Sub-chronic toxicity study of a novel herbal-based formulation (Semelil) on dogs

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ABSTRACT

Semelil (ANGIPARS™), a novel herbal-based compound containing extract of *Melilotus officinalis*, was formulated for treatment of chronic wounds, especially diabetic foot ulcer. The purpose of this study was to investigate safety and toxicity effects of intramuscular administration of Semelil in dogs.

Preliminary one-month study with Semelil was performed on 8 male and female dogs divided into 2 groups, test and control, four animals each. Semelil was administered intramuscularlyat a dose of 0.07 ml/kg body wt. once a day to the animals of the test group, while the control group received sterile saline. During experiments, general state of the animals including the dynamics of body weight changes, appetite, motor activity and behavior, hair condition, ECG parameters, rectal temperature of animals and data of hematological and biochemical tests were monitored for signs of toxicity and side-effects. Finally, morphology and histology analyses were performed using standard methods.

No adverse health or toxicity effects were observed through the course of the study. No damaging consequences of Semelil injections on the functional state of main organs of the experimental animals were found. This observation gave a good evidence of a favorable safety profile compatible with potential therapeutic use of Semelil.

Keywords: Semelil, ANGIPARS™, *Melilotus Officinalis*, Toxicology, Dogs

INTRODUCTION

Diabetes mellitus is one of the most important causes of disability and death in the world (1-3). Serious long-term complications including microvascular dysfunctions cause poor healing wounds, particularly in the feet and legs which result in amputation. Diabetes can dull the sensitivity of nerves called peripheral neuropathy and thus patients might not become aware of a sore spot. The ulcer can become infected and the area may not readily heal because of poor blood circulation. Proper treatment can help ulcers heal and prevent new ones from developing (4-6). Therefore, appropriate interventions for wound care for reducing amputation rates are essential. Herbal extracts are among the medications which can be used easily on wounds. There are several evidences about the putative clinical usefulness of *Melilotus* sp. with both internal and external administration routes for ameliorating skin aging, micro-circulation improvement, and anti-inflammatory effects. Its combination with *Ginkgo biloba* has been helpful for treatment of thrombosis, circulation and sub-acute degenerative problems (7-9).

Prior to the initiation of human clinical trials of novel drugs, the safety of their application is to be proved. Generally this is accomplished by the implementation of general preclinical toxicity experiments to uncover potential poisonous effects of any drug in animals. The aim of the present study was to evaluate the animal safety of Semelil (ANGIPARS™) as a new phytotherapeutic candidate for wound healing.

MATERIALS AND METHODS

Semelil herbal extract (ANGIPARS™) was prepared and delivered by ParsRoos Co. (Tehran, Iran).

A set of reagents of the Labsystems (Finland) was applied for measurement the glucose level. Total protein, creatinine, urea, total bilirubin, the

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activities of lactate dehydrogenase and alkaline phosphatase were determined by a set of reagents of Diakom-Sinteco, Co. (Russia). Triglyceride and cholesterol levels were measured by a set of reagents from DiaSys Co. An automatic cell counter Pikoskel (Hungary) was used for counting blood elements. The activity of alanine aminoteransferase was determined using a set of reagents of Corway (Poland). A biochemical semiautomatic analyzer FP-901 Labsystems (Finland) was utilized for assessment of other biochemical parameters and enzyme activities. The ECG examination of dogs was performed by a Biograph device (USA).

Drug solution preparation

The Semelil was freshly diluted in sterile solution of sodium chloride 0.9% at 1:10 ratio and then was administered to animals**.**

Animals

Healthy young dogs, 12-14.5 kg (4 males and 4 females), which had been acclimated to laboratory conditions and had not been subjected to previous experimental procedures, were enrolled. Conventional laboratory diet was used with an unlimited supply of drinking water. Artificial lighting, with the sequence being 12 hours light, 12 hours dark was used (10-12). Animals were handled in accord with the laboratory animal welfare guidelines.

Sub-chronic toxicity evaluation

The protocol of the study was approved by the Institute Animals Ethics Committee. Sub-chronic toxicity tests were performed on 8 male and female dogs divided into 2 groups of test and control, 4 animals in each, and Semelil was administered at a dose of 0.07 ml/kg once a day intramuscularly to the test group for a period of one month. The control group received sterile saline solution.

During the experiments, general state of the animals including the dynamics of body weight changes, appetite, motor activity, hair condition, the ECG parameters, rectal temperature and behavior of the animals were recorded (13,14). At the baseline of the study and one month after the first administration, a blood sample (2-2.5 ml) was taken from subcutaneous vein of each animal and the hematological, and biochemical analyses were conducted.

Gross necropsy

At the end of the sub-chronic experiments, the animals were euthanized by over-dosage of thiopental with pre-medication of droperidol in order to conduct histopathological examination of the internal organs and tissues. All animals in the study were subjected to a full, detailed gross necropsy which included careful examination of the external surface of the body, all orifices, and the cranial, thoracic and abdominal cavities and their contents. The tissues were preserved in the appropriate fixation medium for intended subsequent histopathological examination.

Statistical Analyses

All determinations were subjected to calculation of mean and SD for the outcome variable. Statistical analyses were performed by using SPSS software, v. 11.5. The probability levels of significance were based on non-parametric tests. Statistically significance level was set at $p<0.05$.

RESULTS AND DISCUSSION

Sub-chronic toxicity evaluation: The data for all tests showed that I.M. injection of Semelil at the dose of 0.07 ml/kg had no effects on the general state and behavior of the animals. We did not detect any significant changes in hematological (Tables 1 and 2) and biochemical parameters including total serum proteins, serum bilirubin, serum glucose, triglycerides, cholesterol, blood urea and creatinine (Table 3), in test group compared to control group after 1 month ($p>0.05$). The ECG data (Table 4) and activities of hepatic enzymes (Table 5) of test and control groups did not show any difference and corresponded to the physiological range of these parameters. The body weight and rectal temperature of animals in experimental group, during all period of study were not significantly different ($p>0.05$) from that of control animals (Table 6).

Although small animal, like mice and rats, are most frequently used animals in preclinical drug developments, phenotypic characterization of the behavior, physiology and biochemistry tests on rodent models have some limitations. For this reason, usage of bigger animals, like dogs, is very important in advanced preclinical studies.

Pathomorphological examination of animals

Microscopic data together with the data of macroscopic evaluation of the animals' organ showed that both test and control groups were practically healthy. According to the data of histological examination, no toxic or allergic effects of Semelil were detected in the test group. No pathological changes of the internal organs and no local irritating effects of the drug preparation were observed in the test group during the study period.

Our experiments showed that Semelil administrated I.M. in dogs has a low-toxicity. We did not observe any significant change in total

Table 1. Hematological parameters in dogs (1-month study) after I.M. injection of Semelil at the dose of 0.07 ml/kg. The control group received 0.9% sodium chloride.

Observation period	Control	Semelil (0.07 ml/kg)
Erythrocytes, 10^{12} /l		
Before introduction	7.2 ± 0.4	6.8 ± 0.6
1 month after introduction	6.9 ± 0.4	7.4 ± 0.4
Leukocytes, 10^{9} /l		
Before introduction	9.4 ± 0.6	9.9 ± 0.6
1 month after introduction	9.8 ± 0.4	9.6 ± 0.4
Thrombocytes, 10^{9} /l		
Before introduction	386 ± 46	413 ± 72
1 month after introduction	396 ± 58	388 ± 58
Hemoglobin, g /l		
Before introduction	106 ± 6	108 ± 6
1 month after introduction	108 ± 8	109 ± 6

Data are means ± standard deviation, All significance levels between groups were p>0.05.

Table 2. The percentage of leukocytic formula of the blood of dogs (after 1 month study), I.M. injection of Semelil at the dose of 0.07 ml/kg. The control group received 0.9% sodium chloride.

Animal groups	Basophils	Eosinophils	Neutrophils		Lymphocytes	Monocytes
			Stab neutrophils	Segmented neutrophils		
Control	0.6 ± 0.4	5.5 ± 1.0	4.2 ± 0.6	61.9 ± 4.8	32.2 ± 3.2	4.6 ± 0.6
Semelil 0.07ml/kg	0.5 ± 0.4	5.8 ± 0.8	4.6 ± 0.6	59.2 ± 6.4	25.2 ± 3.6	4.7 ± 1.0

Data are means, ± standard deviation, All significance levels between groups were p>0.05.

Table 3. Biochemical parameters in dogs (1-month study) after I.M. injection of Semelil at the dose of 0.07 ml/kg. The control group received 0.9% sodium chloride.

Observation periods	Control	Semelil (0.07 ml/kg)
Total serum protein (g/l)		
Before introduction	69.12 ± 11.5	70.06 ± 4.1
1 month after introduction	68.81 ± 12.1	64.65 ± 5.2
Total bilirubin µmol /l		
Before introduction	6.88 ± 0.65	7.59 ± 0.7
1 month after introduction	7.02 ± 0.55	6.87 ± 2.75
Glucose (mmol/l)		
Before introduction	6.26 ± 0.8	6.02 ± 0.55
1 month after introduction	6.33 ± 0.44	6.42 ± 0.56
Urea (mmol /l)		
Before introduction	9.69 ± 0.9	8.78 ± 0.56
1 month after introduction	9.53 ± 0.86	9.35 ± 0.22
Creatinine (µmol /l)		
Before introduction	59.62 ± 9.86	54.86 ± 8.34
1 month after introduction	61.32 ± 9.86	65.43 ± 14.54
Total cholesterol (mmol /l)		
Before introduction	2.39 ± 0.24	2.59 ± 0.44
1 month after introduction	2.67 ± 0.52	2.23 ± 0.58
Triglyceride (mmol /l)		
Before introduction	0.79 ± 0.12	0.65 ± 0.14
1 month after introduction	0.74 ± 0.12	0.43 ± 0.1

Data are means ± standard deviation, All significance levels between groups were p>0.05.

Table 4. ECG parameters in dogs (1-month study) after I.M. injection of Semelil at the dose of 0.07 ml/kg. The control group received 0.9% sodium chloride.

ECG parameters	Before administration		After 1 month	
	Control	Semelil (0.07 ml/kg)	Control	Semelil (0.07 ml/kg)
R-R, msec	467 ± 76	458 ± 70	466 ± 64	462 ± 44
P-Q, msec	86 ± 24	89 ± 32	85 ± 42	89 ± 32
Q-T, msec	139 ± 38	146 ± 36	138 ± 30	139 ± 30
QRS, msec	40 ± 12	39 ± 6	39 ± 8	39 ± 12
ST, msec	0.4 ± 0.4	0.3 ± 0.2	0.3 ± 0.2	0.4 ± 0.2
TP, msec	189 ± 38	192± 56	186 ± 44	194 ± 46
P, msec	0.30 ± 0.06	0.29 ± 0.1	0.26 ± 0.08	0.28 ± 0.08
R, msec	1.38 ± 0.24	1.39 ± 0.48	1.42 ± 0.44	1.38 ± 0.46
T, msec	0.30 ± 0.12	0.32 ± 0.12	0.29 ± 0.12	0.28 ± 0.1
Heart rate/min	139 ± 30	145 ± 30	138 ± 30	146 ± 24

Data are means ± standard deviation, All significance levels between groups were p>0.05.

Table 5. Hepatic enzyme activity in dogs (1-month study) after I.M. injection of Semelil at the dose of 0.07 ml/kg. The control group received 0.9% sodium chloride.

Observation periods	Control	Semelil (0.07 ml/kg)
Alkaline phosphatase unit /l		
Before introduction	199.54 ± 20.5	204.62 ± 37
1 month after introduction	189.17 ± 25	194.28 ± 36
Lactate dehydrogenase unit /l		
Before introduction	163.24 ± 22.8	182.43 ± 37.3
1 month after introduction	164.28 ± 21.5	172.33± 35.7
Aspartate aminotransferase unit /l		
Before introduction	40.22 ± 6.4	49.77± 13.7
1 month after introduction	39.70 ± 7.6	34.33± 4.8
Alanine aminotransferase unit /l		
Before introduction	59.22 ± 6.7	64.99 ± 8.3
1 month after introduction	61.37 ± 7.1	60.05 ± 4.9

Data are means ± standard deviation; All significance levels between groups were p>0.05.

Table 6. The dynamics of body weight changes and rectal temperature in dogs (1-month study) after I.M. injection of Semelil at the dose of 0.07 ml/kg. The control group received 0.9% sodium chloride.

Observation periods	Control	Semelil (0.07 ml/kg)
Body weight, % initial weight		
After 1 month	103.6± 5.6	105.4 ± 5.2
Rectal temperature, °C		
Before introduction	38.8 ± 0.2	38.8 ± 0.4
After 1 month	38.6 ± 0.4	38.7 ± 0.2

Data are means ± standard deviation, All significance levels between groups were p>0.05.

hematological and biochemical parameters in test groups compared to control group. The data indicated the stability of protein-productive function of liver, hepatic enzyme activity, carbohydrate metabolism, pancreas function, lipid metabolism and excretory function of kidneys during the study period.

CONCLUSIONS

The present study is an evidence of good tolerance and safety of Semelil (ANGIPARS™) and without undesirable effects on the functional state of the main organs of these animals after one-month treatment. Taking these results into account, Semelil could be recommended for clinical trials in humans. Since antioxidants are believed to be useful in the management of diabetes and its complications (15) antioxidant potential of Samelil should be focus of the next step of trials.

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