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**PJBS**

ISSN 1028-8880

**Pakistan  
Journal of Biological Sciences**

**ANSI***net*

Asian Network for Scientific Information  
308 Lasani Town, Sargodha Road, Faisalabad - Pakistan

## Effect of Decoction of *Satureja khuzestanica* Jamzad on Blood Coagulation Time, Triglyceride and Glucose Levels in Rats

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**Abstract:** The present study was designed to evaluate the effects of *S. khuzestanica* Jamzad decoction (SKJD) on blood coagulation in male rats. Effects of SKJD and *S. khuzestanica* Jamzad essential oil (SKJEO) on fasting blood glucose and triglyceride levels were evaluated as well. The SKJD significantly prolonged the Prothrombin Time (PT) and Activated Partial Thromboplastin Time (APTT) and decreased the blood concentration of glucose, but not triglyceride. The extract reduced the animal body weight as well. Meanwhile, SKJEO significantly reduced both glucose and triglyceride levels of blood.

**Key words:** *Satureja khuzestanica* jamzad, medicinal plant, blood coagulation, hypoglycemic activity

### INTRODUCTION

*Satureja Khuzestanica* Jamzad (Lamiaceae) is an annual plant, native to Iran, which grows widely in the southern and western parts of the country, especially Khuzestan and Lorestan provinces. It is famous for its therapeutic value as an analgesic and antiseptic in Iranian traditional medicine<sup>[1]</sup>. An essential oil of the aerial parts of this plant exhibited antioxidant, antidiabetic, antihyperlipidemic and reproduction stimulatory effects in rats<sup>[2]</sup>. There are some reports about antiviral<sup>[3]</sup>, anti-HIV-1<sup>[4]</sup>, antinociceptive and anti-inflammatory<sup>[5]</sup>, antibacterial and antifungal<sup>[6-9]</sup>, antispasmodic and anti-diarrhea<sup>[10]</sup>, antioxidant<sup>[11-13]</sup> and vasodilatory<sup>[14]</sup> effects of the other species of genus *Satureja* in different parts of the world. Analysis of chemical composition by GC and GC/MS has been showed the presence of p-cymene (39.6%), carvacrol (29.6%),  $\gamma$ -terpinene (18.9%), linalool (2.4%), 1, 8-cineol (1.8%), limonene (1.3%),  $\delta$ - 3-careen and myrcene (0.8%),  $\beta$ -pinene (0.4%),  $\alpha$ -pinene (0.3%),  $\beta$ -bisabadiene (0.3%),  $\beta$ -caryophyllene (0.2%), terpinene-4ol (0.2%),  $\alpha$ -thujene (0.1%),  $\alpha$ -phellandrene (0.1%), sabinene (0.1%) and thymol (<0.05%) in the essential oil of this plant<sup>[15]</sup>. Increase in bleeding after oral administration of SKJD to treatment of dysmenorrhea in young women has been reported in a hospital in Lorestan provinces; so this study was

designed to investigate the effect of SKJD on blood coagulation time in rats. The body weight, blood glucose and triglyceride levels of animals were checked in parallel too.

### MATERIALS AND METHODS

**Plant material:** The aerial parts of the *Satureja Khuzestanica* Jamzad plant were collected during the flowering stage in June 2003 from Khoram-abad in Lorestan province of Iran. The plant has been identified by the Department of Botany of Research Institute of Forests and Rangelands in Tehran. A voucher specimen (No. 58416) has been deposited at the TARI herbarium.

**Preparation of the total extract:** Dried and powdered aerial parts of the *S. Khuzestanica* Jamzad was macerated in distilled water (0.5 g/100 cc distilled water) and then it was placed in water bath in 100°C for 2 h. The supernatant was filtered; residue was concentrated by rotary evaporator apparatus and then dried at room temperature, corresponding to an 8% yield. The dried extract was diluted with distilled water to the required concentration.

**Preparation of essential oil:** The aerial parts of plant were hydro-distilled using a Clevenger type apparatus for 5 h, giving yellow oil in a 0.9% yield. The oils were

re-dried over anhydrous sodium sulfate. Both aqueous extract and essential oil were stored at 4°C throughout the experiments.

**Animals:** Male Wistar rats (Institute Pasteur of Iran), weighting 220-310 g were housed in standard cages with free access to food (standard laboratory rodents chow) and water. The animal house temperature was maintained at 23±3.0°C. All efforts were made to minimize animal suffering and to reduce the number of animals used.

**Effect of extract on coagulation tests:** Three groups of rats, each 12, received SKJD (50 and 150 mg kg<sup>-1</sup> day<sup>-1</sup>) and water for two weeks. In the day 15, animals were anesthetized intraperitoneally with Ketamine hydrochloride 100 mg kg<sup>-1</sup> (Rotex Medica, Germany) and Xylazine 2%, 16 mg kg<sup>-1</sup> (Alfasan Woerden Holland). Blood samples (1 mL) were collected from the hearts of the rats into tubes containing sodium citrate 3.5% (0.9 cc blood mixed to 0.1 mL citrate), thereafter mixture centrifuged at 2000 rpm (Eppendorf 5810R, Germany) to separate plasma. Prothrombin time (PT) was determined according to the method of Quick *et al.*<sup>[16]</sup>, using lyophilized thromboplastin prepared from rabbit's brain with calcium. APTT was determined according to the method of Bell *et al.*<sup>[17]</sup>, using Silimat (Cephalin with activator) and calcium chloride 0.025 M.

**Effect of extract on Thrombus Weight (TW):** After blood sampling of anesthetized rats for determination of PT and APTT, venous stasis of the inferior venacava was induced as following<sup>[18]</sup>. Briefly, the abdomen was opened and the vena cava was carefully dissected. A tight ligature (with cotton thread) was placed around it just below the left renal vein. Thereafter, the abdominal incision was closed and after 2 h the abdomen was reopened under anesthesia, the thrombus, if present, was removed, rinsed in distilled water, blotted on filter paper and placed in a desiccator and 24 h later the weight of the dry thrombosis was recorded.

**Effect of extract and essential oil on blood glucose and triglyceride levels:** The animals were distributed into control and treated groups with 8 animals in each group. Twelve to sixteen hours before the administration of test samples, food was withdrawn but the animals were allowed free access to water. The treated animals received SKJD (75, 150 and 250 mg kg<sup>-1</sup> day<sup>-1</sup>) and essential oil (SKJEO) (1000 ppm) dissolved in drinking water for 15 days. On the day 15, blood was taken from the heart with a sterile syringe to determine the fasting blood glucose and triglyceride. Glycaemia and triglyceride were determined by a commercial diagnostic kit (PARS AZMUN. Co, Tehran) using spectrophotometer.

**Statistical analysis:** Data are expressed as mean±SD and analyzed by One-way analysis of variance (ANOVA) which followed by the Tukey-Kramer multiple comparison tests. P-value less than 0.05 was the critical criterion for statistical significance.

## RESULTS AND DISCUSSION

**Effect of extract on coagulation tests:** As shown in the Table 1, SKJD could dose-dependently prolong PT and APTT, but this prolongation was significant only at the dose of 150 mg kg<sup>-1</sup> day<sup>-1</sup> (15.7%, p<0.05 and 19.7%, p<0.01, respectively for PT and APTT).

**Effect of extract on Thrombus Weight (TW):** The SKJD didn't have significant effect on TW (Table 1).

**Effect of extract on body weight:** The body weight was also affected by SKJD treatment; So SKJD at the dose of 150 mg kg<sup>-1</sup> day<sup>-1</sup> could significantly reduce the rat body weight compare to control group (Table 1).

**Effect of extract and essential oil on blood glucose and triglyceride levels:** SKJD could dose-dependently decrease the blood glucose of normal rats and this effect was significant only at the dose of 250 mg kg<sup>-1</sup> day<sup>-1</sup> (Table 2). SKJEO could also significantly reduce the glucose level at the dose of 1000 ppm. Decreasing the blood triglyceride was also observed with SKJEO at the dose of 1000 ppm, but not with SKJD (Table 2).

In this study, hypoglycemic and hypolipidemic effects of the *Satureja khuzestanica* Jamzad has been proved. Both SKJD and SKJEO could decrease

Table 1: Effect of *S. khuzestanica* Jamzad decoction (SKJD) (p.o.) for two weeks on Prothrombin Time (PT), Activated Partial Thromboplastin Time (APTT), Thrombus Weight (TW) and body weight of normal male rats

	Control	SKJD (50 mg kg <sup>-1</sup> day <sup>-1</sup> )	SKJD (150 mg kg <sup>-1</sup> day <sup>-1</sup> )
PT (s)	14.1±1.1	14.3±1.1	*15.64±2.0
APTT (s)	17.5±3.4	16.4±2.6	** 2.30±3.5
TW (mg)	2.5±1.4	1.7±0.8	1.20±1.5
Body weight (g)	275.0±29.0	265.1±25.7	***251.20±25.2

Values are mean±SD, n= 8, \*p<0.05, \*\*p<0.01 and \*\*\*p<0.001 vs. control

Table 2: Effect of *S. khuzestanica* Jamzad decoction (SKJD) and essential oil (SKJEO) (p.o.) for two weeks on blood glucose and triglyceride in normal rats

Treatment	Blood glucose (mg dL <sup>-1</sup> )	Blood triglyceride (mg dL <sup>-1</sup> )
Control (Drinking water)	166.0±44.0	90.0±22.0
SKJD (75 mg kg <sup>-1</sup> day)	152.0±21.0	93.0±21.0
SKJD (150 mg kg <sup>-1</sup> day)	141.0±16.0	92.5±13.0
SKJD (250 mg kg <sup>-1</sup> day)	*116.8±24.0	89.8±18.8
SKJEO (1000 ppm)	*97.0±19.0	*43.0±11.0

Values are mean±SD, n= 8. \* p<0.001 vs. control

blood glucose in normal rats. But In controversy to SKJEO, the SKJD could not decrease blood triglyceride. GC/MS analysis of SKJEO has been showed presence of  $\gamma$ -terpinene (a monoterpene which widely occurs in essential oils, but not in aqueous extracts) in relatively large portion (17.9%)<sup>[14]</sup> and there is a study which showed the hypoglycemic effects of  $\gamma$ -terpinene in rats<sup>[19]</sup>. Gathering together of these evidences, the decreasing triglyceride effects of *S. khuzestanica* Jamzad can be attributed to  $\gamma$ -terpinene ingredient of this plant. The similar effects of SKJD and SKJEO to decrease blood glucose level may be attributed to the presence of compound(s) with identical entities in them.

The body weight reduction effect of SKJD is most likely due to its effect on the blood carbohydrate levels.

In conclusion, the findings reported in this study indicate that *Satureja khuzestanica* Jamzad given by normal rats may contain novel bioactive principles with hypoglycemic, hypolipidemic and anticoagulant properties; so further studies are needed to determine the exact principles responsible to each effect.

#### ACKNOWLEDGMENTS

The authors acknowledge the support of this study by the Faculty of Medicine, Lorestan University of Medical Science, Iran. We are grateful to Mrs. Sepahvand and Abdali, Mr. Zarenjuoe and Bahrami for their help throughout the study.

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