Piper sarmentosum Maintains Blood Pressure and Morphological Integrity of Liver in Type 1 Diabetic Rats

Zar Chi Thent and Srijit Das
Department of Anatomy, Faculty of Medicine, Universiti Kebangsaan Malaysia, Bandar Tun Razak, 56000 Cheras, Kuala Lumpur
Email: zarrchii@gmail.com

***Abstract*—*Piper sarmentosum* (*P.s*) is known to possess anti-diabetic property and reduce the complications in diabetes mellitus (DM). In the present study, the effect of *P.s* extract on the blood pressure and liver tissue of experimental diabetic rats was determined. A total of 24 male Sprague-Dawley rats were used. Streptozotocin (STZ) was used for the induction of DM. Following, three days of STZ induction, the animals were equally divided (*n*=6) into; Ctrl: control, Ctrl+*P.s*: control with 0.125 g/kg of *P.s*, DM: untreated diabetic and DM+*P.s*: diabetic with 0.125 g/kg of *P.s*. The treatment with aqueous extract of *P.s* was continued for 28 days. At the end of the study, significantly decreased blood pressure and the morphological disturbances in liver tissue was reverted to normal in the DM+*P.s* group compared to the DM group. It is believed that *P.s* is an effective supplement for improving the organ damage in DM.**

***Index Terms*—*Piper sarmentosum*, Diabetes mellitus, Liver tissue, Blood pressure.**

I. Introduction

Chronic hyperglycaemia, a characteristic feature of diabetes mellitus, tends to cause morphological alterations in many organs, including the liver, an organ which involved in detoxification of the organism [1], [2]. Diabetes mellitus (DM) is associated with an increase in the amount of amino acids in plasma and liver of both rats and humans [3]. Some researchers explained that in the hypoglycaemic condition, diabetic pathogenesis results from increase advanced glycation end products (AGEs) in organs. The AGEs lead to cause free radical overproduction that adds towards the cell damage in DM. The interaction between AGEs and free radical release mediate the tissue and organ lesions [4]. The above factors could be related to the oxidative stress.

Oxidative stress is implicated in the development of morphological alterations in diabetes mellitus, thus anti-oxidative treatment is believed to improve or prevent cellular damage in many organs. Some studies have reported the protective effects of different antioxidants in many organs [5], [6]. It had been already documented that antioxidant rich herbal medicines showed a positive effect on the metabolic disorder like DM [7]. In the present study, piper sarmentosum (*P.s*) was used as an herb which has a protective role in DM and its related complications.

P.s, (Piperacae), herb well known in South East Asian countries like Thailand, Malaysia, Cambodia and Myanmar. Studies have proved that *P.s* is enriched with several properties like antioxidant, anti-diabetic and anti-atherosclerotic properties [8], [9].

However, the effect of *P.s* towards blood pressure changes and morphological changes in liver tissue of STZ-induced diabetic rats were not observed till date. Therefore, the purpose of this study was to observe the effect of *P.s* towards the changes in systolic blood pressure by using non-invasive tail cuff method and the morphological alterations of liver tissue under light microscopes with different histological staining techniques in the control and the experimental diabetic animals.

Figure 1. Photograph of *Piper sarmentosum* leaves

II. Materials and Methodology

A. Plant Materials and Extraction

P.s leaves (5kg) were obtained from Mentah Resources, Malaysia and was identified with a voucher specimen (No.29851). The *P.s* leaves were washed and oven dried at

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50 ℃ then grounded. The *P.s* powder was mixed with 1L of water and boiled for 1 hour. Then it was undergone filtration process and sent to FRIM (Forest Research Institute Malaysia) to obtain the aqueous extract powder form. The freeze-dried powder extract was kept in 4 ℃, until used. The *P.s* powder was suspended in the normal saline. Oral administration dose of 0.125g/kg body weight was used in the present study [10].

B. Experimental Animals

Male Sprague-Dawley rats weighing 200-250 g were obtained from Animal house of Universiti Kebangsaan Malaysia. All the rats were housed in individual cages and with standard condition. The animals received water ad libitum and food pallet throughout the study period. All the rats were acclimatized for 1 week before the commencement of the study. The experiment was carried out with the prior approval of the Institutional Animal Ethics Committee.

C. Induction of Diabetes

The animals were fasted for overnight and diabetes was induced by single intramuscular injection of streptozotocin (50 mg/kg body weight) (Sigma, Germany) [11]. Three days following injection of streptozotocin (STZ), fasting blood glucose was determined and the rats with a blood glucose level > 8 mmol/L were used for the study [11]. The treatment with *P.s* extract was started in the 4th week after STZ injection.

D. Experimental Design

In the experiment, 24 male Sprague-Dawley rats were used. Animals were equally divided into four groups (n=6). Ctrl: control, Ctrl+*P.s*: control with 0.125 g/kg of *P.s*, DM: untreated diabetic and DM+*P.s*: diabetic with 0.125 g/kg of *P.s*. *P.s* extract was given once daily using an intragastric tube for 28 days in both Ctrl+*P.s* and DM+*P.s* groups.

E. Measurement of Blood Pressure

The individual rats were exposed to sunlight before the experiment. Then, the systolic blood pressure was measured at baseline, pre and post treatment (i.e. 0, 4 and 8 weeks) periods by using non-invasive tail cuff method (plethysmograph) with Powerlab Diagnostic system (USA). All the measurements were recorded and compared between the groups [12].

F. Histological Studies

For the histological study, the collected liver specimens from all the groups were weighed. The specimens were fixed under 10% formaldehyde. The tissues were dehydrated with a graded series of alcohol and cleared using xylene and sectioned using a microtome with 5µm thin sections. Then, the specimens were stained with different staining like Haematoxylin and Eosin (H&E) and Periodic Acid-Sciff staining (PAS) staining to observe the morphological hepatocellular changes. All the slides were viewed under a magnification of ×200 using Pix-elink colour camera (USA) with computerized image analysis system Video Test T-morphology 5.1 software with a light microscope (LEICA DMRXA2, Germany).

G. Statistical Analysis

The data were analysed by using ANOVA followed by a Bonferroni test by using SPSS statistical version 21 (SPSS Inc., USA). Continuous variables were shown as mean ± SD and a value of $P < 0.05$ was considered significant.

III. Results

A. Effect of P.s Extract on Blood Pressure

At the beginning of the study, all the groups showed a similar plateau blood pressure level. At the 4th week following STZ induction, experimental diabetic groups were observed to increase in the blood pressure level. At the end of the study (i.e. on 28th day after *P.s* treatment), DM+*P.s* group showed a significant decrease (P<0.05) in blood pressure level compared to DM group. However, there was no significant difference observed between control groups of rats (Fig. 2).

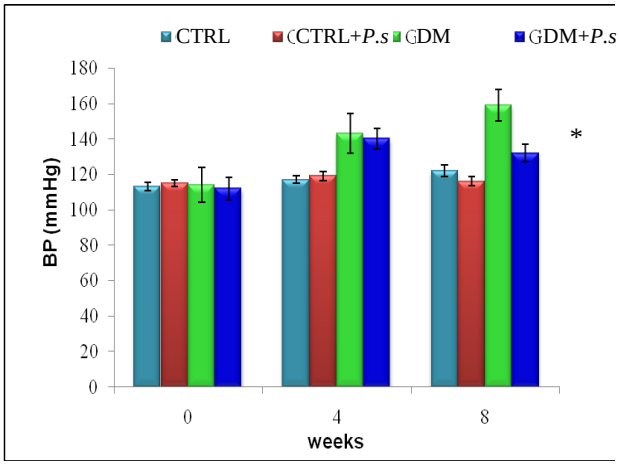

Figure 2. Effect of *P.s* extract on blood pressure (mmHg) in type I diabetic rat model. *indicates P<0.05 significant difference between DM and DM+*P.s* group.

B. Effect of P.s Extract on the Liver Weight

At the end of the study, the whole liver tissues were collected from all groups of rats and weighed. DM group was observed to decrease in the liver weight. DM+*P.s* group showed a significant increase (P < 0.05) in the weight of liver and its ratio with regard to the body weight (Table I).

C. Morphological Changes in the Liver Tissue

Under the light microscope, liver tissues from all the groups were observed by using different staining method (H&E and PAS). There were no morphological changes observed between Ctrl and Ctrl+*P.s* groups (Fig. 3a, Fig. 3b, Fig. 4a, Fig. 4b). Heptocytes were arranged normally, no necrosis or damages were found between these groups. However, the hepatocytes from the liver tissue of the DM group appeared to be larger, and more granular. The nuclei of the hepatocytes were deformed. Furthermore, the dilated

blood sinusoids with red blood cells and congested area were also observed in the DM group (Fig. 3c). It was also found out that there was the area of focal necrosis and vacuolisation with collagen deposits (Fig. 4c) present in the DM group.

The degenerative changes in liver tissues were found to be less in the diabetic group treated with *P.s* extract (DM+*P.s* group). The histological features of hepatocytes seemed to revert to the normal state. The nuclei of hepatocytes showed no deformation and there were less hyperaemic areas found in the sinusoids (Fig. 3d). In addition, the necrosis and vacuolisation were shown to be improved in this group treated with *P.s* extract (Fig. 4d). However, not all the histological damages in the liver tissue of DM+*P.s* group are reverted back to normal. But, it can be observed that the morphological damages of liver tissues were being recovered by treating with *P.s* extract.

TABLE I. EFFECT OF *P.S* EXTRACT ON THE WEIGHT OF THE LIVER TISSUE IN TYPE I DIABETIC RAT MODEL

	Ctrl group	**Ctrl+*P.s* group**	**DM group**	**DM + *P.s* group**
Liver weight (g)	11.05 ± 0.28	12.32 ± 0.22	6.03 ± 0.39	10.23 ±0.27*
Liver weight/100 g body weight	4.26 ± 0.11	4.89 ± 0.19	2.74 ± 0.21	3.96 0.17*

*indicates $P<0.05$ significant difference between DM and DM+*P.s* group.

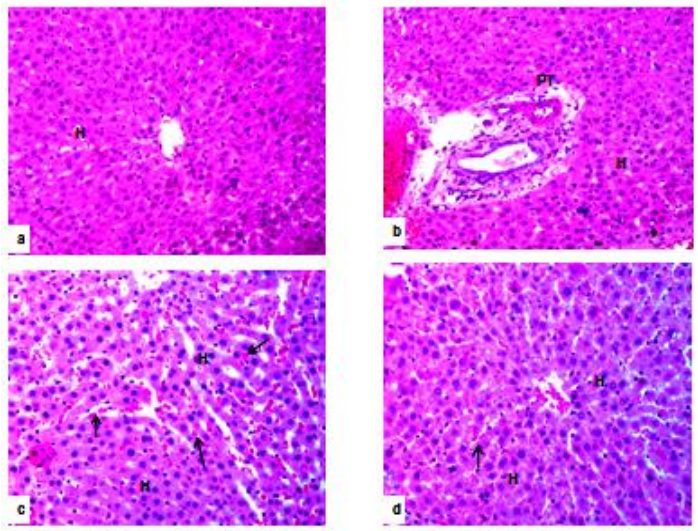

Figure 3. Photomicrograph of liver tissue under H&E staining (×200). a) Ctrl group b) Ctrl+*P.s* group c). DM group d) DM+*P.s* group. H = hepatocytes, dilated sinusoids with red blood cells are shown with arrows.

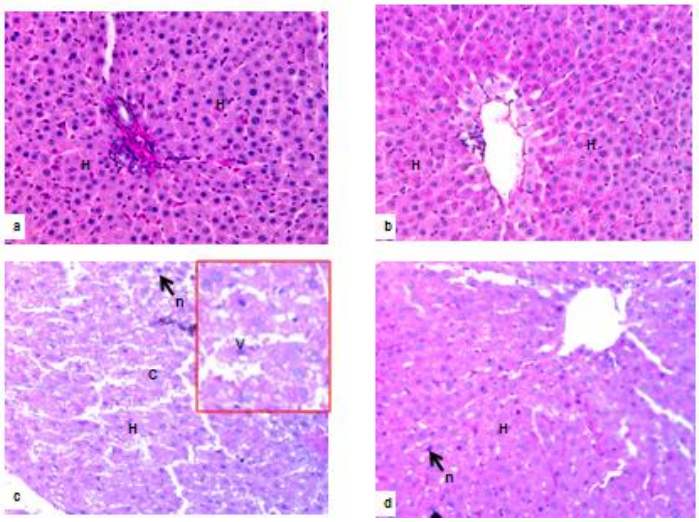

Figure 4. Photomicrograph of liver tissue under H&E staining (×200). a) Ctrl group b) Ctrl+*P.s* group c). DM group d) DM+*P.s* group. H = hepatocytes,, C = collagen deposits, V = vacuolisation, n = necrotic cells, G = glycogen deposits.

IV. Discussion

In the present study, the physiological changes in blood pressure, the weight of the liver tissues and its morphological changes were observed. These changes are commonly seen as in DM related complications. The blood pressure level in the experimental groups was found to be significantly increased ($P<0.05$) compared to the control groups. Similar finding was observed in previous studies which showed increased blood pressure and disruption of endothelial function in aorta of STZ-induced DM rats [13]. Furthermore, in the year 2007, the researchers observed that there was a significant rise in blood pressure and lipid profile in type 1 DM rats [14].

Regarding the hypertension or increase in blood pressure in DM, several mechanisms were suggested. During recent years, some of the investigators described that hypertension to be one of the vascular complications in DM [15]. According to the past researches, AGEs accumulated in the state of chronic hyperglycaemia have also influenced in elevating the blood pressure [16]. On the other hand, it was postulated that increase breakdown of nitric oxide (NO) could lead to increase blood pressure in DM [17].

However, the treatment with 0.125g/kg of *P.s* extract showed decrease blood pressure in DM+*P.s* group. From this finding, it is highlighted that *P.s* could reduce the NO damage or destruction observed in DM. This is similar to the previous report, which showed that *P.s* improved the endothelial dysfunction by enhancing NO synthesis in human umbilical vein endothelial cells [18]. The aqueous extract of *P.s* has been shown to protect nitrogen peroxide from oxidative destruction by hydrogen peroxidation [19]. From our findings, oral supplementation with *P.s* extract for 28 days could reduce hypertension in STZ-induced DM rats were well noticed. It may be due to the presence of anti-oxidant compounds in *P.s* extract, especially narigenin [20], [21].

Regarding the morphological changes in the liver tissues, it has been reported that the hepatocytes of STZ-induced diabetic rats showed cytoplasmic alterations. The effects of DM on hepatic structure include hypertrophy and autophagic vacuoles in hepatocytes. The nuclei of the hepatocytes were generally enlarged with irregular shapes and sizes [22].

In the present study, the hepatocytes in the Ctrl group were similar to those in the Ctrl+*P.s* group, showing the normal histological features of the liver tissue. However, in the DM group showed hepatocellular hypertrophy, disorganized hepatocytes with dilated blood sinusoids. In addition, microvesicular fattening (vacuoles), focal necrosis and collagen deposits were also observed in the DM group under PAS stain. The above disarrangement and disruption of liver tissue occurred in the diabetic untreated rats may be due to the oxidative damage which encountered in DM [11]. The oxidative stress and lipid peroxidation may cause the morphological disruption in the liver tissues of DM rats [23].

However, a decrease in degenerative changes with *P.s* treatment was noted in DM+*P.s* group rats. Treatment with *P.s* extract in DM rats proved to have normal hepatocytes, less dilated sinusoids and vacuolization. It is well noticed that *P.s* extract could reduce the histological changes encountered in DM complication. *P.s* is known to possess anti-oxidant property and enriched with anti-diabetic property [24]. Therefore, it was believed that by reducing the hyperglycaemic conditions, *P.s* could modify the cell structure against damages. It is also agreed with our previous study which proved that *P.s* can be used as a supplement to maintain the histological integrity in both cardiac tissue and proximal aorta of STZ-induced diabetic rats [25]. Eventually, with the presence of antioxidant property, *P.s* extract can be effective at protecting liver tissue from the damage that occurs in STZ-induced diabetic rats.

V. Conclusion

DM leads to develop physiological deterioration such as increasing blood pressure and morphological disruption in the organs including the liver. Based on the present findings, it is concluded that repeated oral administration with aqueous extract of *P.s* for 28 days decreased the blood pressure and maintained the morphological architectural in the liver tissue of STZ-induced diabetic rats. Eventually, aqueous extract of *P.s* showed a positive impact on DM and its related complications.

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Zar Chi Thent was born in Yangon, on 13th November 1984. She obtained MBBS degree from the University of Medicine (2) Yangon, 2008. She did her internship at North Okalapa General Hospital, Yangon. She obtained MMedSc (Anatomy) in the year 2012. At present, she works as a Medical Lecturer in Department of Anatomy, Universiti Kebangsaan Malaysia (UKM). Her mai n research area includes the natural products, diabetes mellitus and cardiovascular complications.

Srijit Das was born in India, on 27th February, 1967. He obtained his MBBS degree in 1992 (from Sambalpur University, India) and MS (Anatomy) degree in 1997 (from Utkal University, India). He is a Professor of Anatomy in Faculty of Medicine, Universiti Kebangsaan Malaysia. His main research area includes clinical anatomy, dia betes mellitus, atherosclerosis, antioxidants and herbal products.