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Research Article
Evaluation of Hematological and Biochemical Activity of Ethanolic Extract of *Zygophyllum simplex* Linn. in Wistar Rats

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Abstract

Background and Objective: *Zygophyllum simplex* is commonly used in Saudi Arabia for the treatment of horny patches of skin and as an anthelmintic, analgesic and anti-inflammatory. The experiment was to aimed at to evaluate the constituents of *Zygophyllum simplex* extract and their effect on blood biochemical parameters in Wistar male rats. **Methodology:** The plant extract was orally administered to the rats (n = 10) at two doses of 250 and 500 mg kg^{-1} b.wt., for 30 days. Its effects on glucose, total cholesterol, triglycerides, aspartate aminotransferase (AST), alanine aminotransferase (ALT), serum creatinine kinase (CK), total protein, total bilirubin and blood urea were investigated. **Results:** The results showed a significant decrease in total serum cholesterol, blood glucose and CK levels. However, levels of AST, ALT, triglycerides, total bilirubin, total protein and blood urea were unaltered. **Conclusion:** In conclusion, the ethanolic extract of *Z. simplex* may act as hypoglycemic and hypolipidimic in rats.

Key words: *Zygophyllum simplex*, cholesterol, glucose, lactate dehydrogenase, triglycerides, urea, creatinine kinase

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Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Medicinal plants have been used as medicines for human disease since centuries. They are being used as medicinal plants in view of the fact that they contain chemical components of therapeutic value[1]. The medicinal value of plants lies in some chemical substances (usually secondary metabolites) that produce a definite physiological action on the human body. The most important of these bioactive compounds are alkaloids, flavanoids, tannins and phenolics[2]. These phytochemicals are non-nutritive plant chemicals produced by the plants to protect themselves as they have disease preventive properties. There are more than thousand known phytochemicals. As per recent studies, these phytochemicals also protect humans against diseases.

The genus Zygophyllum L., the largest genus of Zygophyllaceae, comprises about 100 known species from the Mediterranean to central Asia, South Africa and Australia. It includes perennial shrubs or under-shrubs, with succulent cylindrical rarely flattened leaves, simple or 1-2 foliate[3]. Seven species of Zygophyllum grow in Saudi Arabia are *Zygophyllum album*, *Z. migahidii*, *Z. gaetulum*, *Z. coccineum*, *Z. decumbens*, *Z. mandavillei* and *Z. simplex*[4].

Zygophyllum simplex Linn is an annual, suberect to procumbent, 8-20 cm tall, profusely branched, glabrous herb. Its stem and branches are pale-green or purplish, minutely striate, leaves succulent and simple; seeds smooth and fusiform. It is distributed in Pakistan, Rajasthan, Kutch region of Gujarat, Saudi Arabia, Egypt and South-West Africa[5-7].

The leaf juice of *Z. simplex* acts as antiseptic for skin and useful in treating horny patches of skin[8]. *Zygophyllum simplex* is being used as analgesic and its seeds are anthelmintic[9] and anti-inflammatory[10]. In view of these properties, the present study was aimed to evaluate the constituents of *Zygophyllum simplex* extract and their effect on blood biochemical parameters in Wistar male rats.

MATERIALS AND METHODS

Plant material: Fresh aerial parts of *Zygophyllum simplex* L. (Zygophyllaceae) were collected from Almadina area during the flowering stage. The identity was established by Prof. of Floriculture and Medicinal plants, Department of Biology, Faculty of Science, Taibah University, Al-Madinah Al-Munawwarah, Saudi Arabia.

Ethanol extract of *Z. simplex* was prepared by soaking 100 g air dried powder of aerial plant parts in 1 L of 70% ethanol at 45°C for 2 days. The mixture was filtered to remove particulate matter and lyophilized afterwards, the resulting powder (22 g) was then stored -20°C until use[11].

Screening of the chemical constituents of aqueous extract of *Z. simplex* was done using thin layer chromatography according to the method of Wagner and Bladt[12]. For comparison purposes, a reference solution containing rutin (Fluka, USA), quercetin (Merck, USA), hiperoside (Merck, USA), coumarin (Fluka, USA), eucalyptol (Fluka), menthol (Fluka, USA), caffeic acid (Merck, USA) and chlorogenic acid (Merck, USA) was used.

Animals: Prior to the initiation of the experiment an ethical clearance for performing the experiments on animals was obtained from Institutional Animal Care and Use Committee (IACUC). Thirty mature male Wistar rats (*Rattus norvegicus*) weighing 200-250 g were provided by the animal house. The rats were individually housed in cages and maintained under controlled environmental conditions such as temperature (20±2°C), relative humidity (45-55%) and 12 h dark/light cycle. All rats were fed with rodent pellet diet and water *ad libitum* under strict hygienic conditions. After one week of acclimatization according to set criteria[13] the rats were randomly divided into three different groups, each having ten rats.

Determination of LD_{50} toxicity: Acute toxicity of the plant was determined by the calculation of LD_{50} which represents the dose that can be fatal to 50% of any rats group[14]. Determination of LD_{50} in rats was done to determine the proper treatment dose that should be used in this experiment. For this procedure, 24 albino rats were needed and used, distributed into four different groups each containing 6 albino rats (300 g). Different dosage of ethanol extract of *Z. simplex* root viz., 200, 400, 600, 1000, 3000, 4000 and 5000 mg each dissolved in 0.2 mL normal saline (NaCl 0.9%) was administered intraperitoneally to each corresponding rat group. Rats were then housed in transparent plastic cages under controlled temperature of 24°C and monitored for 24 h representing the time length of this experiment for any toxic symptoms. The number of deceased rats was counted in each group after 24 h and the percentage of mortality was calculated. Treated rats were compared with six controlled rats that had received an intraperitoneal infusion of 1 mL distilled water alone, in the same manner as the experimental rats.

Rat grouping and treatments: Rats were divided in three groups each containing ten rats weighing approximately 225 g each.

- **Group A:** Untreated normal rats (control) receiving 2 mL normal saline
- **Group B:** Rats treated with *Z. simplex* ethanol extract at a dose of 250 mg kg^{-1} b.wt., dissolved in 2 mL normal saline
- **Group C:** Rats treated with *Z. simplex* ethanol extract at a dose of 500 mg kg^{-1} b.wt., dissolved in 2 mL normal saline

Body and organ weights: The initial and final body weight of the animals were recorded. The vital organs viz., pancreas, liver, kidney, heart and spleen were taken out and weighed to assess the effect of *Z. simplex*.

Hematological analysis: The blood pooled from all the animals in each group was collected into bottles containing ethylenediamine tetra acetic acid (EDTA) as anticoagulant. The hematological composition of the blood was measured using all groups of rats (n = 10).

The hematological parameters viz., Red Blood Cells (RBCs) and White Blood Cells (WBCs) were estimated by using the improved neubauer counting chamber as described by Dacie and Lewis[15].

Hemoglobin was measured by using Drabkin's solution as described in (Drabkin's Cynmetheglobin method by Fisher's Haemo-photometer)[16]. Packed Cell Volume (PCV) was determined by Wintrobe method[17].

Biochemical analysis: At the end of the study, rats were fasted overnight, anesthetized with thiopental sodium @ 50 mg kg^{-1} [18]. Blood sample were collected by cardiac puncture; centrifuged at 2000×g for 15 min, after 30 min of collection and stored at -20°C until use. The heart, aorta and liver were collected, cleaned from the fat and adhering connective tissue, weighed and stored at -20°C until use.

Serum was obtained by centrifugation of blood at 3000 rpm for 15 min. Serum obtained was used to examine the following biochemical tests: serum aspartate aminotransferase (AST), serum alanine aminotransferase (ALT), alkaline phosphatase (ALP), serum creatinine kinase (CK), serum creatinine, lactate dehydrogenase (LDH), total protein, creatinine and blood urea using commercial kits (BioSystems S.A. Costa Brava30, Bacelona-Spain). Parameter concentrations were determined using a ultraviolet (UV) visible spectrophotometer (Milton Roy Spectronic 601 Spectrophotometer USA).

Serum triglycerides (Tgs) and Total Cholesterol (TC) were measured colorimetrically using assay kits (Stanbio, Texas, USA) according to the manufacturer instructions using UV-visible spectrophotometer (UV-1601PC, Shimadzu, Japan). The LDL cholesterol was calculated by using Friedewald's formula[19]:

$$\text{LDL (mg dL}^{-1}\text{)} = \text{TC-(HDL+VLDL), VLDL cholesterol was calculated as TG/5 and LDL}$$

Statistical analysis: Data obtained were expressed as Mean±SEM and analyzed using the Statistical Package of Social Sciences (SPSS) program version 17, (Chicago, IL, USA). For all parameters, comparison among groups was carried out using one way analysis of variance (ANOVA) followed by Dunnat's multiple comparison tests. All p values reported are two-tailed and $p<0.05$ was considered significant.

RESULTS

Chemical constituents in aqueous extract of *Z. simplex*: The present phytochemical study of the aerial parts of *Z. simplex* resulted in isolation of seventeen compounds belonging to different chemical classes. The results obtained from the preliminary phytochemical screening of *Zygophyllum simplex* L. revealed the presence of alkaloids, flavonoids and saponins as major components. The presence of carbohydrates and/or glycosides, coumarins, sterols and/or triterpenes, tannins and cardiac glycosides was also recorded.

LD_{50} toxicity: The toxicity study revealed the non-toxic nature of *Z. simplex* extracts at doses up to 1000 mg kg^{-1}. Rats did not show any drug-induced physical signs of toxicity during the whole experimental period and no deaths were reported.

Body and organ weights: During the experimental period, no deaths among experimental animals were recorded. Changes in body weight in treated groups during the experimental period were similar to those in the control group (Table 1). There were no significant ($p<0.05$) changes in the absolute organ weights between various treatment groups (250-500 mg kg^{-1}) as compared to the control group (Table 1).

Hematological analysis: Table 2 shows the various hematological parameters of different groups of rats. There were no significant ($p<0.05$) differences in hematological parameters between control group and various treatment groups (250, 500 and 1000 mg kg^{-1}).

Table 1: Effect of *Zygophyllum simplex* ethanol extracts on body and organ weights of rats

		Organic weight (g)				
Treatment groups	Body weight (g)	Pancreas	Spleen	Heart	Kidney	Liver
Control	213.54±14.17	0.36±0.12	0.18±0.11	0.26±0.14	0.62±0.07	2.89±1.25
Z. simplex ethanolic extraction (250 mg kg^{-1})	233.79±15.72	0.39±0.22	0.15±0.15	0.25±0.12	0.63±0.23	2.94±1.23
Z. simplex ethanolic extraction (500 mg kg^{-1})	224.67±17.36	0.40±0.17	0.14±0.13	0.25±0.22	0.63±0.14	3.04±1.46

Mean±SEM, n = 10

Table 2: Effect of *Zygophyllum simplex* ethanol extract on hematological parameters of rats

	Treatment groups		
Parameters	Control	*Z. simplex* ethanol extract (250 mg kg^{-1})	*Z. simplex* ethanol extract (500 mg kg^{-1})
WBC ($\times10^3$ μL^{-1})	4.12±2.17	4.08±1.55	4.03±2.71
RBC ($\times10^6$ μL^{-1})	6.79±1.65	6.76±2.62	6.77±2.18
PCV (%)	40.50±1.41	39.95±25.9	40.03±25.9
Hb (g dL^{-1})	14.90±0.15	14.50±27.77	14.80±27.77
MCV (fL)	58.10±1.73	59.09±1.47	59.12±1.28
MCH (pg)	21.38±0.66	21.44±0.92	21.86±0.84
MCHC (g dL^{-1})	36.79±1.14	36.29±1.06	36.97±1.12

Mean±SEM, n = 10, WBC: White blood cells, RBC: Red blood cells, PCV: Packed cell volume, Hb: Haemoglobin, MCV: Mean corpuscular volume, MCH: Mean corpuscular haemoglobin, MCHC: Mean corpuscular haemoglobin concentration

Table 3: Effects of *Zygophyllum simplex* ethanol extract on biochemical parameters of rats

	Treatment groups		
Parameters	Control	*Z. simplex* ethanol extract (250 mg kg^{-1})	*Z. simplex* ethanol extract (500 mg kg^{-1})
Glucose (mg dL^{-1})	93.82±2.51	84.26±2.81[a]	78.21±2.11[a]
Cholesterol (mg dL^{-1})	52.47±4.72	44.18±3.86	43.25±6.44[a]
Trigelyceride (mg dL^{-1})	51.67±1.70	50.14±2.19	48.81±2.64
AST (IU L^{-1})	66.55±4.32	63.94±6.33	61.18±3.88
ALT (IU L^{-1})	34.77±4.29	33.12±3.57	35.11±4.91
CK (IU L^{-1})	439.36±4.19	418.72±3.44	415.13±3.66
LDH (IU L^{-1})	216.18±1.30	218.67±1.72	291.00±2.85
Total protein (mg dL^{-1})	7.20±1.44	7.40±1.21	7.70±1.04[a]
Creatinine (mg dL^{-1})	0.31±0.17	0.34±0.24	0.35±1.07
Urea (mg dL^{-1})	26.12±1.48	27.83±1.61	29.14±1.15[a]

Mean±SEM, n =10, [a]Significant at ($p<0.05$) as compared to normal control, AST: Asparate aminotransferase, ALT: Alanine aminotransferase, CK: Creatinine kinase, LDH: Lactate dehydrogenase

Biochemical analysis: Table 3 shows the various biochemical parameters of different groups of rats. There was a significant ($p<0.05$) decrease in the blood glucose levels in rats administered with 250 and 500 mg kg^{-1} b.wt., *Z. simplex* ethanolic extract when compared with the control. Further, there was no significant ($p>0.05$) effect in other parameters when compared with the control group. Also significant increase in total protein and blood urea nitrogen ($p<0.05$) when compared with the control group.

DISCUSSION

Usage of herbal preparations without control dosage coupled with non- availability of adequate scientific studies on their safety has raised concerns on their toxicity[20]. Toxicity studies in animal model are commonly used to evaluate potential health risk in humans caused by intrinsic opposing effects of chemical composites/plant extracts[21].

Organ body weight ratio is a useful index of swelling, atrophy or hypertrophy[22]. Body weight did not show significant difference, but organ weights especially the liver showed a non-significant increase at dose 500 mg kg^{-1}. This increase in liver weight with no change in other parameters related to liver function cannot be considered as toxicological effect and may be due to increased synthesis of protein or incidentally.

Assessment of hematological parameters can be used to determine the extent of deleterious effect of foreign compounds including plant extracts on the blood constituents of an animal. Such toxicity testing is relevant to risk evaluation as changes in the hematological system have higher predictive value for human toxicity, when data are translated from animal studies[23]. It can also be used to explain blood relating functions of chemical compounds/plant extract[24]. The hematological parameters such as RBC and WBC count, hemoglobin concentration, hematocrit value and RBC indices did not show any significant changes (Table 2).

The effect of *Z. simplex* extract on biochemical parameters (Blood glucose, total cholesterol, trigelyceride, total serum protein, etc.) showed that the plant did not affect most of these parameters except the blood glucose levels. The different doses of ethanolic extract of *Z. simplex* produced a significant reduction in blood glucose level in a dose dependent manner. The dose of 250 mg kg^{-1} showed a significant ($p<0.05$) reduction, whereas, dose of 500 mg kg^{-1} showed a highly significant ($p<0.001$) reduction in the blood glucose level. The total serum protein and serum urea, which were increasing to significant values. These elevated values may be due to effect of *Z. simplex* on amino acid synthesis and degradation, which lead to increase the uses of glucose as source of energy instead of protein and result in preserve the protein[25].

High level of transaminases such as ALT and AST is a sign for hepatic damage[26,27] and are measured clinically as a part of a diagnostic evaluation of liver function test. Throughout the entire study, there was no significant changes in the level of transaminases (ALT and AST).

Creatinine Kinase (CK) is an enzyme found primarily in the heart and skeletal muscles and to a lesser extent in the brain[28]. Significant injury to any of these structures will lead to a measurable increase in CK levels[28] but no such increase in CK levels was observed in the present study.

Lactate dehydrogenase (LDH) is a cytoplasmatic enzyme present in major organ systems[29]. The extracellular appearance of LDH is used to detect cell damage or cell death[30]. It is released into the peripheral blood after cell death caused byischemia, excess heat or cold, starvation, dehydration, injury, exposure to bacterial toxins, after ingestion of certain drugs, chemical poisonings etc.[30]. In this study, LDH levels did not show any alteration to significant value among various treatment groups including the control.

CONCLUSION

In conclusion, the results of hematological and biochemical parameters indicated that the ethanolic extract of *Z. simplex* had no adverse effect on various organs of male Wistar rats. The ethanolic extract of *Z. simplex* may act as hypoglycemic and hypolipidimic. It proved to be safe as an oral remedy at the dose rates of 250 and 500 mg kg^{-1} b.wt., with 50 mg kg^{-1} b.wt., dose rate as relatively safe.

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