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Effect of *Eugenia aromatica* and *Archidendron jiringa* on oxidative stress marker in type 1 diabetes rats

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Abstract
This study was conducted to assess two herbs namely *Eugenia aromatica* (clove) and *Archidendron jiringa* on malondealdehyde (MDA) level in streptozotocin-induced diabetic rats. A total of 56 male *Sprague Dawley* rats weighing 150 – 200 g were divided into seven groups. The experiment was carried out over 6 weeks. Blood glucose level, body weight and MDA activity were measured every 3 weeks. Both herbs were able to reduce the oxidative stress of diabetic rats. MDA level decrease significantly (p <0.05) in diabetic group compared to the control at the end of the study. Blood glucose level of both normal and diabetic rats was insignificantly affected by the studied herbs. Further study on effect of these herbs on different oxidative stress marker is needed to support the present finding.

Key words: diabetes, malondialdehyde, herbs, oxidative stress, antioxidants

Introduction

Diabetes is a disease in which the body does not produce or properly use insulin. Insulin is a hormone made in the pancreas. It is needed to convert sugar, starches and other food into energy. According to World Health Organization (WHO), diabetes patients in Malaysia will increase from 942,000 in 2000 to more than 2 million people in 2030.

In modern medicine, several types of clinical treatment are used to treat patients with diabetes. Some examples are insulin therapy, prescription of sulfonylureas, biguanide and thiazolinediones. The use of biologic active components and plants is recommended to decrease the number of diabetes complications and to postpone their development.

Malaysia is rich in natural resources that can be used for medicine. It was reported that at least 15% of these plants had been claimed by various people to have medical usage (Latif et al. 1984). Each plant and herb species has its own role function and uses. Natural herbs have been used by people to treat a wide variety of diseases and conditions. These herbs are called herbal medicine, which are composed of roots, barks, flowers, seeds, fruits, leaves and branches. There are also found to have therapeutic properties.

A number of plants have constituents that have antidiabetic properties when taken orally (Oliver and Zahnd 1979) which include *Eugenia aromatica* and *Archidendron jiringa*. World Health Organization (WHO) suggested investigating traditional methods of treating diabetes (WHO 1980). The objective of this study was to determine the effect of *E. aromatica*

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and *A. jiringa* on MDA level in type 1 diabetes rats.

Materials and methods

The cloves of *E. aromatica* (*cengkih*) and seeds of *A. jiringa* (*jering*) were obtained from Nusantara Herbs Sdn. Bhd. in Bandar Baru Bangi. The used parts were air-dried in an oven for 24 h with temperature range of 40 – 45 °C and then ground into fine powder using blender (Panasonic, Japan).

For sample treatment, 900 g of blended commercial feed was weighed (Vibra weighing balance) and 50 g of *E. aromatica* or 50 g of *A. jiringa* was added into the feed bit by bit and mixed until even. Then, 50 g of starch that had been cooked with 250 ml of water was added slowly into the herb mixture and blended commercial feed. The mixture was placed on a tray, which had been covered with aluminium foil. The mixture was pressed with hand to an even height (~1 cm). Water (around 200 ml) was added bit by bit until the mixture could be shaped. The mixture was pressed until the surface was smooth and cut into cube (~2 cm^2). Then, it was dried in an oven at 60 °C for 2 days.

For normal treatment, the process was the same but the formulation contained only commercial pellet (950 g), 50 g starch and 250 ml water. As for the glibenclamide treatment, the herbs were replaced by glibenclamide (6.72 mg). The process was still the same as the above. Glibenclamide is a medicine that is used to treat diabetes.

Male *Sprague Dawley* white rats of body weight 150 – 200 g and aged 6 – 8 weeks were obtained from Faculty of Veterinary Medicine, Universiti Putra Malaysia. The animals were acclimatised to laboratory environment in animal house for 1 week on standard pellet diet and water *ad libitum*.

There are various clinical methods to induce diabetes such as alloxan and streptozotocin. In this experiment, a freshly prepared solution of streptozotocin (50 mg/kg) in 0.9% normal saline was injected intrapertoneally. Streptozotocin can cause diabetes in experimental animals by specifically destroying the ß-cells of the islet of Langerhans in the pancreas. After 48 h of administration, rats with moderate diabetes (i.e. with blood glucose level more than 7.2 mmol/litre) were selected for experiment. Diabetes was confirmed by measuring the blood glucose level using glucometer kit.

A total of 56 rats (24 normal rats, 32 diabetic rats) were used. The rats were divided into seven groups of eight rats each.

Group A Normal rats given normal feed and water (control group)
Group B Normal rats given feed with *E. aromatica* and water
Group C Normal rats given feed with *A. jiringa* and water
Group D Diabetic rats given normal feed and water (+ve control)
Group E Diabetic rats given feed with *E. aromatica* and water
Group F Diabetic rats given feed with *A. jiringa* and water
Group G Diabetic rats given feed with glibenclamide and water (-ve control)

The rats in each group were given the assigned diet and water once a day (around 10 am). The feed was given at the same time everyday. This experiment was carried out for 9 weeks.

The blood glucose levels of the experimental animals were determined via tail vein using glucometer kit every 3 weeks intervals. The body weights of the experimental animals were also monitored every 3 weeks intervals. Thus, the reading of blood glucose level and body weight were noted in 3 weeks intervals too.

For blood sampling, 1 ml of blood was collected into heparinised tubes every 3 weeks until 6 weeks by intracardiac puncture from rats that were previously anesthetised with diethyl ether.

For plasma preparation, the red blood cells and plasma were then separated and

kept at –20 °C until further analysis. The blood was centrifuged at 3,000 rpm for 10 min (4 °C) to obtain plasma, which was stored at –20 °C. Plasma was used in measurement of MDA level.

Biochemical analysis

Lipid peroxidation was assessed by measuring the concentration of malondialdehyde (MDA) in plasma. The concentration of MDA was measured by thiobarbituric acid reactive substances (TBARS) by the method of Okhawa et al. (1979) with a slight modification.

A mixture was obtained by adding 2.4 ml 1/12 H_2SO_4 and 0.3 ml of 10% Na_2WO_4 to 0.3 ml of plasma and centrifuged (3,500 rpm, 10 min) after being kept at room temperature for 10 min. Then, the supernatant was thoroughly removed by tapping tubes upside-down on several layers of absorbent paper. The reactive mixture was formed by adding 0.5 ml of distilled water, 3.0 ml of 0.05 N HCl and 1.0 ml of 1.0% thiobarbituric acid (TBA), and make up to 5 ml with distilled water.

The well-mixed mixture was then kept in water bath at 95 °C for 60 min within centrifuge tubes covered with glass marble. After cooling, the mixture was again centrifuged, and the supernatant layer was retrieved and its absorbancy was measured at 532 nm.

For the standard control, 0.5 ml of distilled water, 3.0 ml of 0.05 N HCl and 1.0 ml of 1% TBA were added to 0.3 ml of 10 nmol/litre TEP. The absorbency was measured following heating as described earlier. The concentration of MDA was expressed as nmol/ml of plasma.

Statistical analysis

The data were statistically analysed using Analysis of Variance (ANOVA) and Duncan Multiple Range Test (DMRT) using the SPSS, Statistical Computing Program. Only p values of less than 0.05 ($p < 0.05$) were considered significant.

Results and discussion

Blood glucose level

In human, the normal range of blood glucose level is between 4.0 and 6.0 mmol/litre during fasting and after food the value is less than 8.0 mmol/litre (National Diabetes Institute Malaysia). From the result (*Table 1*), blood glucose levels of diabetic rats in groups D, E, F and G were beyond the normal range. Week 0, was the initial stage of the research where the rats were first induced with streptozotocin to obtain the diabetic rats (*Table 1*).

At the 3rd week, there was no significant difference ($p > 0.05$) of blood glucose level between normal groups (A, B, C) and diabetic groups (D, E, F, G) except for group A (normal rats given normal feed), with group D (diabetic rats given normal feed), where it was observed that it was significantly different ($p < 0.05$). This result indicates that the blood glucose level of group D was significantly higher than group A.

Each rat has different immune system. Rats that are being induced with streptozotocin will cause a destruction of pancreatic β-cell. The cytotoxic action of these diabetogenic agents is mediated by reactive oxygen species (Szkudelski 2001), which affect more rats with low immune system and cause immediate high blood glucose level. This happened in group D rats (chronic diabetic group) which had a high blood glucose level since the first week of the experiment (11.5 mmol/litre), compared to group A, the normal group.

Rats from normal groups (A, B and C) were significantly different ($p < 0.05$) from diabetic groups (D, E, F and G) on week 6, which indicate at this week the range of blood glucose level for normal rats was totally different from diabetic groups, which already exceed the normal range. From week 0 to week 6, there was insignificant different ($p > 0.05$) for each group.

From this research, there was no significant difference ($p > 0.05$) in blood glucose level. This indicates that the

treatment that was used is not significantly affected or reduced blood glucose level of rats. Extending period of research is needed.

Body weight

In general, there was no significant increment (p >0.05) throughout the 6 weeks study (*Table 1*). From 0 week to 3rd week, there was no significant increased (p >0.05) in body weight for all groups. For groups A, C, E, F and G except group D, there was significant increased in body weight (p <0.05) from week 0 to week 6. On the other hand, treated diabetic rats gained significantly more weight increment than diabetic controls but the increased remain lesser than the normal controls. From week 6, there was no significant difference (p >0.05) in the body weights of rats among the experimental groups for each week.

From the observation throughout 6 weeks study, feed with *A. jiringa* was the most consumed by either normal rat group or diabetic rat groups. This might be due to the composition of *A. jiringa*, that contained fat which makes it quite delicious to rats compared to other feeds.

During the 6 weeks observation, physical and physiology condition of rats was also important in either the increment or decrement of body weight. Some of the rats, either in normal group or diabetic groups, had decreased body weight due to fighting with each other. The rats got hurt and this affected the food intake. Longer duration of research and proper management of animals were needed to obtain clearer and reproducible results.

Malondealdehyde (MDA) level

The MDA level showed a decreasing trend (*Table 1*). Normal groups (A, B, C) for different treatment showed a decreasing trend from week 0 until week 6. However, consistent significant decreased (p <0.05) was only observed in rats with normal feed. This indicates that normal rats treated with *E. aromatica* and *A. jiringa* insignificantly reduced MDA level.

For all diabetic groups, the MDA level increased from week 0 to week 3. The ever-increasing level of MDA level in diabetic groups is due to immature defence mechanism. After induction with streptozotocin, it will cause diabetes which also cause an increase in free radicals and pro-oxidant as well as lipid peroxidation (Szkudelski 2001). Therefore, their defence system is unable to inhibit peroxidation of PUFA that continuously accumulates in the body.

However the increasing of MDA level from week 0 to week 3 was only significant (p <0.05) in group F (diabetic rats given *A. jiringa*). This may be due to the component in *A. jiringa* that has unusual amino acid-djenkolic acid (S,S' methylenebiscysteine) which may form sharp crystals in the urinary tract, causing pain and can be toxic if taken excessively (Radhiah et al. 2011). Those properties may enhance the increment of MDA level. Significant decreased (p <0.05) in MDA level for group E (diabetic rats with *E. aromatica*, F (diabetic rats with *A. jiringa*) and G (diabetic rats with glibenclamide) from week 3 to week 6 showed that diabetic groups with treatment gave the significant effect towards reducing the oxidative stress.

The antioxidant properties of *E. aromatica* and *A. jiringa* are probably mediated by reducing free radical formation, thus decreasing the lipid peroxidation. The *A. jiringa* seeds contain fibre and alkaloid substance that is claimed to be antidiabetic (Muhamad 1992; Muhamad Sani 1995). The mixture of active component of *E. aromatica* especially eugenol probably reduces the accumulation of MDA level thus decreases oxidative stress in diabetic rats. One of the treatments that is usually used to treat diabetes nowadays is glibenclamide.

Significant decreased (p <0.05) was observed on diabetic group with glibenclamide treatment (*Table 1*). The mechanism probably mediated either by improving insulin secretary capacity of

Table 1. Mean blood glucose level, body weight and MDA level of rats

Group	Duration in treatment		
	0 week	3rd week	6th week
Mean blood glucose level of rats (mmol/litre)			
A (Normal + Normal)	3.9 ± 2.3bc	3.5 ± 1.3b	3.8 ± 1.3b
	A	A	A
B (Normal + *E. aromatica*)	4.6 ± 1.6bc	4.5 ± 0.8ab	4.5 ± 1.6b
	A	A	A
C (Normal + *A. jiringa*)	3.6 ± 0.9c	4.4 ± 0.6ab	3.5 ± 1.0b
	A	A	A
D (Diabetic + Normal)	11.5 ± 3.3ab	9.8 ± 8.5a	7.4 ± 1.1a
	A	A	A
E (Diabetic + *E. aromatica*)	8.6 ± 6.3abc	7.9 ± 4.2ab	8.5 ± 2.8a
	AB	A	AB
F (Diabetic + *A. jiringa*)	15.9 ± 11.1a	8.2 ± 3.0ab	7.7 ± 2.5a
	A	A	A
G (Diabetic + Glibenclamide)	10.5 ± 4.6abc	8.1 ± 5.6ab	7.3 ± 1.9a
	A	A	A
Mean blood glucose level of rats (mmol/litre)			
A (Normal + Normal)	263 ± 29a	298 ± 41a	333 ± 38ab
	B	AB	A
B (Normal + *E. aromatica*))	291 ± 51a	324 ± 56a	286 ± 67b
	A	A	A
C (Normal + *A. jiringa*)	294 ± 22a	323 ± 60a	389 ± 22a
	B	B	A
D (Diabetic + Normal)	278 ± 55a	321 ± 76a	361 ± 72a
	A	A	A
E (Diabetic + *E. aromatica*)	258 ± 95a	296 ± 38a	329 ± 44ab
	B	AB	A
F (Diabetic + *A. jiringa*)	266 ± 45a	298 ± 49a	333 ± 34ab
	C	BC	AB
G (Diabetic + Glibenclamide)	265 ± 24a	300 ± 63a	339 ± 63ab
	B	AB	A
Mean of MDA level (nmol/ml)			
A (Normal + Normal)	10.6 ± 0.9b	8.7 ± 1.8d	5.5 ± 0.5b
	B	C	D
B (Normal + *E. aromatica*)	10.1 ± 0.8b	7.1 ± 1.7d	5.8 ± 2.3b
	B	C	C
C (Normal + *A. jiringa*)	10.4 ± 0.8b	7.1 ± 0.6d	5.9 ± 1.6b
	B	C	C
D (Diabetic + Normal)	11.9 ± 1.2b	13.4 ± 1.8bc	9.3 ± 1.9a
	B	B	B
E (Diabetic + *E. aromatica*)	11.3 ± 0.4b	13.1 ± 1.2bc	7.0 ± 3.0ab
	B	B	C
F (Diabetic + *A. jiringa*)	11.5 ± 0.8b	16.1 ± 0.4a	7.8 ± 1.1ab
	C	B	D
G (Diabetic + Glibenclamide)	10.9 ± 0.7b	12.6 ± 3.2bc	7.2 ± 2.1ab
	B	AB	C

Means within each column with the different letter (small letter) are significantly different at $p < 0.05$
Means within each row with different letter (capital letter) are significantly different at $p < 0.05$

beta cells or by improving the action of insulin. In week 0, the MDA level did not show significant difference between groups (*Table 1*). The stabilisation of MDA level among groups is due to the free radicals formed in diabetic groups from lipid peroxidation is still low.

At the 3rd week, MDA level between normal groups (A, B, C) was insignicantly different ($p > 0.05$). For diabetic groups, only group F, with *A. jiringa* treatment, was significantly different from the other diabetic groups, where the value was quite high compared to other groups. At week 6, only group D (diabetic rats with normal feed) showed significantly different ($p < 0.05$) from other normal groups and diabetic groups. This indicates that, although this group also showed decreasing trend from week 3 to week 6, but the MDA level in this group was still high compared to other groups with treatment. This also indicates that groups with *E. aromatica* and *A. jiringa* treatments are significant in reducing the MDA level of rats or on the other hand reducing the oxidative stress, but no significant decrement of oxidative stress in diabetic groups without treatment.

Conclusion

In general, all body weight of rats was increased insignificantly. Herbal treatment using *Eugenia aromatica* and *Archidendron jiringa* can reduce oxidative stress using MDA level as a marker. Research on the antioxidantive effect of these herbs on different oxidative stress marker can be conducted for further study.

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Abstrak

Kajian dilakukan untuk menilai keberkesanan dua herba iaitu *Eugenia aromatica* (cengkih) dan *Archidendron jiringa* (jering) ke atas paras MDA terhadap tikus diabetik yang telah disuntik dengan streptozotocin. Sejumlah 56 ekor tikus jantan jenis *Sprague Dawley* yang beratnya 150 – 200 g dibahagikan kepada tujuh kumpulan yang berlainan. Eksperimen ini dilakukan selama 6 minggu. Paras kandungan glukosa, berat tikus dan kadar aktiviti malondealdehyde dicatatkan setiap 3 minggu. Dua herba ini dapat menurunkan kadar tekanan oksidatif di dalam tikus diabetik. Tahap kandungan MDA dilihat turun dengan ketara (p <0.05) di dalam kumpulan tikus diabetik berbanding dengan kumpulan kawalan pada akhir kajian. Herba yang digunakan di dalam kajian ini tidak mempengaruhi paras glukosa di dalam darah tikus normal dan tikus diabetik. Kajian seterusnya terhadap jering dan cengkih ke atas penanda tekanan oksidatif yang berlainan diperlukan bagi menyokong penemuan kajian ini.