Effects of treatment with St. John's Wort on blood glucose levels and pain perceptions of streptozotocin-diabetic rats

Özgür Devrim Can [a,*], Yusuf Öztürk [a], Nilgün Öztürk [b], Gianni Sagratini [c], Massimo Ricciutelli [c], Sauro Vittori [c], Filippo Maggi [c]

[a] Department of Pharmacology, Faculty of Pharmacy, Anadolu University, 26470 Eskisehir, Turkey
[b] Department of Pharmacognosy, Faculty of Pharmacy, Anadolu University, 26470 Eskisehir, Turkey
[c] School of Pharmacy, University of Camerino, 62032 Camerino, Italy

ARTICLE INFO

ABSTRACT

This present study was undertaken to examine treating effects of St. John's Wort (SJW) extract on nociceptive perception of STZ-diabetic animals based on its potential antidiabetic and antinociceptive activities. One week administrations of SJW extract (125 and 250 mg/kg) induced significant decrease in high blood glucose levels of three weeks STZ-diabetic rats and improved their dysregulated metabolic parameters. In addition, SJW extract treatment caused restoration in the mechanical hyperalgesia of diabetic animals. These findings provide a rationale for the traditional use of SJW against diabetes and display the potential of this plant as a new drug candidate/source for the treatment of diabetic pain.

© 2011 Elsevier B.V. All rights reserved.

1. Introduction

Diabetes mellitus (DM) is defined as a metabolic disorder of multiple etiologies characterized by chronic hyperglycemia with disturbances of carbohydrate, protein, and fat metabolism resulting from defects in insulin secretion, insulin action, or both [1].

Diabetes can result in numerous acute complications including hypoglycemia, hyperglycemia, ketoacidosis, and hyper-osmolar syndrome. In addition to acute complications, chronic conditions may lead long-term metabolic disorders related to various organ dysfunctions [2–4]. Diabetic neuropathy is one of the most widespread chronic complications of DM and the main cause of diabetic pain [5].

Current first-line treatment for diabetic neuropathic pain is tricyclic antidepressants. Analgesic action of the antidepressants is due to a proposed facilitation of descending inhibitory nociceptive pathways by inhibition of monoamine transporters [6,7]. Other medications include calcium channel ligands (i.e., gabapentin and pregabalin), topical lidocaine, opioid analgesics and tramadol [7,8]. However, effectiveness of current therapeutic regimens is limited. Therefore, discovery and development of new drug candidates for treatment of diabetic pain remains a major challenge in pharmaceutical field [7,8].

SJW preparations, widely used herbal-based antidepressants in many countries [9,10]. SJW extracts interact with monoaminergic system through different mechanisms. For example, hyperforin, phloroglucinol derivative compound in SJW extracts, has been shown to inhibit reuptake of neurotransmitters and reported as the main component responsible from the antidepressant activity [11]. Hypericin, naphthodianthrone derivative compound in SJW extracts, has been reported to cause alterations in the concentration of monoamine neurotransmitters and their metabolites in different brain regions [12]. Rutin, one of the flavonoid compounds in SJW extracts, have been reported to possess antidepressant activity in experimental animals and shown to increase the availability of serotonin

---

* Corresponding author. Tel.: +90 222 3350580x3751; fax: +90 222 3350750.

E-mail address: ozgurdt@anadolu.edu.tr (Ö.D. Can).

and noradrenaline in the synaptic cleft [13]. SJW extracts have been shown to inhibit the neuronal uptake of monoamines similar to tricyclic antidepressants [6,9,14]. This evidence may suggest that similar to TCAs, SJW extracts could also relieve diabetic neuropathic pain.

Although, SJW has also been reported for its traditional use by diabetic patients [15], antidiabetic effect of the plant has not been reported previously. However, some flavonoids such as quercetin, isoquercetin and rutin present in various plants as well as SJW have been searched for their antidiabetic activity potentials. For example, rutin has been reported to enhance insulin release and decrease blood glucose level in diabetic animals [16,17]. Recently, metabolites of rutin have been suggested as useful agents for preventing diabetic complications by inhibiting nonoxidative advanced glycation end products [18]. Quercetin, quercitrin and isoquercitrin have also been associated with some antidiabetic actions. Antihyperglycemic effect has been reported previously in experimentally induced diabetic animals for quercetin [19,20]. Rutin, quercetin, quercitrin have been evaluated as alpha-glucosidase inhibitors in some studies [21,22]. Quercetin and isoquercitrin have been suggested as potent non-competitive inhibitors of the intestinal sugar transporter GLUT2 and decrease intestinal glucose absorption [23].

Based on the above mentioned antinociceptive and antidiabetic activity potentials, this present study was undertaken to investigate effects of SJW extract on blood glucose levels, metabolic parameters and variations of pain tresholds in STZ-diabetic rats.

2. Experimental

2.1. Chemicals

Streptozotocin (STZ), sodium citrate and trisodium citrate were purchased from Sigma (St. Louis, MO). Quercetin, hypericin, hyperforin, chlorogenic acid, rutin and quercitrin were supplied by SIGMA (Milan, Italy), hyperoside and isoquercitrin were supplied by Applied Biosystem (Milan, Italy). Individual stock solutions were prepared by dissolving each compound in methanol and stored in glass-stoppered bottles at 4 °C. Standard working solutions, at various concentrations, were daily prepared by appropriate dilution of aliquots of the stock solutions in methanol. Methanol, acetonitrile and ethyl acetate gradient grade for liquid chromatography were purchased from Merck (Darmstadt, Germany). Deionized water (<18 MΩ cm resistivity) was obtained from a Milli-Q SP Reagent Water System (Millipore, Bedford, MA, USA). All the solvents were filtered through a 0.2 μm cellulose filter (RC 58) from Schleicher and Schuell before use.

2.2. Preparation of extracts

Aerial parts of *Hypericum perforatum* L. were collected in Tahtakuşlar village, Kazdağı, Balıkesir, Turkey in June, 2005. After collection, the plant material was dried at room temperature and its voucher specimen was kept at the Herbarium of the Faculty of Science (OUFE 10337) Osmangazi University, Eskişehir, Turkey. Botanical identification of collected material was made by Dr. Onur Koyuncu from Osmangazi University, Faculty of Science, Department of Biology. The extract was

Table 1
Linear gradient programme for HPLC analysis.

Time (min)	Solvent A (%) [a]	Solvent B (%) [b]	Solvent C (%) [c]
0	85	15	0
10	85	15	0
30	65	35	0
45	10	90	0
60	10	90	0
61	0	0	100
70	0	0	100
75	0	100	0
105	0	100	0
110	85	15	0
130	85	15	0

[a] Solvent A = water and phosphoric acid, pH solution 2.7.
[b] Solvent B = 90% acetonitrile, 10% methanol.
[c] Solvent C = 10% ethyl acetate, 90% mix solvent A and B (10%A, 90%B).

prepared by the department of Pharmacognosy at Anadolu University. Fresh aerial parts of the plant were air-dried at room temperature and powdered. The crude powder was macerated in 50% ethanol (1:10) for one night and extracted for 8 h at 40 °C water bath, then filtered. This process was repeated three times; filtrates were collected and concentrated under reduced pressure in a rotary evaporator at 40 °C to remove ethanol. The remaining aqueous part was freeze-dried at −80 °C and lyophilized. The extract obtained was weighed to determine the yields of soluble constituents. The yield of the extract was calculated as percentages (6.12%).

2.3. Phytochemical analysis

High Performance Liquid Chromatography (HPLC) and diode array detector (DAD) conditions: a Hewlett Packard (Palo Alto, CA, USA) HP-1100 series, equipped with a binary solvent pump, an autosampler, with the volume injection set to 20 μl, and a diode array detector (DAD) coupled with an HPLC/DAD ChemStation (Rev. A. 06. 03) was used. Separation was performed on a LUNA C_{18} (150×4.6 mm) protected by a Security guard cartridge C_{18} (4×2 mm I.D.), both from Phenomenex USA (distributed by Chemtek Analytica, Bologna, Italy). The monitored wavelengths were 210 nm for phenolic compounds, 270 nm for hyperforin, 590 nm for hypericin. The adopted chromatographic method was reported in Table 1. The sample concentration was 3 mg/ml in methanol and the flow rate was 1 ml/min. The method used for identification of components was the comparison of their retention times with respect to those of standards, chromatographed under the same

Table 2
Amounts of certain phenolic compounds (as ppm) in alcoholic extract of SJW.

Flavonoid	Amounts (ppm) [a]
Rutin	2225.45 ppm
Quercetin	741.82 ppm
Isoquercitrin	556.36 ppm
Quercitrin	556.36 ppm
Hyperosid	370.91 ppm
Chlorogenic acid	137.05 ppm
Hyperforin	42.65 ppm
Hypericin	40.71 ppm

[a] Values are means of three determinations (n = 3).
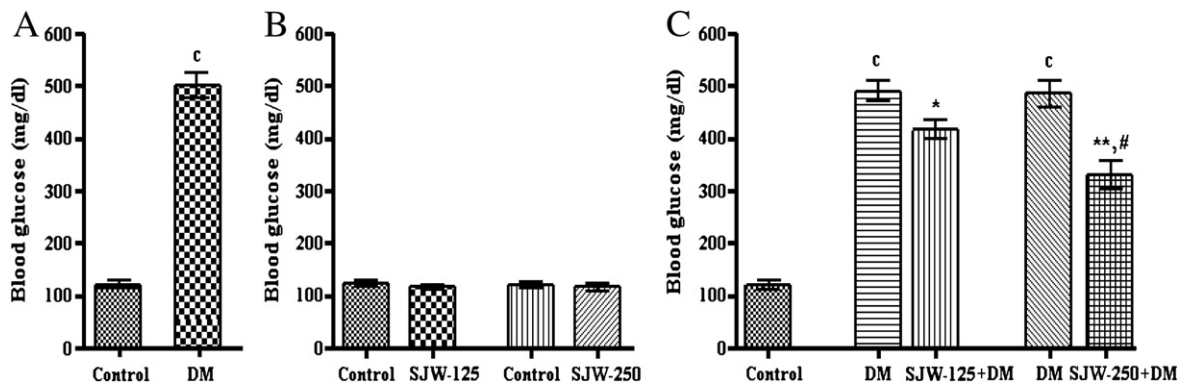
Fig. 1. Blood glucose levels of test groups. (A) Blood glucose levels of rats after STZ injection. Significance against control values $^c p<0.001$. Paired sample t-test, n = 7 (B) Blood glucose levels of normoglycemic rats before and after one week administrations of 125 and 250 mg/kg SJW extract. Paired sample t-test, n = 7 (C) Blood glucose levels of STZ-diabetic rats before and after one week administrations of 125 and 250 mg/kg SJW extract. Significance against control values $^c p<0.001$; significance against diabetes $^* p<0.05$, $^{**} p<0.01$; significance against SJW125 $^\# p<0.05$. One-way ANOVA, post-hoc Tukey's test. Values are mean ± SEM, n = 7.
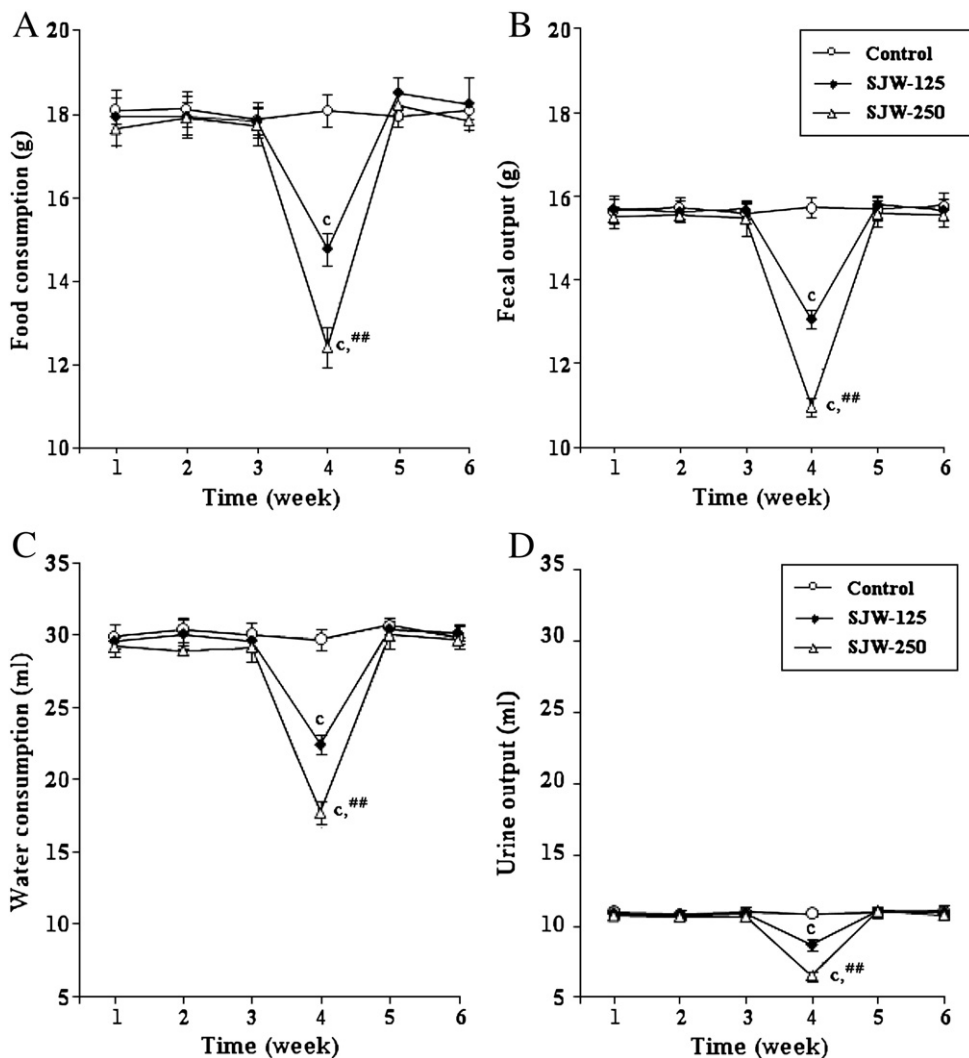
Fig. 2. Effects of SJW extract treatment on metabolic parameters of normoglycemic rats. (A) Effects of one week SJW extract on food consumption of normoglycemic rats. Significance against control values $^c p<0.001$; significance against SJW 125 group $^{\#\#} p<0.01$. (B) Effects of one week SJW extract on fecal output of normoglycemic rats. Significance against control values $^c p<0.001$; significance against SJW 125 group $^{\#\#} p<0.01$. (C) Effects of one week SJW extract on water consumption of normoglycemic rats. Significance against control values $^c p<0.001$; significance against SJW 125 group $^{\#\#} p<0.01$. (D) Effects of one week SJW extract on urine output of normoglycemic rats. Significance against control values $^c p<0.001$; significance against SJW 125 group $^{\#\#} p<0.01$. One-way ANOVA, post-hoc Tukey's test. Values are mean ± SEM, n = 7.

conditions. In addition, UV spectra of both samples and standards were compared using the DAD. Moreover, the confirmation of the identified compounds was obtained injecting standard and sample solutions in a HPLC-MS equipped with an ESI interface in negative ionization mode using the same chromatographic conditions (formic acid was used instead of phosphoric acid at the same pH).

2.4. Pharmacology

2.4.1. Animals

Wistar male rats (n = 7 in each group) with body weights ranging from 200 to 250 g were placed in individual metabolic cages in an air-conditioned room (24 ± 1 °C) with a 12-h light and 12-h dark cycle. Food and water were provided *ad libitum*. The experimental protocol described herein has been approved by the Local Ethical Committee on Animal Experimentation of Eskişehir Osmangazi University, Turkey.

2.4.2. Experimental design

Rats were divided into four groups as control (normoglycemic), SJW extract-treated normoglycemic, STZ-diabetic, and SJW extract-treated STZ-diabetic animals. Seven rats were placed in each of the groups.

SJW extract was administered to animals in SJW extract-treated normoglycemic group via intraperitoneal route at 125 and 250 mg/kg per day during one week. Control-normoglycemic group was treated with physiological saline since SJW extract dissolve in it.

To obtain two diabetic experimental groups, animals fasting for 24 h injected intravenously by a single dose of STZ. STZ was dissolved in the citrate buffer (pH = 4.5, 0.1 M) and immediately injected at the dose of 60 mg/kg. 72 h after the STZ injection, glucose was determined in blood samples obtained by pricking the tail, using Glukotrend® (Roche, Basel, Switzerland). Animals blood glucose levels higher than 300 mg/dl were accepted as diabetic [24]. Diabetic animals were allocated into two groups to form STZ-diabetic and SJW extract-treated STZ-diabetic groups.

SJW extract was administered to animals in SJW extract-treated STZ-diabetic group in the same way with SJW extract-treated normoglycemic animals. In order to permit development of nociceptive perception deficits in diabetic rats, SJW extract treatment was initiated three weeks after the induction of diabetes [25,26]. STZ-diabetic group was treated with physiological saline.

2.4.3. Metabolic cage measurements

Fecal and urine output collections as well as water and food consumptions were evaluated using metabolic cages (Ugobasile, 41700, Verase, İtaly). Rats were individually housed (1 rat/cage) in metabolic cages for 2 or 3 days before the induction of diabetes. Metabolic parameters were begun to measure three days after the STZ injection. Following this acclimation period, urine discharge (ml), fecal output (g), water (ml) and food (g) consumptions were measured daily [27]. Measurements were continued for 6 weeks and data of the each week was the average value of seven days measurements. Body weights of the rats were recorded weekly.

Normoglycemic rats were also kept in metabolic cages for six weeks and effects of one week SJW extract treatment on metabolic parameters and body weights of normoglycemic animals were recorded in the same way with diabetics.

2.4.4. Nociceptive tests

2.4.4.1. Tail-pinch test. Tail-pinch test has been applied as described previously [28]. Rats were placed in a clean cage, and an alligator clamp was placed on the tail approximately 5 to 10 cm from the tip. The latency for vocalization, biting or flicking the clamp was recorded by a stopwatch. Cut-off time was chosen as 90 s in order to prevent tissue damage [29].

2.4.4.2. Tail-flick test. Thermal nociceptive perception of animals was investigated using the tail-flick assay [30]. Radiant heat was applied to the tail at 2.5–5 cm from the tip using a tail-flick apparatus (Ugo Basile, Verase, Italy). When the animal felt pain and flicked its tail, light fell on the photocell and then the timer was automatically stopped. The apparatus was calibrated to produce tail-flick latencies of approximately 2–6 s in control animals. Cut-off time was chosen as 20 seconds in order to prevent tissue damage [31].

2.5. Statistical analysis

The data used in statistical analysis was acquired from seven animals for each group. Statistical evaluation of the data was performed using GraphPad Prism 4.03 (GraphPad Software, San Diego, CA, USA). Experimental data obtained from the same group of animals during 6 weeks were analyzed by repeated measures ANOVA followed by Tukey HSD test. Data comes from different groups of animals were analyzed by one-way ANOVA followed by Tukey HSD test for multiple comparisons.

Experimental results were expressed as mean ± standard error of mean (SEM). Differences between given sets of data were considered as significant when p value was less than 0.05.

3. Results

3.1. Phytochemical analysis

The main flavonoid component of the SJW extract used in the present study was rutin. Other high concentrated flavonoids in the extract were quercetin, quercitrin and isoquercitrin. Chlorogenic acid, especially hyperforine and hypericin were detected in lower amounts. Results were summarized in Table 2.

3.2. Pharmacology

3.2.1. Blood glucose values

Neither 125 nor 250 mg/kg doses of SJW extract caused significant change in the blood glucose levels of normoglycemic animals.

Blood glucose levels of STZ-diabetic animals were significantly increased after the injection of STZ, as expected. Administration of SJW extract caused dose-dependent decrease in the high blood glucose levels of diabetic animals (Fig. 1).

3.2.2. Metabolic cage measurements

Fig. 2 exhibited the change of metabolic parameters by SJW extract treatment in normoglycemic rats. SJW extract caused significant and dose-dependent decrease in water and food consumptions as well as fecal and urine outputs. Body weights of normoglycemic animals were also decreased in dose-dependent manner (Fig. 3).

Urine discharge, fecal output, water, and food consumptions of diabetic animals were increased when compared to control group, as expected. SJW extract treatment caused statistically significant decrease in all these metabolic parameters (Fig. 4). Weight loss of diabetic animals was partially restored with the extract administrations (Fig. 5).

3.2.3. Nociceptive tests

In tail-pinch test, one week after the induction of diabetes, statistically significant decrease was observed in the response latencies of STZ-diabetic animals. This progressive decline was continued in the following weeks. At weeks two and three, response latencies were significantly shorter than week one. Similarly, measurements at week four were significantly shorter than week three. However, after fourth week no more reductions were observed in the response latencies (Fig. 6).

At week one and two after the induction of diabetes the response latencies in the tail-flick test of STZ-diabetic animals were decreased, however the values returned to that of normoglycemic animals at week three and four. At week five and six, response latencies were increased significantly compared to values of normoglycemic rats (Fig. 7).

SJW extract at doses of 125 and 250 mg/kg, prolonged the response latencies of normoglycemic animals in tail-pinch and tail-flick tests. 250 mg/kg dose was more effective than 125 mg/kg (Figs. 6 and 7). Similarly, response latencies of diabetic animals in both of the tail-pinch and tail-flick tests were also prolonged dose-dependently by the extract administrations (Figs. 6 and 7).

4. Discussion

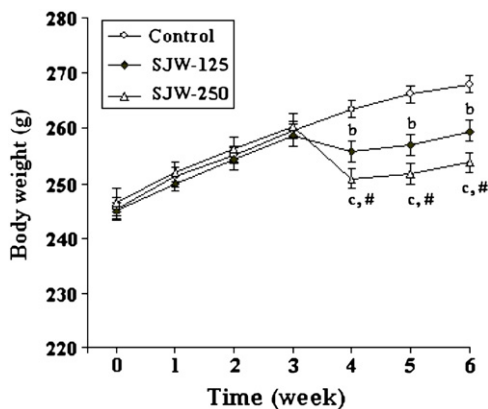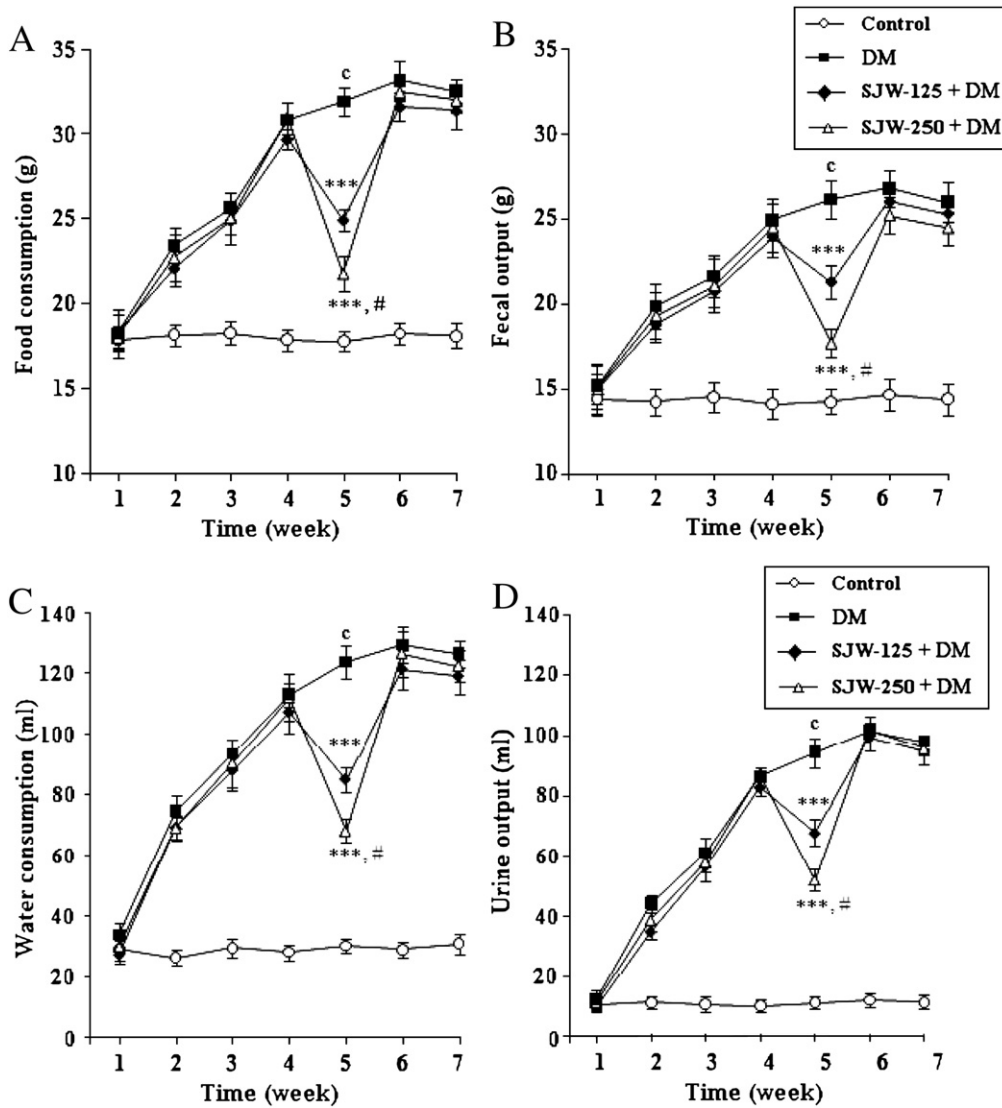The aim of this work was examining the effects of one week SJW extract treatment on metabolic parameters, blood glucose levels and nociceptive thresholds of STZ-diabetic animals.

First part of this present study was investigating the effects of the SJW extract in non-diabetic animals. In this normoglycemic group, SJW extract administrations caused significant and dose-dependent decrease in food intake, water consumptions, faces, and urine outputs. It is known that, monoamines acting on noradrenergic, serotonergic and dopaminergic receptors can reduce food intake of animals [32]. In addition, some antidepressant drugs, especially selective serotonin reuptake inhibitors (SSRI), increasing the monoamine levels in synapses, have been reported to decrease food intake, cause weight loss, and use for the treatment of eating disorders [32–34]. On the other hand, SSRI drugs have been reported for their excess releasing effect on vasopressin, which regulates volume of urine [35]. Serotonergic mechanisms also known to involved in the regulation of water intake [36]. Therefore, based on the brain monoamine levels increasing effect of SJW extract, the antidypsogenic, antidiuretic, hypophagic activities and weight loss observed in the present study may suggested to caused by increased brain monoamine levels. On the other hand, no difference was observed in blood glucose levels of normoglycemic animals by the extract treatment.

In normoglycemic animals, extract administrations dose-dependently prolonged the response latencies of animals in both tail-pinch and tail-flick tests. These results supported the findings of previous studies reporting the antinociceptive activity of SJW extracts against mechanical and thermal nociceptive stimuli [37,38]. Nociceptive effect of SJW extract seem to be related with the increase of monoamine levels in synaptic cleft since drugs increasing monoamine levels in synapses possess analgesic activity by activating descending inhibitory nociceptive pathways [39]. On the other hand rutin, the main component of the tested extract in the present study, may also be one of the responsible constituent from the exhibited analgesic activity due to its own analgesic activity potential [40,41].

The other part of this present study was examining the effects of one week SJW extract treatment in diabetic animals. In three weeks STZ-diabetic group, SJW extract administrations caused significant and dose-dependent decrease in increased food intake, water consumptions, faces and urine outputs of diabetic animals. However, reduced body weight of diabetic animals was significantly increased by the extract administrations. Tested extract induced significant and dose-dependent decrease in the high blood glucose levels of STZ-diabetic animals. Decrease in the high blood glucose levels, restoration in the metabolic parameters and improvement of the decreased body weights together indicated the antidiabetic effect of SJW extract in STZ-diabetic rats. These findings supported the previous papers reporting the antidiabetic effects of rutin, quercetin, quercitrin and isoquercitrin flavonoids, which were also detected in our tested extract [16,17,19–23]. Although flavonoids seem to be related with the antidiabetic activity exhibited in this study, responsible active constituents and their exact mechanism of actions should be clarified with further detailed studies. Studies for searching responsible component/s or possible synergic interactions are proceeding in our laboratory.

Fig. 3. Effects of SJW extract treatment on body weights of normoglycemic rats. Significance against control values [b]$p<0.01$, [c]$p<0.001$; significance against SJW 125 group [#]$p<0.05$. One-way ANOVA, post-hoc Tukey's test. Values are mean ± SEM, n = 7.
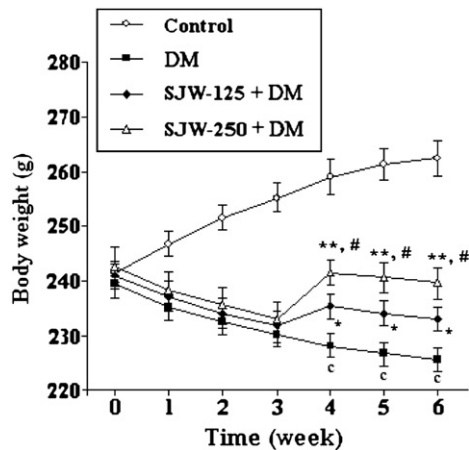
Fig. 4. Effects of SJW extract treatment on metabolic parameters of STZ-diabetic rats. (A) Effects of one week SJW extract on food consumption of STZ-diabetic rats. Significance against control values [c]$p < 0.001$; significance against diabetic group [***]$p < 0.001$; significance against SJW 125 group [#]$p < 0.05$. (B) Effects of one week SJW extract on fecal output of STZ-diabetic rats. Significance against control values [c]$p < 0.001$; significance against diabetic group [***]$p < 0.001$; significance against SJW 125 group [#]$p < 0.05$. (C) Effects of one week SJW extract on water consumption of STZ-diabetic rats. Significance against control values [c]$p < 0.001$; significance against diabetic group [***]$p < 0.001$; significance against SJW 125 group [#]$p < 0.05$. (D) Effects of one week SJW extract on urine output of STZ-diabetic rats. Significance against control values [c]$p < 0.001$; significance against diabetic group [***]$p < 0.001$; significance against SJW 125 group [#]$p < 0.05$. One-way ANOVA, post-hoc Tukey's test. Values are mean ± SEM, n = 7.

In nociceptive tests, obtained data clearly demonstrated significant decrease in the response latencies of diabetic rats in tail-pinch test indicating the occurrence of mechanical hyperalgesia. These findings supported the previous papers reporting on the mechanical hyperalgesia of animals at early stages of experimental diabetes [5,25,42]. Different from the constant mechanical hyperalgesic responses, thermal nociceptive thresholds of diabetic animals were observed as quite variable. Decreased response latencies of diabetic rats in tail-flick test in first two weeks following the induction of diabetes, were returned to that of normoglycemic animals at week three and four; and then increased significantly compared to the values of normoglycemic rats in last two weeks. This data exhibited the changes of thermal nociceptive

threshold from hyperalgesia to hypoalgesia during our study period.

Mechanical allodynia and thermal hyperalgesia observed in experimental diabetes have been reported to associate with the weakening of descending inhibitory nociceptive pathways suppressing the transmission of nociceptive stimuli in medulla spinalis [43]. In addition, augmentation of Ca^{+2} signaling in dorsal root ganglion neurons [44,45]; enhancement of NO production in periaquaductal gray [46]; and increase in glutamate, substance P and cholecystokinin releasing in medulla spinalis [42,47,48] have also been suggested to contribute to diabetic hyperalgesia. Furthermore, decrease in stimuli thresholds of primer sensory neurons [49], high spontaneous activities of spinal dorsal stem neurons [50], and

Fig. 5. Effects of SJW extract treatment on body weights of STZ-diabetic rats. Significance against control values [c]$p<0.001$; significance against diabetic group [*]$p<0.05$, [**]$p<0.01$; significance against SJW 125 group [#]$p<0.05$. One-way ANOVA, post-hoc Tukey's test. Values are mean $\pm$ SEM, n = 7.

ectopic discharges in sensory C fibers [51] have also been reported as some additional factors participating hyperalgesia of diabetic animals.

One week administrations of SJW extract prolonged the reduced response latencies of diabetic animals in tail-pinch test, meaning partial restoration of the mechanical hyperalgesia. Thermal response latencies of diabetic animals were also increased by the extract administrations. Antinociceptive effects of SJW extracts on diabetic animals may be directly related to antinociceptive activity of the extract, which was also exhibited in normoglycemic animals. On the other hand, partial restoration of mechanical hyperalgesia after the administrations may also be related with the antidiabetic activity of the extract. Rutin, potential analgesic and antidiabetic agent [16–18,22,40,41], which is found in quite high amount in the tested extract, is thought to be related with the exhibited pharmacological activity in the present study.

As well as the antihyperglycemic activity, some other potential mechanisms can also be suggested for the anti-nociceptive activity mechanism of the SJW extract in diabetic animals. For example, augmentation of the monoamine levels in central nervous system after SJW extract treatment [14,52] may probably cause improvement of monoaminergic neuro-transmission in descending antinociceptive pathways that are impaired in diabetes [43,53,54]. Therefore, rutin and other constituents such as hyperforine, hypericin, and pseudohy-pericin may probably play roles in the observed antinocicep-tive action by increasing the availability of serotonin and noradrenalin levels in supraspinal descending inhibitory nociceptive pathways [13,14,55,56]. Furthermore, inhibition of nitric oxide synthase (NOS) enzyme by the SJW extract [57,58], which may cause reduction in elevated NO levels in diabetic animals, may be suggested as another factor contributing the exhibited antinociceptive effect. Enhance-ment of GABAergic neurotransmission with SJW extract administration [59,60] may also be proposed as another possible mechanism for the exhibited antinociceptive activity in this work, based on the therapeutic effect of GABAergic drugs on neuropathic pain. Moreover, reduction in the ionic currents of NMDA receptors as well as Na^+ and Ca^{+2} channels [61], inhibition of the substance P induced cytokines synthesis [62], inhibition of the cyclooxygenase-1, 5-lipox-ygenase [63] and cyclooxygenase -2 [64] enzyme activities by SJW extract treatments can be hypothesized as some other mechanisms probably participating exhibited antinociceptive activity in this present study.

The extract tested in the present study was applied at 125 and 250 mg/kg doses which are relatively high compared to the current clinical doses of SJW extract as antidepressant drug. Therefore, SJW extracts at higher than antidepressant doses may be used as a novel therapeutic modality for the clinical management of neurological complications of diabe-tes including diabetic neuropathy, etc.

In summary, the results of the present study exhibited the capability of a SJW hydro-alcoholic extract for improvement of the mechanical hyperalgesia developed in STZ-diabetic
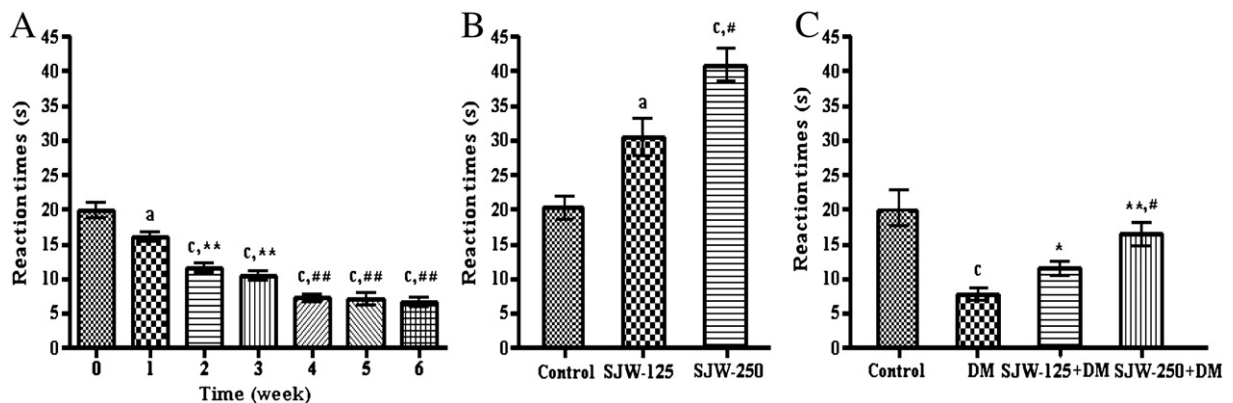
Fig. 6. Tail-pinch responses of test groups. (A) Alterations in response latencies of STZ-diabetic rats during six weeks. Significance against normoglycemic rats [a]$p<0.05$, [c]$p<0.001$; significance against 1st week [**]$p<0.01$; significance against 3rd week [##]$p<0.01$. (B) Effects of SJW extracts on response latencies of normoglycemic rats. Significance against control [a]$p<0.05$, [c]$p<0.001$; significance against SJW 125 group [#]$p<0.05$. (C) Effects of SJW extracts on response latencies of STZ-diabetic rats. Significance against control [c]$p<0.001$; significance against diabetic group [*]$p<0.05$, [**]$p<0.01$; significance against SJW 125 group [#]$p<0.05$. Values are mean $\pm$ SEM. One-way ANOVA, post-hoc Tukey's test, n = 7.
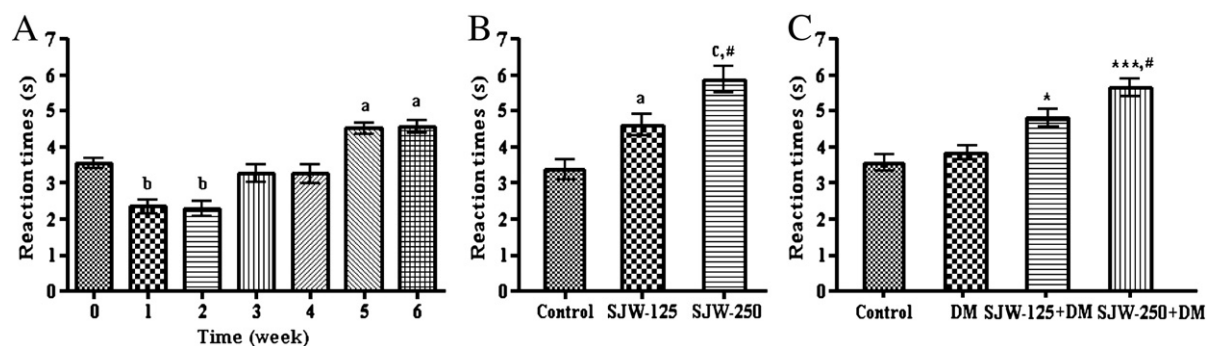
Fig. 7. Tail-flick responses of test groups. (A) Alterations in response latencies of STZ-diabetic rats during six weeks. Significance against normoglycemic rats [a]$p<0.05$, [b]$p<0.01$. (B) Effects of SJW extracts on response latencies of normoglycemic rats. Significance against control [a]$p<0.05$, [c]$p<0.001$; significance against SJW 125 group [#]$p<0.05$. (C) Effects of SJW extracts on response latencies of STZ-diabetic rats. Significance against diabetic group [*]$p<0.05$, [***]$p<0.001$; significance against SJW 125 group [#]$p<0.05$. Values are mean ± SEM. One-way ANOVA, post-hoc Tukey's test, n = 7.

rats. Our results also supported the finding of a recent study indicating the improving effect of SJW extract on various neuropathic pain models of rats [65].

5. Conclusion

This present study exhibited the antidiabetic activity of SJW extract for the first time. This finding provides a rationale for the use of this plant against diabetes as reported in the folk medicine. Besides, analgesic activity was also revealed for the SJW extracts in diabetic animals as well as normoglycemic group. Therefore, SJW extract can be suggested as a new drug candidate/source for the treatment of diabetic pain because of both its antihyperglycemic and antinociceptive actions.

References

[1] World Health Organization. Department of Noncommunicable Disease Surveillance, Definition, Diagnosis and Classification of *Diabetes mellitus* and it's Complications, Geneva; 1999.
[2] Tripathi BK, Srivastava AK. *Diabetes mellitus*: complications and therapeutics. Med Sci Monit 2006;12:130–47.
[3] Venkatraman R, Singhi SC. Hyperglycemic hyperosmolar nonketotic syndrome. Indian J Pediatr 2006;73:55–60.
[4] Wolfsdorf J, Glaser N, Sperling MA. Diabetic ketoacidosis in infants, children, and adolescents: a consensus statement from the American Diabetes Association. Diab Care 2006;29:1150–9.
[5] Khan GM, Chen SR, Pan HL. Role of primary afferent nerves in allodynia caused by diabetic neuropathy in rats. Neuroscience 2002;114:291–9.
[6] Nathan P. The experimental and clinical pharmacology of St. John's Wort (*Hypericum perforatum* L.). Mol Psychiatry 1999;4:333–8.
[7] Mico JA, Ardid D, Berrocoso E, Eschalier A. Antidepressants and pain. Trends Pharmacol Sci 2006;27:348–54.
[8] O'Connor AB, Dworkin RH. Treatment of neuropathic pain: an overview of recent guidelines. Am J Med 2009;122:S22–32.
[9] Yager J, Siegfried SL, Dimatteo TL. Use of alternative remedies by psychiatric patients: illustrative vignettes and a discussion of the issues. Am J Psychiatry 1999;156:1432–8.
[10] Rang HP, Dale MM, Ritterb JM. Pharmacology. London: Churchill Livingstone; 2003.
[11] Singer A, Wonnemann M, Müller WE. Hyperforin, a major antidepressant constituent of St. John's Wort, inhibits serotonin uptake by elevating free intracellular Na^{+}. J Pharmacol Exp Ther 1999;290: 1363–8.
[12] Butterweck V, Böckers T, Korte B, Wittkowski W, Winterhoff H. Long-term effects of St. John's Wort and hypericin on monoamine levels in rat hypothalamus and hippocampus. Brain Res 2002;930:21–9.
[13] Machado DG, Bettio LE, Cunha MP, Santos AR, Pizzolatti MG, Brighente IM, et al. Antidepressant-like effect of rutin isolated from the ethanolic extract from *Schinus molle* L. in mice: evidence for the involvement of the serotonergic and noradrenergic systems. Eur J Pharmacol 2008;587:163–8.

[14] Müller WE. Current St. John's Wort research from mode of action to clinical efficacy. Pharmacol Res 2003;47:101–9.
[15] Duke JA. Handbook of Medicinal Herbs. Florida: CRC Press; 1985.
[16] Kamalakkannan N, Prince PS. Antihyperglycaemic and antioxidant effect of rutin, a polyphenolic flavonoid, in streptozotocin-induced diabetic wistar rats. Basic Clin Pharmacol Toxicol 2006;98:97–103.
[17] Prince P Stanley Mainzen, Kamalakkannan N. Rutin improves glucose homeostasis in streptozotocin diabetic tissues by altering glycolytic and gluconeogenic enzymes. J Biochem Mol Toxicol 2006;20:96–102.
[18] Pashikanti S, de Alba DR, Boissonneault GA, Cervantes-Laurean D. Rutin metabolites: novel inhibitors of nonoxidative advanced glycation end products. Free Radic Biol Med 2010;48:656–63.
[19] Coşkun O, Kanter M, Korkmaz A, Oter S. Quercetin, a flavonoid antioxidant, prevents and protects streptozotocin-induced oxidative stress and β-cell damage in rat pancreas. Pharmacol Res 2005;51: 117–23.
[20] Kanter M, Altan MF, Donmez S, Ocakci A, Kartal ME. The effects of quercetin on bone minerals, biomechanical behavior, and structure in streptozotocin-induced diabetic rats. Cell Biochem Funct 2007;25:747–52.
[21] Matsuda H, Morikawa T, Yoshikawa M. Antidiabetogenic constituents from several natural medicines. Pure Appl Chem 2002;74:1301–8.
[22] Li YQ, Zhou FC, Gao F, Bian JS, Shan F. Comparative evaluation of quercetin, isoquercetin and rutin as inhibitors of alpha-glucosidase. J Agric Food Chem 2009;57:11463–8.
[23] Kwon O, Eck P, Chen S, Corpe CP, Lee JH, Kruhlak M, et al. Inhibition of the intestinal glucose transporter GLUT2 by flavonoids. FASEB J 2007;21:366–77.
[24] Aydın S, Öztürk Y, Altan VM, Yıldızoğlu-Arı N, Özçelikay AT. Effect of experimental diabetes and insulin on smooth muscle calmodulin levels in the rats with longterm streptozotocin-diabetes. Mol Cel Endocrinol 1996;116:67–71.
[25] Daulhac L, Mallet C, Courteix C, Etienne M, Duroux E, Privat AM, et al. Diabetes-induced mechanical hyperalgesia involves spinal mitogen-activated protein kinase activation in neurons and microglia via N-methyl-D-aspartate-dependent mechanisms. Mol Pharmacol 2006;70:1246–54.
[26] Tomić MA, Vucković SM, Stepanović-Petrović RM, Micov AM, Ugresić ND, Prostran MS, et al. Analysis of the antinociceptive interactions in two-drug combinations of gabapentin, oxcarbazepine and amitriptyline in streptozotocin-induced diabetic mice. Eur J Pharmacol 2010;628:75–82.
[27] Pepato MT, Keller EH, Baviera AM, Kettelhut IC, Vendramini RC, Brunetti IL. Anti-diabetic activity of *Bauhinia forficata* decoction in streptozotocin-diabetic rats. J Ethnopharmacol 2002;81:191–7.
[28] Bianchi C, Franceschini J. Experimental observations on Haffner's method for testing analgesic drugs. Br J Pharmacol 1954;9:280–4.
[29] Cannon KE, Hough LB. Inhibition of chemical and low-intensity mechanical nociception by activation of histamine H3 receptors. J Pain 2005;6:193–200.
[30] D'Amour FE, Smith DL. A method for determining loss of pain sensation. J Pharmacol Exp Ther 1941;72:74–9.
[31] Yarushkina N, Bogdanov A, Filaretova L. Somatic pain sensitivity during formation and healing of acetic acid-induced gastric ulcers in conscious rats. Auton Neurosci 2006;126–127:100–5.
[32] Bray GA. A concise review on the therapeutics of obesity. Nutrition 2000;16:953–60.
[33] Hainer V, Kabrnova K, Aldhoon B, Kunesova M, Wagenknecht M. Serotonin and norepinephrine reuptake inhibition and eating behavior. Ann NY Acad Sci 2006;1083:252–69.

[34] Di Francesco V, Sacco T, Zamboni M, Bissoli L, Zoico E, Mazzali G, et al. Weight loss and quality of life improvement in obese subjects treated with sibutramine: a double-blind randomized multicenter study. Ann Nutr Metab 2007;51:75–81.

[35] Rottmann CN. SSRIs and the syndrome of inappropriate antidiuretic hormone secretion. Am J Nurs 2007;107:51–8.

[36] Oliveira Margatho L, Pereira Barbosa S, Antonio De Luca L, Vanderlei Menani J. Central serotonergic and adrenergic/imidazoline inhibitory mechanisms on sodium and water intake. Brain Res 2002;956:103–9.

[37] Kumar V, Singh PN, Bhattacharya SK. Anti-inflammatory and analgesic activity of Indian *Hypericum perforatum* L. Indian J Exp Biol 2001;39: 339–43.

[38] Abdel-Salam OM. Anti-inflammatory, antinociceptive, and gastric effects of *Hypericum perforatum* in rats. ScientificWorld J 2005;8: 586–95.

[39] Korzeniewska-Rybicka I, Płaźnik A. Supraspinally mediated analgesic effect of antidepressant drugs. Pol J Pharmacol 2000;52:93–9.

[40] Calixto JB, Beirith A, Ferreira J, Santos AR, Filho VC, Yunes RA. Naturally occurring antinociceptive substances from plants. Phytother Res 2000;14:401–18.

[41] Lapa Fda R, Gadotti VM, Missau FC, Pizzolatti MG, Marques MC, Dafré AL, et al. Antinociceptive properties of the hydroalcoholic extract and the flavonoid rutin obtained from *Polygala paniculata* L. in mice. Basic Clin Pharmacol Toxicol 2009;104:306–15.

[42] Chen SR, Pan HL. Hypersensitivity of spinothalamic tract neurons associated with diabetic neuropathic pain in rats. J Neurophysiol 2002;87:2726–33.

[43] Malcangio M, Tomlinson DR. A pharmacologic analysis of mechanical hyperalgesia in streptozotocin/diabetic rats. Pain 1998;76:151–7.

[44] Hall KE, Sima AAF, Wiley JW. Opiate-mediated inhibition of calcium signaling is decreased in dorsal root ganglion neurons from the diabetic BB/W rat. J Clin Invest 1996;97:1165–72.

[45] Voitenko NV, Kruglikov IA, Kostyuk EP, Kostyuk PG. Effect of streptozotocin-induced diabetes on the activity of calcium channels in rat dorsal horn neurons. Neuroscience 2000;95:519–24.

[46] Jang MH, Shin MC, Koo GS, Lee CY, Kim EH, Kim CJ. Acupuncture decreases nitric oxide synthase expression in periaqueductal gray area of rats with streptozotocin-induced diabetes. Neurosci Lett 2003;337 (3):155–8.

[47] Dougherty PM, Willis WD. Enhancement of spinothalamic neuron responses to chemical and mechanical stimuli following combined microiontophoretic application of N-methyl-D-aspartic acid and substance P. Pain 1991;47: 85–93.

[48] Kamei J, Zushida K. The role of spinal cholecystokinin B receptors in thermal allodynia and hyperalgesia in diabetic mice. Brain Res 2001;892:370–5.

[49] Suzuki Y, Sato J, Kawanishi M, Mizumra K. Lowered response threshold and increased responsiveness to mechanical stimulation of cutaneous nociceptive fibers in streptoxotocin-diabetic rat skin in vitrocorrelates of mechanical allodynia and hyperalgesia observed in the early stage of diabetes. Neurosci Res 2002;43:171–8.

[50] Pertovaara A, Wei H, Kalmari J, Ruotsalainen M. Pain behavior and response properties of spinal dorsal horn neurons following experimental diabetic neuropathy in the rat: modulation by nitecapone, a COMT inhibitor with antioxidant properties. Exp Neurol 2001;167: 425–34.

[51] Chen X, Levine JD. Hyperresponsivity in a subset of C-fiber nociceptors in a model of painful diabetic neuropathy in the rat. Neuroscience 2001;102:185–92.

[52] Hirano K, Kato Y, Uchida S, Sugimoto Y, Yamada J, Umegaki K, et al. Effects of oral administration of extracts of *Hypericum perforatum* (St John's Wort) on brain serotonin transporter, serotonin uptake and behaviour in mice. J Pharm Pharmacol 2004;56:1589–95.

[53] Omiya Y, Suzuki Y, Yuzurihara M, Murata M, Aburada M, Kase Y, et al. Antinociceptive effect of shakuyakukanzoto, a Kampo medicine, in diabetic mice. J Pharmacol Sci 2005;99:373–80.

[54] Morgado C, Terra PP, Tavares I. Neuronal hyperactivity at the spinal cord and periaqueductal grey during painful diabetic neuropathy: effects of gabapentin. Eur J Pain 2010;14:693–9.

[55] Chavez ML, Chavez PI. Monographs on alternative therapies: Saint John's Wort. Hosp Pharm 1997;32:1621–32.

[56] Duncan MG. The effects of nutritional supplements on the treatment of depression, diabetes, and hypercholesterolemia in the renal patient. J Ren Nutr 1999;9:58–62.

[57] Lu YH, Tan RX, Sun Q, Luo L, Mao Y. Inhibitory effects of flavonoids from *Hypericum perforatum* on nitric oxide synthase. J Ethnopharmacol 2004;93:221–5.

[58] Uzbay IT, Coşkun I, Kayır H, Öztürk N, Öztürk Y. Extract of *Hypericum perforatum* blocks caffeine-induced locomotor activity in mice: a possible role of nitric oxide. Phytother Res 2007;21:415–9.

[59] Butterweck V. Mechanism of action of St John's Wort in depression: what is known? CNS Drugs 2003;17:539–62.

[60] Cott JM. In vitro receptor binding and enzyme inhibition by *Hypericum perforatum* extract. Pharmacopsychiatry 1997;30:108–12.

[61] Chatterjee S, Filippov V, Lishko P, Maximyuk O, Noldner M, Krishtal O. Hyperforin attenuates various ionic conductance mechanisms in the isolated hippocampal neurons of rat. Life Sci 1999;65:2395–405.

[62] Fiebich BL, Hollig A, Lieb K. Inhibition of substance P-induced cytokine synthesis by St. John's Wort extracts. Pharmacopsychiatry 2001;34: 26–8.

[63] Albert D, Zundorf I, Dingermann T, Müller WE, Steinhilber D, Werz O. Hyperforin is a dual inhibitor of cyclooxygenase-1 and 5-lipoxygenase. Biochem Pharmacol 2002;64:1767–75.

[64] Raso GM, Pacilio M, Di Carlo G, Esposito E, Pinto L, Meli R. In-vivo and in-vitro anti-inflammatory effect of *Echinacea purpurea* and *Hypericum perforatum*. J Pharm Pharmacol 2002;54:1379–83.

[65] Galeotti N, Vivoli E, Bilia AR, Vincieri FF, Ghelardini C. St. John's Wort reduces neuropathic pain through a hypericin-mediated inhibition of the protein kinase C gamma and epsilon activity. Biochem Pharmacol 2010;79:1327–36.