ORIGINAL ARTICLE

TOXICITY AND ANTI-INFLAMMATORY ACTIVITY OF *ZIZIPHUS JUJUBA* MILL. LEAVES

MARILENA-VIORICA HOVANEŢ[1], ROBERT VIOREL ANCUCEANU[1]*, MIHAELA DINU[1], ELIZA OPREA[2], EMMA ADRIANA BUDURA[3], SIMONA NEGREŞ[4], BRUNO ŞTEFAN VELESCU[4], LIGIA ELENA DUŢU[5], IULIANA ADRIANA ANGHEL[1], IRINA ANCU[6], ELENA MOROŞAN[7], OANA CRISTINA ŞEREMET[4]

[1]*Department of Pharmaceutical Botany, Faculty of Pharmacy, University of Medicine and Pharmacy, "Carol Davila", 6 Traian Vuia Street, Bucharest, Romania*
[2]*Department of Organic Chemistry, Biochemistry and Catalysis, University of Bucharest, Faculty of Chemistry, 4-12 Regina Elisabeta Boulevard, Bucharest, Romania*
[3]*Department of Pharmaceutical Technology Faculty of Pharmacy, University of Medicine and Pharmacy, "Carol Davila", 6 Traian Vuia Street, Bucharest, Romania*
[4]*Department of Pharmacology and Clinical Pharmacy, Faculty of Pharmacy, University of Medicine and Pharmacy, "Carol Davila", 6 Traian Vuia Street, Bucharest, Romania*
[5]*Department of Pharmacognosy, Phytochemistry and Phytotherapy, Faculty of Pharmacy, University of Medicine and Pharmacy, "Carol Davila", 6 Traian Vuia Street, Bucharest, Romania*
[6]*Research Institute for Fruit Growing, Rilomania, OP 1, CP 73, Argeş County, Romania*
[7]*Department of Clinical Laboratory and Food Safety, Faculty of Pharmacy, University of Medicine and Pharmacy, "Carol Davila", 6 Traian Vuia Street, Bucharest, Romania*

**corresponding author: robert.ancuceanu@gmail.com*

Manuscript received: February 2016

Abstract

A dried ethanolic extract, obtained from *Ziziphus jujuba* Mill. (*Rhamnaceae*) leaves, was tested in order to evaluate the effects on plant cell division, its acute oral toxicity and anti-inflammatory properties. Two inflammation experimental models on rats, with carrageenan and kaolin as inflammatory agents, were used. The experimental group was treated by gavage for 7 days with 5 % aqueous suspension of the extract (500 mg/kg b.w.) and the control group with 1 mL/100 g b.w. distilled water. The reference group, in the 7th day, was treated with 0.1 % indomethacin solution (10 mg/kg b.w.). No toxic symptoms or mortality were observed in any animals. In both inflammation models a very weak anti-inflammatory effect was detected, a little better in the kaolin model but generally inferior to indomethacin. A statistically significant inhibitory effect on the growth of *Triticum* radicles was observed at 0.5% and 1% concentrations, accompanied by microscopic signs of cytotoxicity.

Rezumat

Un extract etanolic uscat obţinut din frunzele de *Ziziphus jujuba* Mill. (*Rhamnaceae*) a fost testat pentru a se evalua efectele asupra diviziunii celulei vegetale, toxicitatea orală acută şi proprietăţile antiinflamatoare. Au fost folosite 2 modele experimentale de inflamaţie la şobolani, cu carrageenan şi caolin ca agenţi de inducere a inflamaţiei. Grupul testat a fost tratat timp de 7 zile, prin gavaj, cu 500 mg/kg corp extract (suspensie apoasă 5 %), iar grupul de control cu 1 mL/100 g apă distilată. Grupul de referinţă, în a 7 zi de tratament, a fost tratat cu 10 mg/kg indometacin (soluţie 0,1 %). La animalele utilizate pentru evaluarea toxicităţii acute nu s-au înregistrat mortalitate sau manifestări toxice. În ambele modele experimentale a fost detectat un foarte slab efect antiinflamator, puţin mai intens în modelul cu caolin dar inferior indometacinului. La concentraţiile 0,5% şi 1% s-a observat un efect inhibitor semnificativ statistic asupra creşterii radiculare, însoţit la nivel microscopic de semne de citotoxicitate.

Keywords: *Ziziphus jujuba* Mill. leaves, ethanolic extract, toxicity, anti-inflammatory properties

Introduction

Ziziphus jujuba Mill. (*Rhamnaceae*), known as jujube red date, is a thorny tree distributed in the tropical and subtropical regions of Asia. Its fruit, named dazao in China, is widely used as a food and food additive and has a high nutritional value [18]. This species has been used as a remedy in oriental folk medicine for thousands of years as a tonic and antispastic, hipotensive, antinephritic, anticancer, antifungal, antibacterial, antiulcer, anti-inflammatory, cognitive, hypnotic-sedative, antioxidant, immuno-stimulant and wound healing agent [21, 28].

Several triterpenes, cyclopeptide alkaloids, saponins, and flavonoids have been isolated from its fruit, bark and leaf [27]. Traditionally dried and fresh leaves are applied externally for healing wounds and to relieve the burning sensation. The infusion

from the leaves mixed with cumin has been used to treat urinary infections [10, 12].

The leaves were the object of several clinical and preclinical studies. The leaf extract (5 - 50 µg/mL) was reported to stimulate the activity of human neutrophils [11]. *Ziziphus* leaves possess significant anti-inflammatory properties against carrageenan-induced rat paw oedema at doses of 200, 400 and 600 mg/kg b.w. [17]. The results of studies by pylorus ligation model in rats suggest antiulcer activity due to its cytoprotective and anti-secretory action [10]. *Ziziphus* leaves were shown to have strong anti-allergic properties [25]. A *Ziziphus jujuba* leaf extract has shown anti-diarrhoeal activity in rats, in an experimental model where diarrhea was induced with castor oil [13]. This herbal product led to increased HDL levels and significantly decreased glucose, triglyceride and VLDL blood levels in rats [24]. *Ziziphus jujuba* leaf extract demonstrated a significant antipyretic effect in rats after Brewer yeast injection. The effect was comparable with that of paracetamol used as a positive control [2]. The alcoholic extract of leaves demonstrated anti-obesity properties comparable with sibutramine by decreasing body weight, food intake, serum glucose and lipid levels in obese rats [9]. The leaves contain triterpenoid sweetness inhibitors (jujubasaponins II, III, IV, V VI, jujuboside B) that supress the sweet taste of sucrose [26].

The leaves have also been used for the control of fever and obesity [19], improving sleep, for wound dressing and treatment of urinary infections [22].

The aim of this paper was to report the possible toxicity and the anti-inflammatory potential of jujube leaves.

Materials and Methods

Jujube leaves were supplied by the Research Institute for Fruit Growing, Pitești - Mărăcineni (Romania), and were harvested in June 2015. In order to obtain the extract, finely ground dried leaves (sieve IV) were refluxed with ethanol 70%, for three times (half an hour each time, 70°). The ratio between raw material and solvent was 1:10 (w/w). The resulted solutions were pooled and concentrated at 60 °C using a rotary evaporator (Ingos RVO 004). Then the concentrated solution was freeze-dried at (-) 55 °C using a Scanvac CoolSafe Freeze Dryer. Finally, a dry ethanolic leaf extract of *Ziziphus jujuba* was obtained.

The effects of the extract on plant cell division were evaluated using the *Triticum* bioassay (Constantinescu method). The method has been described in detail elsewhere [1]. Six concentrations (1%, 0.5%, 0.1%, 0.05%, 0.01% and 0.001%) and a negative control (distilled water) were used.

Inferential statistical analyses were performed on the values measured on the third day, using the computing and statistical programming environment, R v. 3.1.3, and several software packages for R, "car" [8], "fBasics" [30]. The inhibition index was calculated based on median values, using the formula published previously [1, 3]. The normality of residuals was assessed visually by q-q plots, histograms and boxplots, and additionally, for an objective assessment, by the d'Agostino-Pearson test; the homogeneity of variance was evaluated by applying the Levene test. Because neither the assumption of normality of residuals, nor the homoscedasticity were confirmed (besides there were numerous tie values at the higher concentrations), multiple comparisons (with concentration as an independent factor) were performed using Welch ANOVA on ranked values, because similar investigations have indicated that this approach ensures the best control of type I error with a minimal impact on the type II error [5]. For further assurance, a robust, heteroscedastic ANOVA procedure for medians was also applied (the *med1way* function from the "WRS2" package) and the results were consistent. For subgroup analysis we have used nonparametric multiple contrast tests based on ranks, with simultaneous confidence intervals, as proposed by F. Konietschke, L. A. Hothorn and E. Brunner in 2012 [15] and implemented in the R package "nparcomp" [16] (the mctp function, which returns results identical with those of the Brunner-Dette-Munk test, based on ranks, implemented in the R package "asbio" [14]). These tests have the advantage of allowing the computation of effect size (as relative effects) for each subgroup.

The animals (mice and rats) were supplied by the rodent farm of "Cantacuzino" Institute, Bucharest, Romania, and housed in plexiglass cages. Drinking water and food were provided *ad libitum* throughout the experiment. All animals were habituated for 5 days prior to the experiment to the testing environment and maintained on a 12 h light/dark cycle. The temperature and relative humidity were continuously monitored using an electronic hygro-thermometer. The temperature was between 21 - 24ºC and the relative humidity was generally maintained at 35 - 45%.

All procedures were carried out in accordance with the DIRECTIVE 2010/63/EU of 22 September 2010, regarding the protection of animals used for experimental and other scientific purposes [34].

The acute oral toxicity of the extract was evaluated in mice according to the procedures outlined by the Organization for Economic Cooperation and Development, OECD 420 [33]. The study was approved by the "Carol Davila" University Ethics Committee.

Adult male NMRI mice (22 ± 1.5 g, n = 5) were used. The animals were fasted 4 h prior to treatment and 2 h after, with free access to drinking water. A single dose of 2000 mg/kg b.w. of dry extract (suspended in distilled water) was administered to a single mouse by gavage. After 48 h, the other 4 mice received the same treatment. The mice were observed in detail for any signs of toxicity within the first 4 h after the treatment period, and daily, for a period of 14 days. The animals were weighed initially, 7 and 14 days after the beginning of the experiment. Visual observations for mortality, behavioural pattern, changes in physical appearance, injury, pain and signs of illness were conducted daily during this period.

Male Wistar rats (303 ± 32 g) were used for the anti-inflammatory assay. Two inflammation experimental models using carrageenan and kaolin as inflammatory agents were used [4]. The inflammation was induced by intraplantar administration of 0.1 mL aqueous suspension of carrageenan (1%) and 0.2 mL aqueous suspension of kaolin (10%) into the rat's inferior right paw. The experimental group was treated for 7 days by gavage with 500 mg/kg b.w. of 5 % aqueous suspension of the extract. The anti-inflammatory effect was compared with a control group, treated by gavage with distilled water 1 mL/100 g b.w. for 7 days, and a reference group, treated by gavage with distilled water 1 mL/100 g b.w. for 6 days, and in the 7th day treated by gavage with 0.1 % indomethacin solution, 10 mg/kg b.w. In the 7th day of the experiment, after the substances administration, all animals were anaesthetized by intraperitoneal administration of 1300 mg/kg b.w. urethane, 13 % aqueous solution. The initial paw volume was measured using Ugo Basile 7140 Plethysmometer. The paw edema was further evaluated at 30 min, 1, 2, 3, and 4 h, after the inflammatory agents administration.

Results were statistically processed using the R language and environment for statistical computing, version 3.2.4. They are expressed for descriptive purposes as mean ± standard deviation. The Gaussian distribution was assessed graphically (histograms, boxplots, quantile-quantile plots) and by means of the d'Agostino omnibus test. For both t-student and ANOVA tests the Welch adjustment (assuming unequal variance) was employed, at a confidence level of 95%. ANOVA was used for global comparisons among the test, reference (indomethacin) and the control group, but because of the small sample size and henceforth statistical power, no correction for multiplicity was applied; instead a t-student test was used to compare both the reference and the test groups with the control one. Effect size was estimated by Hedges' g (unbiased estimate of d) using the R package "compute.es" [6]. The evolution of paw oedema was calculated using the following formula (where V_0 is the initial paw volume):

$$\% = \frac{V_{xh} - V_0}{V_0} \times 100.$$

Results and Discussion

Triticum bioassay

The extract inhibited the growth of *Triticum* radicles in a concentration-dependent manner ($p < 0.001$, Welch ANOVA and robust ANOVA for medians). The inhibitory effect on radicles growth was only discernible for the 0.5% and 1% concentrations ($p < 0.001$, Tukey-type, non-parametric test; inhibition index 98.94% and 100%) (Figure 1). For concentrations lower than 0.5% either a minimal inhibition (inhibition index 5.31% for the 0.1% concentration) or a slight stimulation (inhibition index 11.70 - 32.98%, for concentrations between 0.05% and 0.001%), but the effects were not statistically significant compared to the control group ($p > 0.65$ for all four lower concentrations). Relative effects for each subgroup are shown in Table I. Because the experiment was performed using a dry extract, the 0.5% concentration of the latter is roughly equivalent to that of a fluid extract (e.g. infusion) of about 2.5% concentration (w/v), and thus the extract is phytotoxic only at a relatively high concentration. At the highest concentration (1.0%) of the extract, obvious signs of cytotoxicity were observed by microscopically examination, such as nuclei with altered shapes (reniform, triangular) and condensed nuclear material.

Table I

Nonparametric relative effects for the influence of the tested concentrations on *Triticum* root growth, computed based on global ranks ("mctp" function from "nparcomp", package, using the Fisher transformation function)

Concentration	Sample size	Relative effect	95% confidence interval (simultaneous)
Control	10	0.5821	0.4852 - 0.6731
0.001%	10	0.6779	0.5859 - 0.7579
0.01%	10	0.7193	0.6174 - 0.8027
0.05%	10	0.6493	0.5269 - 0.7547
0.1%	10	0.5857	0.4848 - 0.6799
0.5%	10	0.1686	0.1453 - 0.1947
1 %	10	0.1171	0.0946 - 0.1442

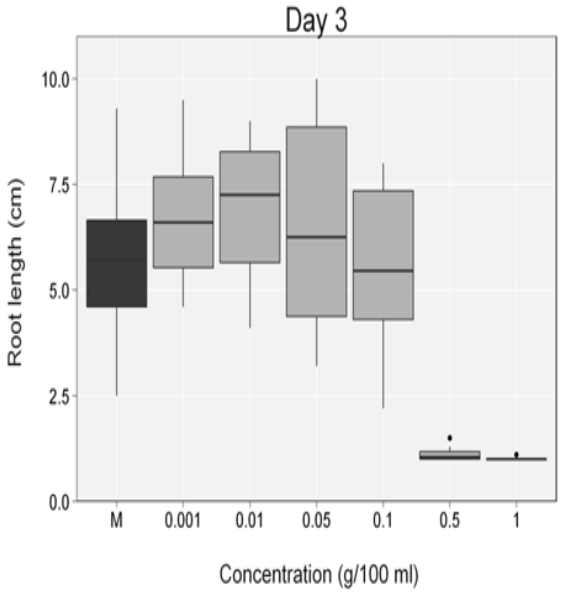

Figure 1.
Boxplot ("box and whiskers") showing the variation of root length as a function of concentration in the 3rd day

Acute Oral Toxicity Study

No toxic symptoms or mortality were observed in any animals. Skin, fur, eyes, mucous membranes, behavioural pattern, salivation and sleep pattern parameters of the treated animals were found to be normal. The body weight of all the mice increased after the administration of the extract, the changes showed statistical significance from day 7 of observation, indicating the disappearance of clinical signs of toxicity (Figure 2).

According to OECD 420 guideline, the dry ethanolic leaf extract of *Ziziphus jujuba* was placed in the GHS category 5 (LD50 > 5000 mg/kg b.w., p.o.) [20, 32].

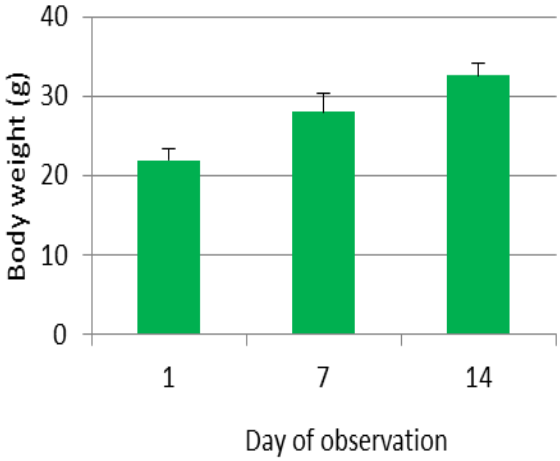

Figure 2.
The effect of the dry ethanolic leaf extract on the body weight of mice at 2000 mg/kg b.w. dose

Anti-inflammatory assay

The anti-inflammatory activity after oral administration for 7 days of the dry ethanolic leaf extract of *Ziziphus jujuba* (500 mg/kg b.w.) was determined (Table II).

Table II

The effect of the administration of *Ziziphus jujuba* dry ethanolic leaf extract on paw oedema in rats and the statistical significance (computed with the initial paw volume in each group)

Group	**Oedema rate percentage (mean % ± s.d.)**				
	30 min.	1 h	2 h	3 h	4 h
Carrageenan-induced paw oedema					
Control group	24.71 ± 13.26	35.79 ± 16.38	39.32 ± 14.37	36.80 ± 12.95	35.28 ± 14.97
Indomethacin (Welch t-test *versus* control)	11.42 ± 5.40 (p = 0.028)	26.65 ± 8.40 (p = 0.195)	27.00 ± 8.57 (p = 0.064)	30.01 ± 11.48 (p = 0.301)	31.22 ± 13.41 (p = 0.590)
Ziziphus jujuba extract (Welch t-test *versus* control)	29.95 ± 11.06 (p = 0.396)	36.89 ± 11.28 (p = 0.875)	36.30 ± 15.53 (p = 0.683)	33.31 ± 13.10 (p = 0.589)	36.94 ± 13.64 (p = 0.816)
Welch ANOVA	p = 0.002	p = 0.133	p = 0.123	p = 0.587	p = 0.714
Kaolin-induced paw oedema					
Control group	30.62 ± 11.92	36.88 ± 9.13	46.94 ± 10.25	43.30 ± 9.31	42.81 ± 9.30
Indomethacin (Welch t-test *versus* control)	9.48 ± 6.81 (p = 0.002)	28.51 ± 14.30 (p = 0.214)	31.51 ± 17.46 (p = 0.069)	33.76 ± 14.25 (p = 0.161)	31.83 ± 14.09 (p = 0.109)
Ziziphus jujuba extract (Welch t-test *versus* control)	25.70 ± 10.72 (p = 0.388)	32.23 ± 11.92 (p = 0.378)	37.01 ± 10.29 (p = 0.065)	36.42 ± 10.41 (p = 0.171)	40.61 ± 14.28 (p = 0.710)
Welch ANOVA	p = 0.002	p = 0.408	p = 0.094	p = 0.254	p = 0.265

The results showed that both carrageenan and kaolin injection stimulate local inflammation and induce oedema of paw tissues, evidenced by the increase in paw volume of rats from the control group. Indomethacin (10 mg/kg b.w.), used as a reference, decreased the paw oedema induced by carrageenan and kaolin, when compared with the control group. Rather surprisingly, though, in both models this effect was statistically significant only for the measurements performed at 30 minutes and

two hours, whereas for the other time points, the differences *versus* the control group were not statistically significant, although in most cases they were larger for indomethacin than for the *Ziziphus jujuba* extract (Table II).

For the carrageenan - induced inflammation model, unlike indomethacin, for the *Ziziphus jujuba* dry ethanolic leaf extract, no statistically significant anti-inflammatory activity against the control group was observed. Moreover, rather unexpectedly, in the first 30 minutes and one hour, the average paw oedema was larger (although not statistically significant) for the animals treated with *Ziziphus jujuba* extract than for the control group. After four hours, the average oedema was again higher for the *Ziziphus jujuba* group than for the control one. Although the absence of a consistently significant effect for indomethacin imposes caution in interpreting these results, they tend to indicate that the *Ziziphus jujuba* extract is either fully devoided of any anti-inflammatory effect, or if such an effect is present, it is very modest. Even assuming that the non-significant outcome observed at two and three hours reflects a real effect (which in the light of these results in this experimental model is doubtful), it would be of very short duration, because it was neither evident before two hours, nor after four hours. Long ago it has been shown that the carrageenan-induced oedema has three distinct phases involving different biological mediators: in the first 1.5 hours the main mediators are histamine and serotonin (5-HT); between 1.5 hours and 2.5 hours kinins and the kinin system is involved, and after that a third, longer phase intervenes (2.5 - 6 hours), where the major mediators are prostaglandins [7]. When considering this phased occurrence of the paw oedema, the experimental data suggest that the *Ziziphus* extract might - if active at all - only influence the median phase, inhibiting the kinins. But, considering the very small effect size (g = 0.19), leaving aside any statistical significance/power considerations, if such a hypothetical effect exists, it seems of very limited practical significance.

For the kaolin - induced inflammation model, also, at no time point was the conventional threshold of statistical significance of 0.05 reached, although unlike the carrageenan model in this one the average effect was consistently better for the extract than for the control group throughout the experiment, but the effect it is also generally smaller in comparison with the indomethacin - treated animals (in all cases the difference in favour of indomethacin is statistically significant) (Figure 3).

However, at two hours, the p value in the Welch t-test was very similar for the indomethacin and the *Ziziphus* extract, as it was also for the measurements carried out at three hours (although only close to the 0.05 threshold at two hours and 0.16 - 0.17 at three hours). The oedema induced by kaolin seems to be mediated mainly by activation of kallikrein and by kinins [29], and thus the only faint effect discernible for the carrageenan model at 2 - 3 hours (when a mediating role of kinins is hypothesized) would be consistent with the somewhat more pronounced effects observed in the kaolin model.

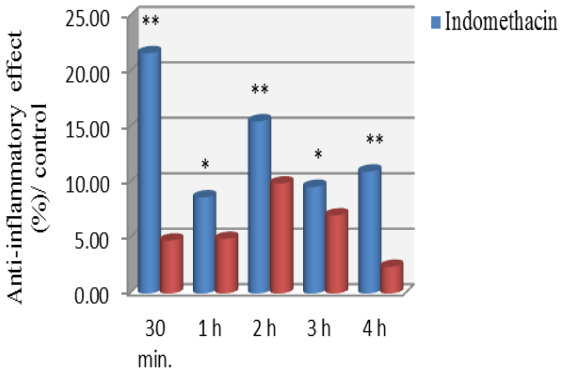

Figure 3.
Intensity of anti-inflammatory effect (%) against control group in the kaolin - induced inflammation model (difference between the average effect (%) seen for indomethacin or *Ziziphus* extract and the control group)
(*p < 0.05; **p < 0.01)

The results of this study, showing a very weak anti-inflammatory effect, if at all, are conflicting with an older study [17], which reported an inhibitory effect of 44.5%, 62.2% and 81.8% compared with the control, for doses of 200, 400 and 600 mg/kg b.w. of a *Ziziphus* leaf ethanolic extract, comparable with the effects of diclofenac sodium. In that study the extract was possibly obtained with 90% ethanol, a solvent with lower polarity than the one used in this study and a lower temperature, as the method used for its preparation was percolation.

Conclusions

Despite a previous study with a slightly different extract reporting anti-inflammatory effects for *Ziziphus jujuba* leaves, in the present study performed on two experimental inflammation models in rat, only a weak anti-inflammatory effect, if at all, was detected. In the animal model the extract is virtually non-toxic after single dose administration. The *Triticum* assay suggested that the toxicity of this extract on vegetal cell is low.

Acknowledgement

This work was supported by UMF "Carol Davila" a project number 33894/11.11.2014.

References

1. Anghel A.I., Ilie M., Olaru O.T., Dinu M., Ancuceanu R.V., Istudor V., HPTLC qualitative and quantitative detection of sterols in species of the *Portulaca genus* from Romania. *Farmacia*, 2015; 63(5): 969-699.
2. Balakrishnan A., Balasubramaniyam P.D., Natesan S.K., Antipyretic activity of *Ziziphus jujuba* leaves. *JASR*, 2012; 3(3): 40-42.
3. Bădiceanu C.D., Poesina N.D., Missir A.V., Stecoza C.E., Dinu M., The phytobiological testing of some new thiourea derivatives. *Farmacia*, 2014; 62(1): 23-33.
4. Castardo J.C., Prudente A.S., Ferreira J., Guimarães C.L., Delle Monache F., Cechinel Filho V., Otuki M.F., Cabrini D.A., Anti-inflammatory effects of hydroalcoholic extract and two bi-flavonoids from *Garcinia gardneriana* leaves in mouse paw oedema. *J. Ethnopharmacol.*, 2008; 118(3): 405-411.
5. Cribbie, R.A., Wilcox R.R., Bewell C., Keselman H.J., Tests for Treatment Group Equality When Data are Nonnormal and Heteroscedastic. 2007; 6. http://digitalcommons.wayne.edu/jmasm/vol6/iss1/12.
6. Del Re A.C., compute.es: Compute Effect Sizes. 2013; R package version 0.2-2. URL.
7. Di Rosa M., Biological properties of carrageenan. *J. Pharm. Pharmacol.*, 1972; 24(2):89-102.
8. Fox J., Weisberg S., An {R} Companion to Applied Regression, Second Edition. Sage, 2011; Thousand Oaks, CA. http://socserv.socsci.mcmaster.ca.
9. Ganachari M.S., Kumar S., Alagawadi K.R., Anti-obese activity of *Ziziphus jujuba* leaves extract in dietary obese rats. *Journal of natural remedies*, 2007; 7(1): 102-108.
10. Ganachari M.S., Kumar S., Anti-ulcer properties of *Ziziphus jujuba* leaves in rats. *Journal of natural remedies*, 2004; 4(2): 103-108.
11. Ganachari M.S., Shiv K., Bhat K.G., Effect of *Ziziphus jujube* leaves extract on phagocytosis by human neutrophils. *Journal of natural remedies*, 2004; 41: 47-51.
12. Hadkarni K.M., Indian Materia Medica, Popular Prakashan, Bombay, 1986; 1315-1319.
13. Jagannadha G.H., Iakshmi P., Anti-dirrhoeal activity of *Ziziphus jujuba* leaf extract in rats. *Int. J. Pharm. Biol. Sci.*, 2012; 3(1): 6275-6299.
14. Ken Aho., asbio: A Collection of Statistical Tools for Biologists. R package version 1.1-5. 2015. http://CRAN.R-project.org/package=asbio.
15. Konietschke F., Hothorn L.A., Brunner E., Rank-based multiple test procedures and simultaneous confidence intervals. *Electron. J. Stat.*, 2012; 6: 738-759.
16. Konietschke F., Placzek M., Schaarschmidt F., Hothorn L.A., nparcomp: An R Software Package for Nonparametric Multiple Comparisons and Simultaneous Confidence Intervals. *Journal of Statistical Software*, 2015; 64: 1-17.
17. Kumar S., Ganachari M.S., Nagoor V.S., Anti-inflammatory activity of *Ziziphus jujuba* leaves in rats. *Journal of natural remedies*, 2004; 4(2): 183-185.
18. Li J.W., Fan L.P., Ding S.D., Ding, X.L., Nutritional composition of five cultivars of chinese jujube. *Food Chem.*, 2007; 103: 454-460.
19. Mahajan, R.T., Chobda, M.Z., Phyto-pharmacology of *Ziziphus jujuba* Mill. Aplant review. *Pharmacogn. Rev.*, 2009; 3: 320-329.
20. Negreş S., Dinu M., Ancuceanu R.V., Olaru T.O., Ghica M.V., Şeremet O.C., Zbârcea C.E., Velescu B.S., Ştefănescu E., Chiriţă C., Correlations *in silico*/*in vitro*/*in vivo* regarding determinating acute toxicity in non-clinical experimental trial, according to bioethic regulations inforced by the European Union. *Farmacia*, 2015; 63(6): 877-885.
21. Outlaw W.H., Zhang S.Q., Riddle K.A., Womble A.K., Anderson L.C., Outlaw W.M., Outlaw N.N., Outlaw E.C., Thistle A.B., The jujube (*Ziziphus jujuba* Mill.), a multipurpose plant. *Econ. Bot.*, 2002; 56: 198-200.
22. Preeti T., Shalini T., *Ziziphus jujuba*: a phyto-pharmacological review. *Int. J. Res. Dev. Pharm. L. Sci.*, 2014; 3: 959-966.
23. Ritz C., Streibig J.C., Bioassay Analysis using R. *J. of Statistical Software*, 2005; 12(5): 1-22.
24. Shirdel Z., Madani H., Mirbadalzadeh R., Investigation into the hypoglycemic effect of hydroalcoholic extract of *Ziziphus jujuba* leaves on blood glucose and lipids in alloxan-induced diabetes in rats. *Iranian Journal of diabetes and lipid disordes*, 2009; 13-19.
25. Su B., Farnsworth N.R., Fong H.H., Pezzuto J.M., Kinghorn A.D., Activity-guided fractionation of the seeds of *Ziziphus jujuba* using a cyclooxygenase-2 inhibitory assay. *Planta Med.*, 2002; 68: 1125-1128.
26. Suttisri R., Lee I.S., Kinghorn A.D., Plant-derived triterpenoid sweetness inhibitors. *J. Ethnopharmacol.*, 1995; 47(1): 9-26.
27. Tripathi M., Pandey M.B., Jha R.N., Pandeya V.B., Tripathi P.N., Singh J.P., Cyclopeptide alkaloids from *Zizyphus jujube*. *Fitoterapia*, 2001; 72: 507-510.
28. Tripathi P., Tripathi S., *Ziziphus jujuba*: a phyto-pharmacological review. *Int. J. Res. Dev. Pharm. L. Sci.*, 2014; 3(3): 959-966.
29. Udegbunam R.I., Udegbunam S.O., Anosa G.N., Analgesic and anti-inflammatory effects of *Cyphostemma vogelii* (Hook. f.) Desc. root extract in mice. *Afr. J. Biotechnol.*, 2013; 12(17): 2288-2292.
30. Wuertz D., Setz T., Chalabi Y., fBasics: Rmetrics - Markets and Basic Statistics. R package version 3011.87. 2014. https://CRAN.R-project.org.
31. Zhang R., Chen J., Shi Q., Li Z., Peng Z., Li Z., Wang X., Phytochemicalanalysis of Chinese commercial *Ziziphus jujube* leaf tea using high performance liquid chromatography–electrospray

ionization-time of flight mass spectrometry. *Food Res. Int.*, 2014; 56: 47-54.
32. *** ENV/JM/MONO(2001)6. Harmonized Integrated Hazard Classification System For Human Health And Environmental Effects Of Chemical Substances. Adopted on 14 august 2001.
33. *** OECD Guideline 420. Acute Oral Toxicity – Fixed Dose Procedure. Adopted on 17 decembrie 2001.
34. *** Directive 2010/63/EU of the European Parliament and of the Council of 22 September 2010 on the protection of animals used for scientific purposes, Official Journal of the European Union