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## Effects of *Piper sarmentosum* Water Extract on 11- $\beta$ Hydroxysteroid Dehydrogenase Type 1 Bioactivity in Ovariectomy-Induced Obese Rats

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**Abstract:** The 11 $\beta$ -hydroxysteroid dehydrogenase type 1 (11 $\beta$ -HSD1) convert inactive circulating 11-keto steroids into active glucocorticoids, amplifying local glucocorticoid action. It is elevated in adipose tissue in obese humans and rodents, suggesting that adipose tissue glucocorticoid excess may be the causative factor for obesity. This study was conducted to evaluate the effects of *Piper sarmentosum* (PS) water extract and glycyrrhizic acid (GCA) on 11 $\beta$ -HSD1 bioactivity in ovariectomized induced obese rats. Forty-two female *Sprague-Dawley* rats were randomly divided into six groups; four treatments (PS, GCA, CTRL and SHM) and two basal (B-CTRL and B-SHM). All groups underwent ovariectomy excluding SHM and B-SHM which underwent sham operation. Basal groups were sacrificed on the first day of treatment, while ovariectomized groups were given PS extract (0.125 g kg<sup>-1</sup>), GCA (0.120 g kg<sup>-1</sup>) and water (CTRL), respectively, while SHM received only water. Blood pressure was measured monthly while body weight weekly. After five months, rats were sacrificed and liver, heart and visceral adipose tissues were taken for analysis. *Piper sarmentosum* (PS) and GCA group showed a significant reduction in enzyme activity but no difference in body weight compared to CTRL group. Meanwhile only the blood pressure in GCA group was significantly higher after three months of treatment as compared to CTRL group but no difference after five months. In conclusion, both PS water extract and GCA have the ability to reduce 11 $\beta$ -HSD1 enzyme activity but only GCA cause an increased in blood pressure.

**Key words:** *Piper sarmentosum*, obese, 11 $\beta$ -HSD1, ovariectomy, body weight

### INTRODUCTION

Obesity can be defined as an accumulation of body fat (Gallagher *et al.*, 1996) and it is associated with many diseases like hypertension, diabetes mellitus, coronary heart disease and others. It occurs as a result of either by hypertrophy of existing adipocytes or differentiation of preadipocytes through to adipocytes. A series of epidemiological studies have suggested that for a given body mass index, mortality is higher if adipose tissue is deposited centrally (visceral or omental obesity) as compared with generalized obesity (Björntop, 1991). It has been reported that visceral obesity is caused by excessive production of glucocorticoid exclusively within visceral adipose tissue (Masuzaki *et al.*, 2001).

Glucocorticoids are regulated at the pre-receptor level by the microsomal enzyme 11 $\beta$ -hydroxysteroid dehydrogenase (11 $\beta$ HSD), which interconverts active glucocorticoids (cortisol in men; corticosterone in

rodents) to inactive glucocorticoids (cortisone in men; 11-dehydrocorticosterone) (Stewart and Krozowski, 1999). Two different isoforms of 11 $\beta$ HSD enzymes have been identified; the 11 $\beta$ -HSD1 has a low affinity NADP(H)-dependent enzyme that activates the glucocorticoid, while the 11 $\beta$ -HSD2 is a high affinity NAD-dependent enzyme that inactivates the glucocorticoid.

The 11 $\beta$ -hydroxysteroid dehydrogenase type 1 (11 $\beta$ -HSD1) acts *in vivo* as a reductase, converting inactive to the active glucocorticoid (Katz *et al.*, 1999). The link between obesity and the 11 $\beta$ -HSD1 enzyme had been investigated in many studies. It had been reported that 11 $\beta$ -HSD1 activity is increased in animal models of obesity (Livingstone *et al.*, 2000). The 11 $\beta$ -HSD1 knock-out mice were found to be resistant to high fat diet-induced metabolic syndrome (Kotelevtsev *et al.*, 1997; Morton *et al.*, 2001) and mice with specifically over expressing 11 $\beta$ -HSD1 in adipose tissue showed all the typical features of the metabolic syndrome

(Masuzaki *et al.*, 2001, 2003). Moreover, both activity (Rask *et al.*, 2001, 2002) and mRNA expression (Paulmyer-Lacroix *et al.*, 2002) of 11 $\beta$ -HSD1 have been reported to be elevated in subcutaneous adipose tissues of obese human subjects. Therefore, the present study investigates the effects of water extracts of *Piper sarmentosum* and glycyrrhizic acid (GCA) on 11 $\beta$ -HSD1 bioactivity in ovariectomized induced obese rats.

For centuries, people have used herbs for the treatment of many ailments including chronic disease because of its trusted remedies that have been passed from generation to generation. One of the herbs that have great potential is *Piper sarmentosum*. *Piper sarmentosum* or known as 'kaduk' has been used widely in folk medicine especially in Southeast Asia region but very few studies have been documented scientifically.

In Malay traditional society, water decoction of the *Piper sarmentosum* leaves are being used for the treatment of cough, headache, waist pain and arthritis, while the water decoction of the roots are used in treatment of menstrual pain and to improve urination. Moreover, the pounded leaves are useful in the treatment of eczema. Most parts of the plant have potential benefits. The water extract of the whole plant had been shown to induce hypoglycemic effect in streptozocin-diabetic rats (Peungvicha *et al.*, 1998). While the methanolic extract of the leaves was found to possess a marked neuromuscular blocking activity in the rat phrenic nerve-hemidiaphragm preparation. Furthermore, the chloroform and methanol extracts of the leaves have the ability to act as an antiplasmodial agent against *Plasmodium falciparum* and *Plasmodium berghei* parasites. In addition, the methanolic extracts of the leaves was found to have higher level of antioxidant activity compared to other traditional medicine plants. Therefore, this study was conducted to evaluate the effects of *Piper sarmentosum* (PS) water extract on 11 $\beta$ -HSD1 bioactivity, blood pressure and body weight in ovariectomy-induced obese rats.

## MATERIALS AND METHODS

**Preparation of *Piper sarmentosum* water extract:** Fresh leaves (3 kg) of *Piper sarmentosum* were collected from the Ethnobotanic Garden, Forest Research Institute Malaysia (FRIM) after being identified and confirmed by a plant taxonomist from the Medicinal Plant Division (voucher specimen, FRI 45870), FRIM. All the extraction procedures were performed at the FRIM laboratory. Fresh leaves of the plants were cleaned with tap water and dried at room temperature before been chopped into small

pieces. The leaves were then boiled with distilled water (90%, v/v) at 80°C for 3 h. The water extract was then concentrated and followed by freeze-drying to form powder. The powdered extract was stored at 4°C until further use.

**Animal preparation:** All procedures were carried out in accordance with the institutional guidelines for animal research of the Universiti Kebangsaan Malaysia (FAR/2006/AZLINA/19-APRIL/168-MARCH-2007). Forty-two female Sprague-Dawley rats weighing 180-200 g were used in this study and placed in Animal House at Faculty of Medicine, Universiti Kebangsaan Malaysia. The rats were maintained in a temperature-controlled room (24°C) and illuminated for 12 h daily (lights on from 0700 to 1900). Two rats were kept in each cage and allowed free access to rat chow (Gold Coin, Klang, Selangor, Malaysia) and tap water *ad libitum*. The rats were randomly divided into six groups; four treatment groups (PS, GCA, CTRL and SHM) and two basal groups (B-CTRL and B-SHM). All groups underwent an experimental ovariectomy excluding SHM and B-SHM groups which underwent sham operation. Basal groups (B-SHM and B-CTRL) were sacrificed on the first day of treatment started. The ovariectomized groups were given *Piper sarmentosum* water extract (PS), glycyrrhizic acid (GCA) and water (CTRL), respectively, while the sham-operated (SHM) group received only water by oral feeding daily for 5 months.

**Experimental protocol:** The procedures were carried out in accordance with the institutional guidelines for animal surgery of the Universiti Kebangsaan Malaysia Animal Ethics Committee (UKMAEC). The rats were anaesthetized with intramuscular injection of ketamine (Troy Laboratories, Australia) and xylazine (Troy Laboratories, Australia) cocktail (1:1). The hairs on the both sides of the body were shaved off from the lowest rib to the hip region. For the rats which underwent a sham operation, only skin and muscles were cut but the ovaries were spared. Meanwhile for rats in the ovariectomized groups, bilateral ovariectomy was performed with an incision 1.5 cm inferior to the palpated rib cage. The ovaries and surrounding tissue were removed; the incision was closed by suturing the muscles and stapling the skin. Antibiotic cream was applied on the wound and the animal was placed on paper towels in a cage until awake. The treatment period was started after 2 weeks post-ovariectomy. *Piper sarmentosum* and GCA (Sigma, USA) were administered by oral gavage at a dose of 1.25 mg kg<sup>-1</sup> (Peungvicha *et al.*, 1998) and 1.20 mg kg<sup>-1</sup> (Al-Wahaibi *et al.*, 2007), respectively for

five months. After five months of treatment, the rats were sacrificed and the liver, heart and visceral adipose tissues were taken for bioactivity analysis.

**Bioactivity of 11 $\beta$ -HSD1 measurement:** Enzyme activity was measured according to the method of Al-Wahaibi *et al.* (2007) with some modification. The liver, heart and visceral adipose tissues were homogenized in 2 mL Krebs-Ringer buffer. Two-hundred  $\mu$ mol of NADP (Sigma, USA) for 11 $\beta$ -HSD1 activity and 12 nM [ $^3$ H]-corticosterone (Amersham, England) were added to tissue homogenates containing 0.5 mg protein. The total protein content was estimated calorimetrically (Bio-Rad, USA) on aliquots of each homogenates. Krebs-Ringer buffer containing 0.2% glucose (Sigma, USA) and 0.2% bovine serum albumin (Sigma, USA) were added to make up the total assay volume of 250  $\mu$ L. The mixture was incubated in a shaking water bath at 37°C for 10 min (JE10 TECH, Korea). The reaction was terminated by the addition of 1 mL of ethyl acetate (Merck, Germany), steroids were then extracted. The organic layer was separated by centrifugation at 4°C, 3000 rpm for 10 min (IEC Centra EC4R, USA). The top layer was then transferred into test tube and evaporated using vacuum concentrated (Hetotrap VR10, USA). Steroid residues were dissolved in ethanol (Merck, Germany) containing 11-dehydrocorticosterone (Sigma, USA) and kept in 4°C overnight. The following day, it was separated by thin layer chromatography (Merck, Germany) in chloroform (Merck, Germany) and 95% ethanol (Merck, Germany) in the ratio of 92:8. The fractions corresponding to the steroid were located under ultraviolet lamp absorption 240 nm. It were scraped and transferred into scintillation vials and counted in scintillation fluid using fluid scintillation  $\beta$ -counter. Enzyme activity was calculated as the percentage conversion of [ $^3$ H]-corticosterone to [ $^3$ H]11-dehydrocorticosterone.

**Body weight and blood pressure measurement:** Body weight was taken every week by using the electronic weighing scale (Tanita Model 1144, Japan). Blood pressure was measured by using a physiograph machine. The rat was anaesthetized with diethyl ether (Merck, Germany). After which, the transducer was put at the tail. Then the cuff was inflated and the reading was recorded. This step was performed in triplicate and the average reading was taken as final reading.

**Statistical analysis:** All data were tested for normal distribution and presented as Mean $\pm$ SD. Differences in the body weights and blood pressure were analyzed by a one-way Analysis of Variance (ANOVA) followed by Tukey test for multiple group comparisons. A value of

$p < 0.05$  was taken as significant. All statistical analysis was conducted using the SPSS version 11.5 software.

## RESULTS

After ovariectomy, the 11 $\beta$ HSD1 activity in B-CTRL group were increased in all the three organs especially in liver 5% ( $p = 0.715$ ) (Fig. 1) followed by visceral adipose tissues 2% ( $p = 0.055$ ) (Fig. 3) and heart 1% ( $p = 1.000$ ) (Fig. 2) as compared to B-SHM group.

However, after 5 months of treatment both PS and GCA treated group showed reduction in the 11 $\beta$ HSD1 activities as compared to CTRL group. For PS treated

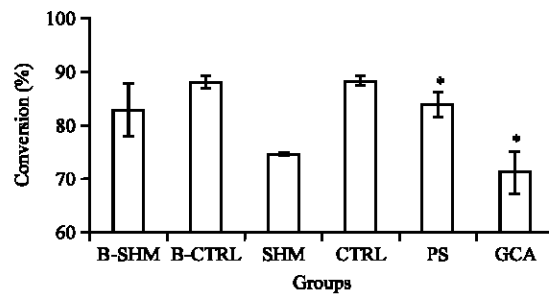


Fig. 1: Effects of the oral administration of the water extract of *Piper sarmentosum* (PS) on the 11 $\beta$ HSD-1 enzyme activity in the liver of ovariectomized induced obese rats. B-SHM and B-CTRL were sacrificed on the day 0 of the treatment, SHM, CTRL, PS and GCA were sacrificed after 5 months of treatment. \*Significant different compared with CTRL (\*,  $p < 0.05$ ). N = 7 in each group. The data are the Mean $\pm$ SD

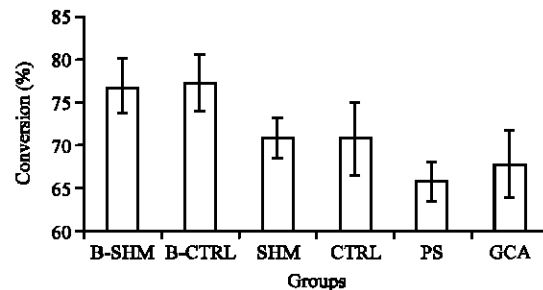


Fig. 2: Effects of the oral administration of the water extract of *Piper sarmentosum* (PS) on the 11 $\beta$ HSD-1 enzyme activity in the heart of ovariectomized induced obese rats. B-SHM and B-CTRL were sacrificed on the day 0 of the treatment, SHM, CTRL, PS and GCA were sacrificed after 5 months of treatment. \*Significant different compared with CTRL (\*,  $p < 0.05$ ). N = 7 in each group. The data are the Mean $\pm$ SD

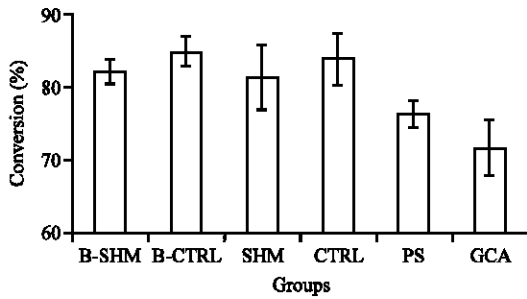


Fig. 3: Effects of the oral administration of the water extract of *Piper sarmentosum* (PS) on the 11 $\beta$ HSD-1 enzyme activity in the visceral adipose tissue of ovariectomized induced obese rats. B-SHM and B-CTRL were sacrificed on the day 0 of the treatment; SHM, CTRL, PS and GCA were sacrificed after 5 months of treatment. \*Significant different compared with CTRL (\*,  $p < 0.05$ ). N = 7 in each group. The data are the Mean $\pm$ SD

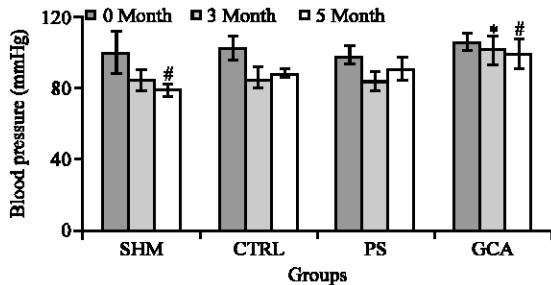


Fig. 4: Comparisons of blood pressure between groups. Blood pressure was measured on 0 month, 3 month and 5 month of treatment. \*Significant different compared with 3MONTH CTRL (\*,  $p < 0.05$ ), #Significant different compared with 5MONTH CTRL (#,  $p < 0.05$  N = 7 in each group. The data are the Mean $\pm$ SD

group, the most of the reduction in the activity occurred was in the liver 7% ( $p = 0.006$ ) (Fig. 1) followed by the visceral adipose tissues 5% ( $p = 0.144$ ) (Fig. 3) and the heart 5% ( $p = 0.161$ ) (Fig. 2). Meanwhile in GCA treated group, the most of the reduction in the activity occurred was in the visceral adipose tissues 17% ( $p < 0.001$ ) (Fig. 3) followed by the liver 12% ( $p < 0.001$ ) (Fig. 1) and the heart 3% ( $p = 0.688$ ) (Fig. 2).

The blood pressure measurement, at 0 month of treatment, showed no significant difference between all the four groups. However, only GCA treated group showed a significant higher blood pressure readings after 3 months ( $p = 0.001$ ) and 5 months ( $p = 0.014$ ) of treatment as compared to CTRL group (Fig. 4).

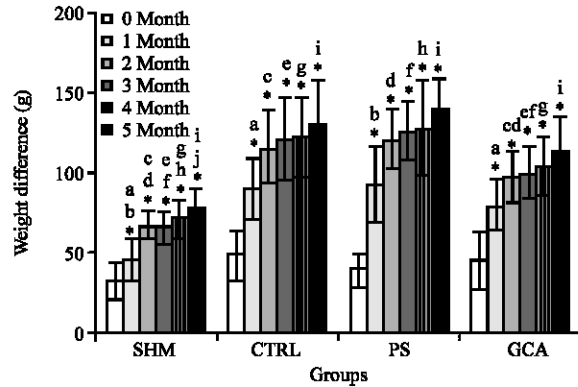


Fig. 5: Weight difference between group over time. Same letters are statistically significant ( $p < 0.05$ ) between groups, \*Significant difference between 0 MONTH (\*,  $p < 0.05$ ). N = 7 in each group. The data are the Mean $\pm$ SD

Figure 5 shows that ovariectomy procedure caused obesity in the rats because the body weight increase was almost up to 20% as compared to the SHM group after 1 month of surgery. After 5 months of treatment, only GCA-treated group showed a significant ( $p < 0.05$ ) reduction in body weight difference as compared with CTRL group (Fig. 5).

## DISCUSSION

This presence study was done to investigate the effect of ovariectomy on body weight and also on the activity of the 1 $\beta$ -hydroxysteroid dehydrogenase type 1 (11 $\beta$ -HSD1) enzyme in the liver, visceral adipose and heart tissues. It is known that ovariectomy procedure will cause increase in body weight which results in obesity (Chu *et al.*, 1999). Present finding showed that the weight differences increased was almost up to 20% as compared to the SHM group after one month of surgery, which is comparable with Kano and Doi (2006) study. This observation is supported with the finding by Heymsfield *et al.* (1994) and Poehlman *et al.* (1995) which had suggested that menopause increased central adiposity. This is consistency with the view that it is associated with changes in fat distribution and estrogen deficiency that is believed to play a role in postmenopausal women fat distribution (Reubinoff *et al.*, 1995). Moreover, estrogen has been found to influence eating behavior and cause weight gain in animals (Heymsfield *et al.*, 1994).

It became evident that excessive glucocorticoid action plays a causal role in the pathogenesis of metabolic syndrome (Rosmond, 2005; Asensio *et al.*, 2004). In

particular, the enhanced local conversion of inactive glucocorticoids (cortisone, 11-dehydrocorticosterone) to active glucocorticoids (cortisol, corticosterone) by 11 $\beta$ -HSD1 in metabolically active tissues such as liver, adipose tissue and skeletal muscle has been implicated in these disorders (Tomlinson, 2005; Peterson *et al.*, 2005; Wang, 2006). The pioneer study in 11 $\beta$ -HSD1 have purified the enzyme from the rat liver, followed by other studies that reported that it's widespread of tissues distribution. In human, 11 $\beta$ -HSD1 was identified in samples from liver, testis, lung, foreskin fibroblast, ovary, colon and kidney with the highest level of expression in the liver and the lowest in the kidney (Tischer *et al.*, 1991). Meanwhile in rats, 11 $\beta$ -HSD1 also has wide tissue distribution including liver, kidney, testis, lung, heart and colon (Agarwal *et al.*, 1989; Krozowski *et al.*, 1992; Brereton *et al.*, 2001).

The effect of ovariectomy on 11 $\beta$ -HSD1 in the liver, visceral and heart tissues was investigated in this study. The enzyme activity was increased after ovariectomized in liver and visceral adipose but no difference in the heart. The increment in visceral adipose tissues was consistence with Rask *et al.* (2001) reported that 11 $\beta$ -HSD1 activity has increase in adipose tissues from obese human. In another study it was also reported that visceral adipose tissues has more higher 11 $\beta$ -HSD1 activity than subcutaneous adipose tissues and therefore preadipocytes from visceral adipose tissues are more prone to differentiated into adipocytes compared to subcutaneous adipose tissues (Berger *et al.*, 2001). Similarly, the increment of hepatic 11 $\beta$ -HSD1 activity was comparable with Low *et al.* (1993) study where they reported that gonadectomy resulted in a significant increased in enzyme activity in the female rat liver. Explanation for the unaffected 11 $\beta$ -HSD1 activity in the heart was most probably because of the 11 $\beta$ -HSD1 activity in the heart was restricted to the interstitial cells of the endocardium and isolated cells surrounding cardiac vessels (Brereton *et al.*, 2001).

There are minimum numbers of scientific studies which reports on the effects of *Piper sarmentosum* although it is widely been used as alternative medicine. The results of the present study demonstrated that the water extract of *Piper sarmentosum* had the ability to reduce the 11 $\beta$ -HSD1 enzyme activity in the liver, visceral adipose tissues and a minute effect in the heart tissues of ovariectomy-induced obese rats. Several studies have reported that *Piper sarmentosum* water extract had hypoglycemic effects in rats and in alloxan diabetic rabbits (Peungvicha *et al.*, 1998). In our earlier study we found that *Piper sarmentosum* water extract reduced the blood glucose level and increase the adiponectin

plasma level in ovariectomy-induced obese rats (Aida Azlina *et al.*, 2009). Treatment of obese mouse strains with selective 11 $\beta$ -HSD1 inhibitors resulted in significant reduced visceral fat deposits, accompanied with improvement of glucose tolerance and insulin sensitivity (Alberts *et al.*, 2002, 2003). As hypothesized, the activity of the 11 $\beta$ -HSD1 enzyme in PS and GCA treated groups were significantly ( $p < 0.05$ ) reduced in all the three organs as compared to CTRL group. Therefore, there is a possibility that *Piper sarmentosum* water extract has the ability to reduce the activity of the 11 $\beta$ HSD1 enzyme.

After five months of treatment, significant ( $p < 0.05$ ) reduction in body weight only occurred in GCA treated group. For PS treated group, there was no dissimilarity as compared to CTRL group. This result was not shown to be correlated with the bioactivity of 11 $\beta$ HSD1. Explanation for this probably because of the reduction of 11 $\beta$ HSD1 enzyme activities is site specific which is occurs more at the visceral adipose tissues compared with subcutaneous fat, when in fact, body weight measurement were included the subcutaneous adipose tissues as well. This finding were supported with other studies (Hauner *et al.*, 1989; Fried *et al.*, 1993) which have reported that the expression of 11 $\beta$ HSD1 enzyme was highest occurred in the visceral compared with the subcutaneous adipose tissues.

Glycyrrhetic acid, the main metabolite of glycyrrhizic acid (GCA) is potent but not selective inhibitors for 11 $\beta$ -HSDs isoforms (Monder *et al.*, 1989). It has been reported that it can reduce the thickness of subcutaneous thigh fat through topical application (Armanini *et al.*, 2005). However, prolonged ingestion of glycyrrhizic acid can cause hypertension because it may develop pseudo aldosterone-like effects (Heikens *et al.*, 1995).

Present finding on blood pressure agrees with Heikens *et al.* (1995) finding that glycyrrhizic acid may induce hypertension by inhibition of cortisol metabolism which results in the pseudo-aldosterone-like effects. On the zero month of treatment, there was no significant difference between the sham-operated and ovariectomized rats. However, the blood pressure of the GCA treated group was significantly higher after three months of treatment compared to other group which confirms the hypothesis that glycyrrhizic acid increases the blood pressure. Krozowski and Funder (1983), Edwards *et al.* (1988) and Funder *et al.* (1988) proposed that conversion of cortisol (corticosterone for rodents) to cortisone (11-dehydrocorticosterone) represent the physiological mechanism conferring specificity for aldosterone upon the mineralocorticoid receptors. Since, cortisol normally circulates at levels 100-1000 time

compared to aldosterone, this leads to signs of mineralocorticoid excess, even though aldosterone secretion is suppressed. Thus, in prolonged glycyrrhizic acid ingestion, it permits cortisol to occupy the mineralocorticoid receptors which leads to hypertension.

### CONCLUSION

As a conclusion, both *Piper sarmentosum* water extract and glycyrrhizic acid reduces the activity of the 11 $\beta$ -hydroxysteroid dehydrogenase type 1 enzyme, which suggest that *Piper sarmentosum* water extract may have the ability to inhibit the 11 $\beta$ HSD1 enzyme activity. However, only glycyrrhizic acid can reduce the body weight as well as cause an increase in the blood pressure in ovariectomized induced obese rats.

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