleiniana* (belongs to *Rosaceae* family) locates mainly as a wild plant in the mountain area in southern China with a great drug resource (Ling and Chen, 1985). The plant is claimed to have a wild range of biological activities, such as malaria, cough, anti-bacterial, hypoglycemic and anti-inflammatory (Ren *et al.*, 2010), and have been used as traditional herbal medicine against

a variety of diseases for hundreds of years. We have been identified the compounds of *Potentilla kleiniana*, such as flavonoid, polyphenols, triterpenes, and steroids (Tomczyk and Latté, 2009; Li *et al.*, 2011).

In order to further validate the beneficial effects of *Potentilla kleiniana* as an antidiabetic natural product, and better understand its action, we investigated the effect of the total flavones of *Potentilla kleiniana* (TFP) extraction in experimental diabetic animals. TFP was administered orally at three different doses (100 mg/kg, 200 mg/kg and 400 mg/kg) to different rat groups (normal groups, diabetic groups, gliclazide groups and TFP groups), with weekly blood glucose levels (BGL) and body weight measurement. Insulin level, total cholesterol (TC), triglyceride (TG) and high density lipoprotein (HDL) cholesterol were measured, as well as superoxide dismutase (SOD), catalase (CAT), malondialdehyde (MDA), glutathione (GSH) and the liver of hepatic glycogen.

## MATERIAL AND METHOD

### *Plant material*

The *Potentilla kleiniana* was locally collected in July 2011 from natural habitat (Hunan Province, China), and authenticated By Professor Xianjin Wu (Department of Bioscience, Huaihua University). The material was dried at 40-50°C for 72h and powdered (the grain size <0.5 mm).

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### **Determination and preparation of TFP**

The total flavones extract from *Potentilla kleiniana* was obtained by ultrasound-assisted extraction with 70% ethanol for 45 min, and then filtrated through filter paper. The filtrate was evaporated till dryness under reduced pressure and weighed. The extract was solved with hot water, added into column loading-treated D101 macro reticular resin (Sigma Chemical Company, St. Louis, MO, USA) for adsorption for 12 h, and then washed with 20% ethanol respectively for getting rid of impurity. TFP in the column was eluted with 60% ethanol. Eluting solution was evaporated till dryness under reduced pressure and weighed.

To calculate the total flavonoids content, a standard curve was firstly established as follows: A calibration rutin (Sigma) solution containing 200.8 $\mu$ g/mL rutin was prepared with 70% ethanol. Next, 0, 1, 2, 3, 4, 5 and 6mL of calibration rutin solution was respectively added to seven 25mL volumetric flasks, with 6mL of 70% ethanol and 1mL 5% NaNO<sub>2</sub>. Each flask was then shaken fully, and placed for 6 min, followed by the addition of 1mL 10% AlCl<sub>3</sub> solution. After fully mixture, and 6 min placement, 10mL 10% NaOH solution was added into each flask. Subsequently, every flask was diluted to the mark with 70% ethanol, shaken fully, mixed and then let rest for 15min. Absorbency (A) of the final solutions were measured at 500nm, with 70% ethanol as a blank control. Total flavones content extracted was calculated according to the regression equation by the standard curve.

### **Experimental animal**

Male Wistar rats aged one and a half month (220 $\pm$ 4.5g) were purchased from the animal house of Xiangya School of Medicine of Central South University. They were kept in departmental animal house in an environmentally control room (25 $\pm$ 2°C), with relative humidity 50 $\pm$ 5% and 12h day/night cycle. All the animals were free to standard laboratory feed and tap water before experiment. All the studies were performed in accordance with the guidelines for the care and use of laboratory animals, as adopted and promulgated by the Institutional Animal Care Committee.

### **Induction of diabetes**

To the overnight fasted rats, streptozocin (STZ; Sigma; 60 mg/kg body weight), dissolved in ice citrate buffer (0.1 M, pH 4.5), was administered intraperitoneally to introduce diabetes. Diabetes was confirmed by the determination of fasting BGL with a glucose meter (Roche) on the third day after administration. Rats with the blood glucose level of 16mmol/L or more were selected and used for further study. All the animals were allowed free access to water and pellet diet, and maintained at from temperature (25 $\pm$ 2°C) in plastic cages.

Animals were divided into six groups with 10 rats each. The six groups were treated with weekly for 4 weeks. Normal control group: rats were given vehicle (distilled water) orally. Diabetic control group: diabetic rats were given vehicle orally. Gliclazide group: gliclazide at 30 mg/kg were administrated orally. One week after STZ injection, the STZ-induced diabetic rats in TFP-1, TFP-2 and TFP-3 group were respectively administrated with TFP at the doses of 100, 200 and 400 mg/kg by oral gavage for four weeks. Body weights were measured once weekly.

Under anesthesia, bloods were collected from the retro-orbital plexus 0, 1, 2, 3 and 4 weeks after treatment to measure the BGL. On the 28th day, fasting blood samples were collected from tail vein of all the groups of rats. Liver and pancreatic tissues were dissected and washed immediately with ice-cold saline to remove blood of all groups of rats.

### **Biochemical determination**

Blood serum was separated by centrifugation (3500rpm  $\times$  10min, RT). Serum insulin levels were measured by using a radioimmunoassay kit (Nanjing Jiancheng Bioengineering Institute, P.R. China), TC, TG and HDL cholesterol were estimated by using respective diagnostic kits (Nanjing Jiancheng Bioengineering Institute, P.R. China). Very low-density lipoprotein (VLDL) cholesterol and low-density lipoprotein (LDL) cholesterol were calculated as per Friedevald's equation:

$$\text{VLDL cholesterol} = \text{TG}/5$$

$$\text{LDL cholesterol} = \text{TC} - \text{VLDL cholesterol} - \text{HDL cholesterol}$$

(Naik *et al.*, 2013).

### **Tissues analysis**

Liver glycogen was measured according to the anthrone-H<sub>2</sub>SO<sub>4</sub> method with glucose as standard (Wood and Bhat, 1988). Tissue homogenate supernatants of experimental rats were used for studying the antioxidant properties of TFP. For this, liver was removed and weighted. Tissues were then homogenized in 0.2M Tris-HCl. The homogenate was centrifuged at 10,000 rpm for 20min at 4°C. Then the supernatant was used for the determinations of total protein, SOD, CAT, MDA, and GSH, which were measured according to the operation manual of diagnostic kits, which were purchased from Nanjing Jiancheng Bioengineering Institute, P.R. China.

The determination of SOD activity was based on the reaction of xanthine with xanthine oxidase to produce super oxide radicals, and super oxide radicals reacted with hydroxylamine to generate nitrite. Then the absorbance was measured at 550 nm by visible spectrophotometer. The determination of MDA activity was based on the reaction of MDA with thiobarbituric acid (TBA) to generate the red mixture, which has a biggest absorbance

at 532 nm. The GSH activity was measured based on the reaction of dithiobisnitrobenzoic acid with hydroxyl compound to generate the yellow mixture, which was measured at 420 nm using colorimetric method. The CAT activity was detected based on the reaction of CAT with  $H_2O_2$  to reduce the content of  $H_2O_2$  in solution, which resulted in decline of the absorbance at 405 nm.

#### **Histopathological studies**

Liver and pancreatic tissues of TFP groups were studied for histopathological changes. The changes were compared with normal control, diabetic control and gliclazide groups. The tissues samples of liver and pancreatic were fixed in 10% formalin for at least 24 h, processed in graded series of alcohol, embedded in paraffin wax, and then cut into 5 $\mu$ m sections. The sections were then stained with haematoxylin and eosin, and photomicrographed with a microscope (IX 51, Olympus, Japan) to detect histopathological changes.

#### **STATISTICAL ANALYSES**

Results were expressed as mean  $\pm$  standard errors of means (SEM). Statistical analysis was performed using one-way analysis of variance (ANOVA), followed by TUKEY's post hoc multiple comparison test using SPSS (VERSION 16.0). The values of  $P < 0.05$  were considered as statistically significant.

#### **RESULTS**

##### ***Extraction and determination of TFP***

TFP was extracted from *Potentilla kleiniana* with 70% ethanol and purified with D101 macroreticular resin. Then, the extracts were dried under reduced pressure and weighed, with the final yield of 1.74%. Regression equation of standard curve for rutin was:  $A = 10.054C - 0.007$ ,  $r = 0.9998$ , with a better linear correlation between 10.86 and 64.35  $\mu$ g/mL. Quantitative analysis indicated that the content of total flavonoids was up to 73.5%.

##### ***Effects of TFP on body weight in STZ-induced diabetic rats***

The mean body weight of the diabetic rats after STZ treatment was lower than that of normal control rats (table 1). One week after STZ injection, body weight was 211.4 $\pm$ 4.6g in diabetic control rats compared with 234.6 $\pm$ 5.6g in normal control rats. Very prominent decreases were also seen at 4 weeks after STZ injection. The 100, 200 and 400 mg/kg supplementation of TFP caused a recovery in loss of body weight caused by STZ after TFP supplementation for 4 weeks, but the reverse effects were lower than that of gliclazide group.

##### ***Effects of TFP on BGL in STZ-induced diabetic rats***

The mean blood glucose of the diabetic rats after STZ treatment was significantly higher than that in normal

control rats (table 2). Diabetic control rats showed increase in BGL levels throughout whole experiment periods, and the BGL (29.6 $\pm$ 1. mmol/L) was 5.3-fold of that in normal control rats at 4 week (5.4 $\pm$ 0.5mmol/L). The blood glucose in the TFP groups lowered significantly, especially in TFP-2 and TFP-3 group. The results showed that TFP possesses remarkable hypoglycemic effect on hypoglycemia to STZ-induced diabetic rats.

##### ***Effects of TFP on insulin level in STZ-induced diabetic rats***

The onset of diabetic in rats resulted in a reduction in plasma insulin (by approximately 48% compared with that normal control rats) 4 weeks after STZ treatment. By treating with gliclazide and different doses of TFP, significant ( $P < 0.05$ ) difference was found in the insulin levels compared with the control group. The insulin levels in TFP groups were higher than that of the diabetic control groups, whereas the 400mg/kg body weight supplementation of TFP significantly increased the concentration of insulin by 65% (fig. 1 A).

##### ***Effects of TFP on liver glycogen in STZ-induced diabetic rats***

As shown in fig. 1B, the liver glycogen level of normal and diabetic control rats were 13.53 $\pm$ 2.3 and 2.6 $\pm$ 0.45 mmol/L. By treating with gliclazide and different doses of TFP, significant ( $P < 0.001$ ) difference was found in the levels of the liver glycogen. The liver glycogen in STZ-induced rats was significantly affected by TFP treatment ( $P < 0.001$ ) (fig. 1 B).

##### ***Effects of TFP on liver serum lipid profile in STZ-induced diabetic rats***

The normal group was a negative control and the diabetic group was a positive control in this study. The levels of serum TC, TG, HDL, LDL and VLDL of the diabetic rats had returned basically to the normal level with gliclazide treatment. With the TFP treatment for 4 weeks, the levels of serum TC, TG, LDL and VLDL of the diabetic rats had a significant ( $P < 0.001$ ) reduction and the level of serum HDL had a significant ( $P < 0.001$ ) increase (table 3).

##### ***Effects of TFP on liver antioxidant in STZ-induced diabetic rats***

In vivo antioxidant activities of TFP in liver are presented in table 4. The administration of TFP at 400 mg/kg was comparable to that of gliclazide ( $P > 0.05$ ). Both SOD and CAT played important roles in defense mechanisms against the harmful effects of reactive oxygen species (ROS) and free radicals in biological systems. GSH content was another important parameter that revealed oxidative damage in liver and MDA was oxidase product that is a hallmark of lipid peroxidation. The TFP treatment reduced elevated MDA ( $P < 0.01$ ) and increased SOD, CAT and GSH levels ( $P < 0.01$ ) back to their control levels, indicating that TFP may prevent the peroxidation

**Table 1:** Effect of TFP supplementation at 100, 200 and 400mg/kg body weight on body weight (g) of STZ-induced diabetic rats

Group	Dose (mg/kg)	Before STZ (g)	Initial (g)	1 Week (g)	2 Week (g)	3 Week (g)	4 Week (g)
Normal	—	224.6±5.6	227.4±8.1	234.6±5.6	239.2±6.4	246.1±3.8	247.4±5.8
Diabetic	—	223.5±4.8	218.3±15.8	211.4±4.6	208.4±7.8	196.6±4.6	184.8±9.3
gliclazide	30	217.4±9.5	206.5±12.7	204.4±8.4	211.9±2.4	218.4±5.7*	229.4±5.2**
TFP-1	100	220.5±11.4	212.6±12.8	210.8±11.2	214.7±1.3	214.3±6.8*	226.5±4.3**
TFP-2	200	221.7±6.4	209.7±11.4	204.7±6.7	211.5±7.5	219.7±5.2*	228.2±6.7**
TFP-3	400	218.5±6.7	216.4±9.5	209.9±4.8	218.2±5.8	223.8±4.2**	234.4±4.7**

\* $P < 0.05$ , \*\* $P < 0.01$  compared with the diabetic control group

**Table 2:** Effect of TFP on blood glucose changes in STZ-induced diabetic rats

Group	Dose (mg/kg)	Initial (mmol/l)	1 Week (mmol/l)	2 Week (mmol/l)	3 Week (mmol/l)	4 Week (mmol/l)
Normal	—	5.9±0.6	5.8±0.9	5.7±0.5	5.6±0.4	5.4±0.5
Diabetic	—	22.4±1.2	25.3±1.2	27.6±0.8	28.6±1.1	29.6±1.5
Gliclazide	30	25.1±0.6	19.5±0.5*	17.6±1.6**	15.6±1.3***	10.9±1.3***
TFP-1	100	26.5±0.5	22.6±0.3	18.6±1.8**	16.5±0.7***	14.6±1.5***
TFP-2	200	24.3±2.1	20.5±1.5*	18.6±1.6**	16.6±1.4***	13.6±1.5***
TFP-3	400	25.4±1.5	19.8±1.3*	17.8±1.2**	14.6±1.6***	11.3±2.4***

\* $P < 0.05$ , \*\* $P < 0.01$ , \*\*\* $P < 0.001$  compared with the diabetic control group

**Table 3:** Effects of TFP on liver serum lipid profile in STZ-induced diabetic rats

Parameters	Normal	Diabetic	gliclazide	TFP-1	TFP-2	TFP-3
TC (mg/dl)	85.6±1.2	223.7±1.6	90.5±2.1***	189.6±3.2*	153.4±3.7**	112.3±2.5***
TG (mg/dl)	76.5±2.3	169.8±1.8	86.5±1.4***	148.6±2.3	136.5±1.5*	98.9±1.8***
HDL (mg/dl)	46.5±1.6	23.6±2.1	43.5±2.5***	30.5±1.9*	38.6±2.7***	41.6±3.6***
LDL (mg/dl)	23.8±1.8	156.2±1.9	29.7±2.6***	129.4±2.3*	87.5±1.4***	50.8±2.5***
VLDL (mg/dl)	15.3±1.2	33.9±0.9	17.3±0.7***	29.7±1.1**	27.3±0.6**	19.9±1.4***

\* $P < 0.05$ , \*\* $P < 0.01$ , \*\*\* $P < 0.001$  compared with the diabetic group

**Table 4:** Effects of TFP on liver antioxidant in STZ-induced diabetic rats

Parameters	Normal	Diabetic	Gliclazide	TFP-100	TFP-200	TFP-400
SOD (U/mg protein)	252.3±18.6	112.5±11.2	232.1±8.9***	156.6±6.9*	187.1±11.6**	214.9±9.5***
CAT (U/mg protein)	82.3±1.6	45.6±2.6	74.9±3.5***	49.4±1.5	59.5±2.4*	68.4±1.8***
GSH (mg/g protein)	36.54±2.6	13.65±3.8	31.56±1.5***	18.62±1.7	21.68±2.1**	27.85±1.2***
MDA (nmol/mg protein)	1.24±0.09	2.08±0.09	1.65±0.12**	1.92±0.23	1.74±0.32*	1.68±0.15**

\*  $P < 0.05$ , \*\* $P < 0.01$ , \*\*\*  $P < 0.001$  compared with the diabetic group

of lipids in STZ-induced rats.

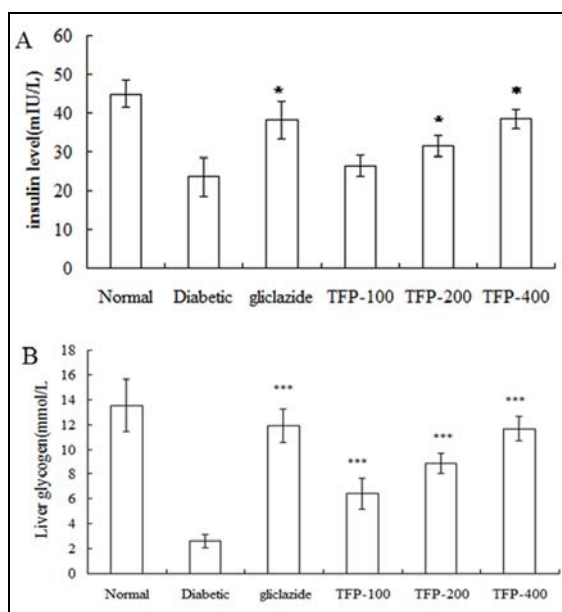
### Histopathological examination

Autopsy revealed remarkable damage in the liver and pancreas of persistently diabetic rats, and normal histological tissue details of normal control rats. Liver of diabetic rats revealed dilated sinusoids, atrophic hepatocytes and some necrotic regions (fig. 2). The TFP and gliclazide treatment brought back the cellular arrangement around the central vein and reduced necrosis. The alleviating response might be due to protective effect of the provided treatments.

Histopathological details of pancreas of STZ-induced diabetic rats showed damaged islets. Atrophic islet of langerhans was the most striking feature in these animals (fig. 3 b). No discernible islets were found in severely and persistently diabetic animals. However, in different dose of TFP (fig. 3 d-f) and gliclazide (fig. 3 c) group, the regenerative effect of the islets cells via exocrine cells of pancreas may restore the activity of islets of diabetic rats and enlighten the positive effect of these agent on the production of insulin (fig. 3).

## DISCUSSION

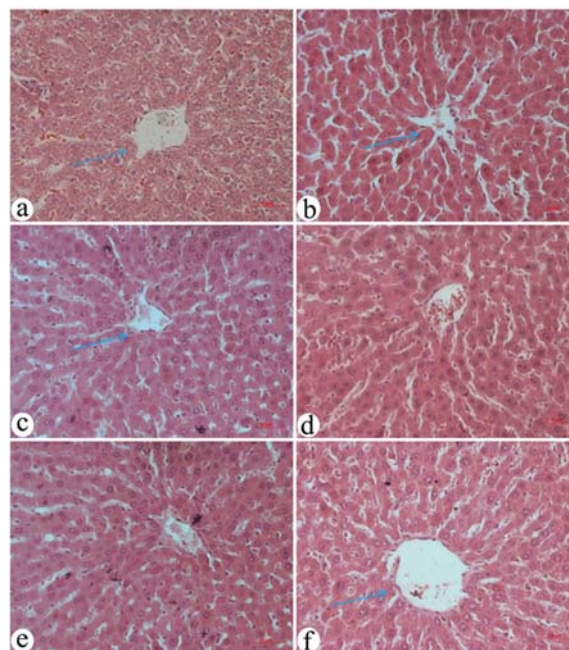
In recent years, traditional medicine and medicinal plants have been gradually used in most developing countries, as a normative basis for the maintenance of good health (Tiwari and Madhusudanarao, 2002). Some plants are found to play a vital role in the treatment of diabetes, used for a range of diabetic complications, and prescribed widely because of effectiveness and less side effects (Venkatesh *et al.*, 2003; Verma *et al.*, 2013).



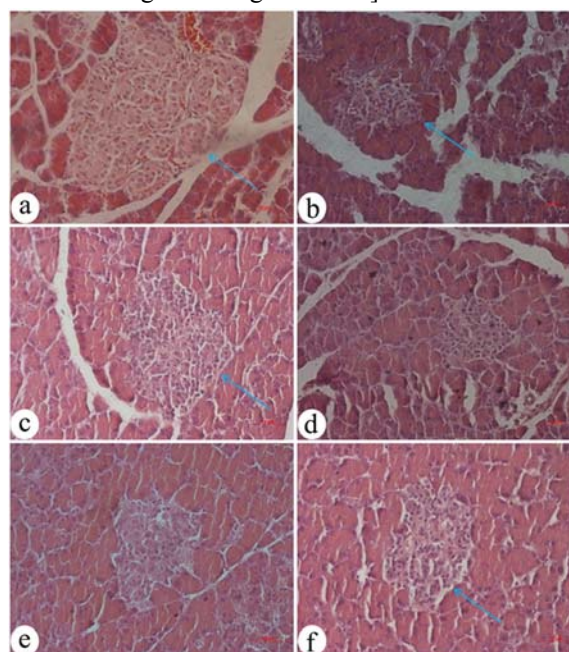
**Fig. 1:** Effect of TFP on insulin levels (A) and liver glycogen (B) in streptozotocin (STZ)-induced diabetic rats. \* $P < 0.05$ , \*\* $P < 0.01$ , \*\*\* $P < 0.001$  compared with the diabetic control group.

Diabetes mellitus is a worldwide problem, including a wide range of patients from those with insulin resistance and relative insulin deficiency, to those with a predominantly secretory defect with some insulin resistance (Home, 1998). The insulin deficiency or ineffectiveness results in increased concentrations of glucose in the blood, and finally in turn damage many of the body's systems, especially the blood vessels and nerves (Verma, Sarwa, 2013). Hyperglycemia, the primary bio-clinical manifestation in diabetic, is associated with the development of certain complications, producing oxygen radicals in the body and pancreatic injury, and could be responsible for increased blood glucose in animals. STZ destroys  $\beta$ -cell of the pancreas which is responsible for the insulin production from endocrine cells and induces hyperglycemia (Palmer *et al.*, 1998; Soltani *et al.*, 2011; Tappia *et al.*, 2013). Hence, STZ-induced hyperglycemia has been described as a useful experimental model to study the activity of hyperglycemic agents (Junod *et al.*, 1969). Gliclazide was commonly considered as the reference drug for the diabetic by stimulating regeneration of  $\beta$ -cell of the

pancreas. The survived animals treatment with gliclazide imply incomplete destruction of  $\beta$ -cell of diabetic animals (Verma, Sarwa, 2013). So the STZ-induced diabetic rat treatment with gliclazide can be used as a control in the study.



**Fig. 2:** Photomicrographs showing histological detail of liver (H&E, 40X) [a. normal liver; b. diabetic control liver; c. gliclazide treated Liver; d. TFP-100 treated liver; e. TFP-200 treated liver; f. TFP-400 treated liver; the arrows: histological changes of liver].



**Fig. 3:** Photomicrographs showing histological detail of pancreas (H&E, 40X) [a. normal pancreas; b. diabetic control liver pancreas; c. gliclazide treated pancreas; d. TFP-100 treated pancreas; e. TFP-200 treated pancreas; f.

TFP-400 treated pancreas; the arrows: histological changes of pancreas].

In the present study, changes in body weight of diabetic rats treatment with TFP at the dose of 100, 200, and 400 mg/kg was measured weekly for 4 weeks. Body weight of the diabetic control rats were significant decreased ( $P < 0.01$ ) compared with the normal controls, and reversed the declination with TFP treatment. The increased BGL in diabetic rats might be due to glycogenolysis or gluconeogenesis. However, treatment with TFP caused a significant reduction in fasting BGL with respect to diabetic control group at the end of 28 days experimental period, indicating that the TFP have the similar effect with gliclazide, as the reference drug, on BGL for the diabetic rats.

Some substances are shown antihyperglycemic effect by influencing  $\beta$ -cell to stimulate insulin secretion and restore insulin sensitivity (Lombardo and Chicco, 2006). Our study showed that significant difference was observed in insulin level between control group with gliclazide group as well as TFP group. Those results implied that the TFP may be increase insulin secretion from the  $\beta$ -cell of the islets of langerhans in diabetic rats because partly damaged  $\beta$ -cell of the islets of langerhans remains active (Soltani, Qiu, 2011; Shu *et al.*, 2012). Hence, the TFP may be attributed to enhanced peripheral utilization, or potentiated the insulin effect by regeneration  $\beta$ -cell of islets in pancreas in the antihyperglycemic action.

Liver glycogen reserves is important for whole body glucose homeostasis and is markedly low in the diabetic state (Hornbrook, 1970; Whitton and Hems, 1975). The decrease in liver glycogen may be caused by insufficient insulin and inactivation of glycogen synthetase in diabetic state (Ghosh and Suryawanshi, 2001). However, with TFP treatment, there was a significant increase in liver glycogen for diabetic rats. Based on this results, it is informed that the TFP treatment have a certain effect for the diabetic rats by the increase of the liver glycogen.

Besides altered carbohydrate metabolism, diabetes is also accompanied by disordered fat and lipid metabolism (Lewis *et al.*, 2002). Diabetes-induced dyslipidemia may be due to excess mobilization of fat from adipose tissue because of underutilization of glucose (Krishnakumar *et al.*, 2000). TFP treatment for 28 days not only decreased the elevation of TC and LDL cholesterol, but also significantly increased the HDL cholesterol level, indicating that TFP may help to increase transport of peripheral tissue cholesterol to liver and thereby decreased blood cholesterol level. These results were consistent with several earlier studies (Annamala and Augusti, 1980; El-Shenawy and Abdel-Nabi, 2006;

Mooradian *et al.*, 2006). The antidyslipidemic action of TFP might be due to inhibition of lipid peroxidation, since DM is associated with an increase in lipid peroxides and a decreased in antioxidant enzymes.

Numerous experimental and clinical observations have indicated that hyperglycemia may directly or indirectly contribute to an increased formation of free radicals and consequently to the onset of oxidative stress which has been implicated in diabetes associated complications (Kayamori and Igarashi, 1994; Mehta *et al.*, 2006). In diabetes, hypoinsulinemia causes lipid per oxidation which in turn impairs membrane function by decreasing membrane fluidity and changing the activity of membrane bound enzyme and receptors, and resultant products which are harmful to the cells in the body (Saha *et al.*, 2008). Oxidative stress is a condition of reduction, such as SOD, GSH, and CAT, which playing a key role in the pathogenesis of diabetes mellitus. MDA was oxidase product that is a hallmark of lipid per oxidation (Gultekin *et al.*, 2000; Irmak *et al.*, 2002). In our study, the level of SOD, GSH, CAT and MDA of the diabetic rats back basically to their normal levels with the TFP treatment for 28 days. In our study, histopathological report showed that the cells in islets of STZ-induced diabetic rats were destroyed and atrophic, which was similar with the study of Soleimani *et al.* (Soleimani *et al.*, 2007). With the TFP and gliclazide treatment, the islet of langerhans was no more atrophic. Inhibition of these histomorphological changes of insulin cells by TFP found to protect from the destruction of these cells by STZ. For those results, we known that the TFP therapy is beneficial for the diabetic rats compared with the gliclazide treatment. The STZ-induced diabetic rats are useful model to study the diabetic for human. Hence we concluded that the TFP treatment may be useful for the diabetic patients by the antioxidant therapy.

But there are some limitations in our study. The glycemia and plasma lipids were significantly affected in the low dose group, while the insulin level and antioxidant capacity were not obviously improved. The reasons for the discrepancy might be due to that the flavonoid compounds have direct effects on insulin sensitivity. However, the effects of flavonoid compounds on insulin stimulated signaling markers were not investigated in this present study. Moreover, the effects of the flavonoid extract on hepatic mRNA expression of gluconeogenic markers were not studied. In our future study, these issues will be investigated to better understand the effects of flavonoid extract on diabetes.

## CONCLUSIONS

In conclusion, the present study demonstrated that the TFP possess significant antiperglycemic and antioxidant

potential. The present investigation has also opened an avenue for further research especially with reference to the development of potent formulation for diabetic mellitus from TFP of *Potentilla kleiniana*.

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