


PHYTOCHEMICAL CONSTITUENT AND SUBCHRONIC TOXICITY EVALUATION OF ETHANOL EXTRACT OF *Saurauia vulcani* Korth Lour. LEAVES IN WISTAR RATS


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ABSTRACT

The objective of this study is to determine phytochemical constituents and to evaluate the subchronic toxic effect in the ethanol extract of *Saurauia vulcani* leaves. Phytochemical screening revealed that the extract consists of flavonoids, tannins, glycosides, saponins and steroids/triterpenoids. The extract was orally administered to the rats at doses of 50, 250, 500, 1000 mg/kg body weight (BW) for 90 days. Signs of toxicity and mortality were observed for 90 days and 118 days. The biochemistry, hematology, macro pathology, relative organ weight and histopathology examination were also performed. The macroscopic and microscopic examinations of liver, lung, kidney, spleen and heart showed normal color and texture after treatment with ethanol extract of *Saurauia vulcani* leaves at doses of 50, 250, 500 and 1000 mg/kg BW. There was no effect on hematological parameters at all doses of the extract. It can be concluded that ethanol extract of *Saurauia vulcani* leaf is nontoxic at doses 50, 250, 500 and 1000 mg/kg BW for long-term use.

Keywords: Subchronic Toxicity, Ethanol Extract *Saurauia vulcani*, Macroscopic, Microscopic

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INTRODUCTION

Diabetes is a disorder impaired metabolism of carbohydrates, proteins, and fats due to inadequate or inefficient activity of insulin. It is characterized by fasting hyperglycemia, postprandial hyperglycemia and hyperlipidemia, resulting from defects in carbohydrate, fat and protein metabolism.¹⁻³ The World Health Organization estimates that the total number of diabetics worldwide will reach 333 million in 2025 from 135 million in 1995. Diabetes mellitus is an enormous public health problem globally, associated with high morbidity and mortality. It is recognized as a wide-reaching chronic disorder affecting almost all age groups. According to a report of IDF 2017, there are 422 million people experiencing diabetes in the world and this figure is expected to rise 629 million in 2045.⁴ There are much research has been performed to manage diabetes and oral hypoglycemic agents have been developed but due to certain limitations of these agents the scientific community is still trying to explore some new oral hypoglycemic agents.⁵

Currently, antidiabetic drugs synthetic hypoglycemic agents such as sulfonylureas group, insulin treatment are given parenterally and specific enzyme inhibitors like acarbose, miglitol are used for patients. However, these drugs are expensive and usually have side effects like insulin resistance, anorexia nervosa, brain atrophy, hepatotoxicity, abdominal pain, and flatulence.⁶⁻⁸ For a long time, diabetes has been treated with medicinal plants and the scope of exploration of new antidiabetic drugs via herbs is still of interest.⁹ The use of medicinal plants is increasing because of the fewer side effects compared to synthetic drugs.¹⁰

Scientific data have indicated that medicinal plant and their products possess antidiabetic properties with less toxicity and side-effect.¹¹

A natural product that has antihyperglycemic activity and has the potential to be developed into antidiabetic is pirdot (*Saurauia vulcani* Korth). Pirdot is an endemic plant that is widely found in Sumatera Utara. Ethanol extract of pirdot leaves at a dose of 200 mg/kg BW has activity in reducing blood glucose levels on glucose tolerance test in mice.^{12,13} In research showed that *Saurauia Roxburghii* has antioxidant and antidiabetic effects.¹⁴

EXPERIMENTAL

Plant and Chemicals Material

Fresh leaves of *Saurauia vulcani* Korth were collected from Kabanjahe district, Karo subdistrict, Sumatera Utara province, Indonesia. Chemicals used were distilled water, Na CMC, ethanol 96%.

Preparation of Extracts

The air-dried and powdered leaves of *Saurauia vulcani* Korth (500 g) were macerated with ethanol 96% (3x3 d, 7.5 L). The filtrate was collected and then evaporated to give a viscous fraction and then freeze-dried to dry.

Animals

Male and female Wistar rats in age 6–8 week old, weighing 150–200 g were used. The animals were acclimatized for 7–14 days in the experiment room.

Phytochemical Screening

Phytochemical screening was conducted using a standard method for detection of alkaloids, flavonoids, tannins, glycosides, saponins and steroids/triterpenoids.¹⁵

Subchronic Toxicity Study

The subchronic toxicity evaluation followed OECD guidelines.¹⁶ There were 7 groups of the animal consists of 5 females and males rats, a negative control was given Na-CMC 0.5%, positive control was given *Saurauia vulcani* ethanol extract 50, 250, 500, and 1000 mg/kg BW, and satellite groups was given Na CMC 0.5% and *Saurauia vulcani* ethanol extract 1000 mg/kg BW with extended observations. The treatments were given orally at a single dose to the rats for 90 days. The body weight, toxic symptoms and mortality were observed for 90 days each week. For the satellite groups, further observations were carried out for 118 days aimed at evaluating the recovery process from the toxic effect that occurred.

Analysis of Hematological and Biochemistry

Blood for hematological and biochemical clinical parameters was collected by the tail, arterial and cardiac arteries of rats. Plasma was used in hematological, and serum was used to determine ALT, AST, creatinine, and BUN levels.

Macroscopic Assessment of the Organs

The organs were taken from rats namely liver, spleen, kidney, lung, and heart) then washed to observe the color, surface, and consistency. After that, the organ was dried and weighed to determine the relative organ weights which are calculated using the following formula:

$$\text{Weight Relative} = [\text{Weight of Organ}] / [\text{Weight of Animal}]$$

Microscopic Study of the Organs

The rat's organs were immersed in a 10% formalin buffer solution and then implanted in paraffin. The microtome was used to slice then stained with hematoxylin and eosin. Then, the organ slices were observed using a microscope.

Statistical Analysis

Data were analyzed with one-way analysis of variance (ANOVA) and Post Hoc Tuckey to see differences with significance ($p > 0.05$) between test groups.

RESULTS AND DISCUSSION

Phytochemical Constituent

The ethanol extract of *Saurauia vulcani* contained flavonoids, glikocyde, saponins, tannins and steroids/triterpenoids in phytochemical screening.

Signs of Toxicity

The observation was made for 90 and 118 days for the signs of toxicity such as salivation, seizure, changes of eye mucosa, diarrhea, changes of fur and skin, walking with the abdomen and walking backward. Clinical signs were evaluated qualitatively (presence or absence of the clinical signs). The clinical signs appeared after treatment by extract for 90 days. Based on the results of qualitative observations, there were no signs of toxicity coming out of all groups, both male and female animals. The activities and habits of the test animals are normal, there is no evidence of hypersalivation, diarrhea, convulsions, or signs of pain.

Body Weight Observation

There were none of the animals tested suffered weight loss in this study. All the treatment and control groups displayed weight gain, so did the females in the control group. The ethanol extract of *Saurauia vulcani* not increased the body weight and did not differ significantly ($p > 0.05$) with the normal group. Some specific indicators for toxicity are clinical symptoms and weight. Every day, animals clinical symptoms were observed and weight was measured regularly. Rapid and significant weight loss is usually a sign of an unhealthy body. Weight loss can be caused by not consuming enough food and drink, signs of a toxic or specific disease.¹⁷

Mortality

There are no rats died among treatment.

Macroscopic and Microscopic Examination

At the end of the experiment over 90 days and 118 days, macropathology and histopathology were observed. The liver and kidney of rats were observed. The microscopic evaluation of the liver on treatment groups at the dose of 50 and 250 mg/kg BW was comparable with control groups. Whereas, at the dose of 500 mg/kg BW there was sinusoidal dilatation and hydrotropic degeneration, at dose 1000 of mg/kg BW there was necrosis of hepatocyte. This condition was reversible at a satellite dose of 1000 mg/kg BW (Fig.-1 and Fig.-2).

The organs observed were Liver (A) and Kidney (B) on; (1) normal group; (2) extract group with a dose of 50 mg/kg BW; (3) extract group with a dose of 250 mg/kg BW; (4) extract group with a dose of 500 mg/kg BW; (5) the extract group with a dose of 1000 mg/kg BW.

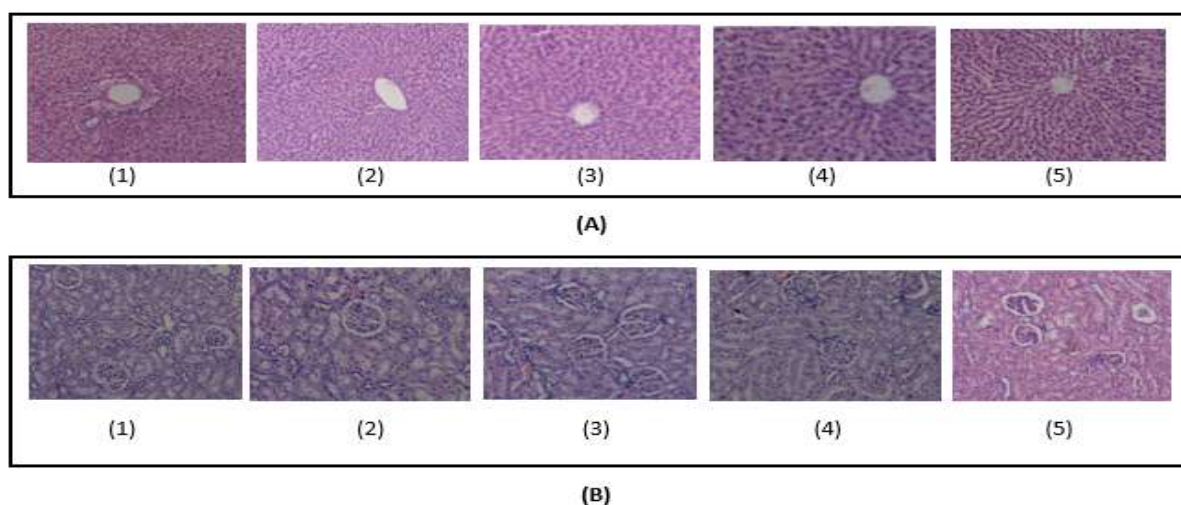


Fig.-1: Representative of microscopic changes after treatment with *Saurauia vulcani* Leaf Extract at doses of 50, 250, 500 and 1000 mg/kg BW in male rats (10×10), scale bars: 10×40 .

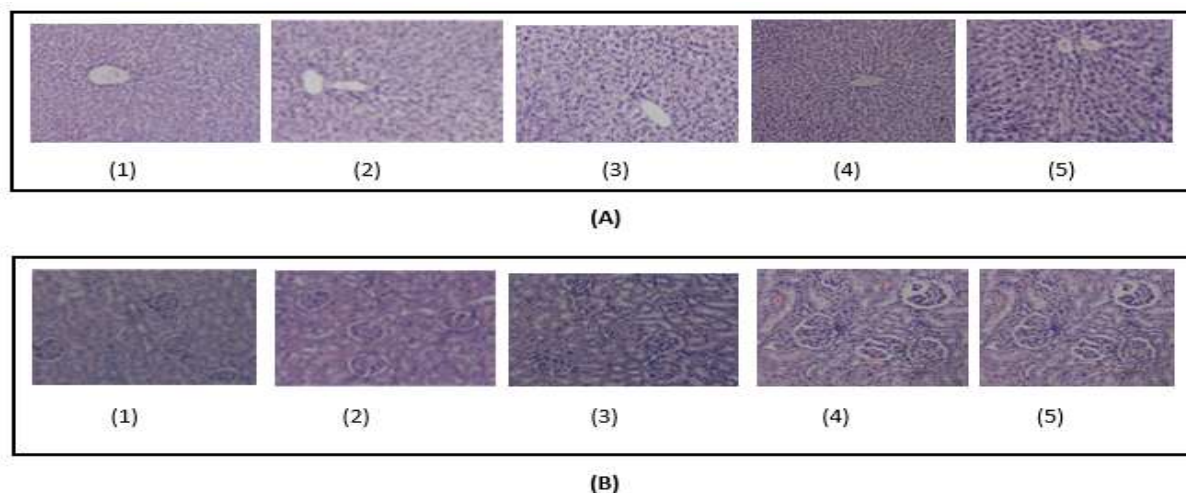


Fig.-2: Representative of microscopic changes after treatment with *Saurauia vulcani* Leaf Extract at doses of 50, 250, 500 and 1000 mg/kg BW in female rats (10 × 10), scale bars: 10 × 40.

Relative Organ Index

The effect of *Saurauia vulcani* leaf extract on male wistar rats relative organ index did not statistically significant with the normal group ($p > 0.05$), so did female wistar rats. There was weight of the liver, kidney, lung, heart, and spleen was measured.

Biochemistry Parameters

The statistical analysis using one-way ANOVA showed that there was no significant difference ($p > 0.05$) (Table-1 and Table-2).

Table-4: Effect of *Saurauia vulcani* Leaf Extract on Hematological Parameters on Male Wistar Rats (Mean ± SD)

Profiles of blood (Mean ± SD)	Group						
	CMC Na 0,5 %	EESL 50 mg/kg bw	EESL 250 mg/kg bw	EESL 500 mg/kg bw	EESL 1000 mg/kg bw	Satellite CMC Na 0,5 %	Satellite EESL 1000 mg/kg bw
WBC	7.04 ± 1.15	7.66 ± 1.29	8.32 ± 0.91	8.43 ± 0.67	9.14 ± 1.61	7.06 ± 1.47	9.18 ± 0.98
RBC	8.37 ± 1.09	8.87 ± 0.94	8.35 ± 0.97	7.95 ± 1.30	7.12 ± 1.45	8.21 ± 1.20	7.03 ± 0.57
Platelet	881.60 ± 146.64	899.40 ± 167.51	892.40 ± 167.17	905.80 ± 120.01	943.40 ± 161.13	895.60 ± 106.66	967.00 ± 102.71
Hemoglobin	15.71 ± 1.28	15.31 ± 0.86	15.56 ± 1.15	14.60 ± 0.78	14.74 ± 1.29	15.12 ± 0.90	16.69 ± 1.96
Hematocrit	48.11 ± 4.53	44.92 ± 4.18	46.60 ± 4.19	48.53 ± 4.67	47.67 ± 4.83	46.48 ± 3.00	51.79 ± 2.36
MCH	17.20 ± 0.86	17.98 ± 1.04	18.22 ± 1.24	18.10 ± 0.94	17.60 ± 1.34	17.32 ± 0.77	17.42 ± 0.90
MCV	54.60 ± 1.41	54.48 ± 2.57	54.12 ± 2.53	55.26 ± 1.56	54.38 ± 2.53	54.82 ± 0.85	52.74 ± 0.60
MCHC	32.74 ± 1.16	34.52 ± 2.12	34.68 ± 2.26	35.86 ± 2.05	35.36 ± 2.35	33.04 ± 1.12	34.72 ± 2.52
Eosinophil	3.20 ± 0.71	3.00 ± 0.53	3.04 ± 0.64	3.08 ± 0.55	3.16 ± 0.68	3.08 ± 0.60	3.18 ± 0.56
Monocyte	1.80 ± 0.53	2.76 ± 0.23	2.16 ± 0.50	2.28 ± 0.65	2.32 ± 0.66	2.00 ± 0.43	2.48 ± 0.54
Basophil	0.38 ± 0.09	0.35 ± 0.09	0.35 ± 0.08	0.36 ± 0.10	0.41 ± 0.09	0.38 ± 0.05	0.41 ± 0.06

Lymphocyte	64.54 ± 15.18	67.10 ± 14.21	71.38 ± 10.51	69.08 ± 14.66	66.86 ± 10.32	63.48 ± 4.27	68.14 ± 7.41
Neutrophil	29.28 ± 6.75	27.34 ± 6.67	31.22 ± 5.93	31.08 ± 7.21	33.52 ± 9.44	29.86 ± 1.58	32.96 ± 5.14

Table-5: Effect of *Saurauia vulcani* Leaf Extract on Hematological Parameters on Female Rats (Mean ± SD)

Profiles of blood (Mean ± SD)	Group						
	CMC Na 0,5 %	EESL 50 mg/kg bw	EESL 250 mg/kg bw	EESL 500 mg/kg bw	EESL 1000 mg/kg bw	Satellit CMC Na 0,5 %	Satellit EESL 1000 mg/kg bw
WBC	6.82 ± 0.53	6.85 ± 1.18	7.78 ± 1.05	8.14 ± 0.71	8.92 ± 1.38	6.90 ± 1.37	8.94 ± 1.25
RBC	8.13 ± 0.68	8.23 ± 0.91	7.46 ± 0.46	7.57 ± 0.80	6.89 ± 0.68	7.78 ± 0.92	6.69 ± 0.93
Platelet	789.60 ± 128.50	839.00 ± 198.49	912.40 ± 195.04	878.00 ± 123.14	795.00 ± 210.86	884.00 ± 94.15	806.20 ± 167.99
Hemoglobin	15.56 ± 0.97	25.75 ± 1.25	15.09 ± 1.09	15.62 ± 1.45	15.18 ± 1.03	15.75 ± 1.45	15.26 ± 0.19
Hematocrit	45.00 ± 4.70	46.84 ± 7.18	45.66 ± 5.64	47.32 ± 6.17	45.31 ± 6.00	46.48 ± 6.15	46.25 ± 6.05
MCH	18.30 ± 1.71	18.66 ± 1.60	18.16 ± 1.86	18.62 ± 1.67	18.36 ± 1.90	18.64 ± 1.45	18.90 ± 1.57
MCV	54.12 ± 2.98	55.37 ± 3.89	56.61 ± 1.04	55.93 ± 1.57	56.45 ± 1.69	54.18 ± 1.74	54.74 ± 2.07
MCHC	33.72 ± 2.34	35.14 ± 2.95	36.00 ± 2.79	35.56 ± 2.74	35.54 ± 2.93	35.18 ± 2.09	35.88 ± 2.96
Eosinophil	3.50 ± 0.71	3.30 ± 0.80	2.86 ± 0.84	3.22 ± 0.88	3.22 ± 0.72	3.24 ± 0.67	3.30 ± 0.62
Monocyte	2.00 ± 0.57	2.28 ± 0.71	2.24 ± 0.76	1.82 ± 0.52	2.40 ± 0.87	2.08 ± 0.64	2.56 ± 0.50
Basophil	0.31 ± 0.07	0.36 ± 0.10	0.35 ± 0.76	0.34 ± 0.09	0.36 ± 1.21	0.35 ± 0.72	0.40 ± 0.06
Lymphocyte	58.98 ± 16.16	60.62 ± 12.06	66.52 ± 11.79	67.36 ± 14.73	72.34 ± 8.38	62.38 ± 9.89	65.16 ± 9.24
Neutrophil	27.1 ± 6.34	25.92 ± 4.84	26.10 ± 5.90	29.04 ± 5.45	31.80 ± 2.68	27.48 ± 3.18	31.72 ± 1.80

The histopathological and biochemical parameters showed the effect on the liver, lung, kidney, and heart. There was not a significant difference in ALT, AST, creatinine and BUN levels between the control and extract groups for all doses 50, 250, 500 and 1000 mg/kg BW. From the result known that the effect of ethanol extract of *Saurauia vulcani* leaf was not affected on biochemistry parameters. In a previous study, it was concluded that the duration and intensity of exposure to toxic substances also can affect the form and toxicity of a particular substance.¹⁸ If adaptive biochemical responses are sustained, it can cause pathological and biochemical change or disorder.¹⁹

CONCLUSION

The ethanol extract of *Saurauia vulcani* leaves did not cause a toxic effect in long-term treatment at the dose of 50, 250, 500 and 1000 mg/kg BW. The result of macroscopic and microscopic, there were no changes observed at the dose of 50, 250, 500 and 1000 mg/kg BW compared to the normal group. In addition, the hematological and biochemistry parameters were still within the normal range. And there was no mortality along with treatment.

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