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# Antioxidant effects and oxidative stress-modulatory activity of *Glycyrrhiza glabra* L. root extract against acute toxicity

Y. D. Karamalakova[1]*, G. D. Nikolova[1], P. Agarwal[2], V. G. Gadjeva[1]

[1]*Department Chemistry and Biochemistry, Medical Faculty, Trakia University, 11 Armeiska Str., 6000 Stara Zagora, Bulgaria*
[2]*Radiation Biotechnology Group, Institute of Nuclear Medicine and Allied Sciences, Brig. S. K. MazumdarMarg, Defence Research and Development Organization, Delhi-110054, India*

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*Glycyrrhiza glabra (G. glabra)*, Indian medicinal plant, exhibited good anti-inflammatory, anti-microbial, anti-oxidative, immunomodulatory, hepatoprotective, anticancer properties and excellent *ex vivo* radical-scavenging capacity against induced acute toxicity. In the present study, using *in vivo* methods, we investigated changes, therapeutic potential and antioxidant action against oxidative stress-induced conditions in tissues isolated from *G. glabra* extract-treated animals (80 mg/kg/ given 14 times/14 days) and in Bleomycin (dose 0.34 U/kg body) induced acute toxicity. After dissection of the liver, lung and spleen, the tissues were homogenates in cold PBS solution and studied by spin-trapping electron paramagnetic (EPR) spectroscopy. Tissue homogenates were prepared for determination of the ascorbate, nitric oxide radical levels and ROS products. It was established that in the *G. glabra* extract-treated animals the biomarker levels were close to the controls ($p<0.05$). Statistically significantly lower levels of nitrite and ascorbate radicals were measured only in spleen. Lipid peroxidation was significantly reduced in the *G. glabra*+BLM group, compared to the BLM-treated group ($p<0.004$). *In vivo* EPR study characterized *G. glabra* root extract as a good antioxidant scavenging free-radical formations with a possibility of neutralization of acute oxidative diseases.

**Keywords:** *G. glabra* root extract; BLM; ROS; acute oxidative stress

## INTRODUCTION

Plant materials (leaves and roots) used in Hindu medicine (*Ayurveda*) are evaluated for antioxidant-pharmacological activities and characterized with low reactive oxygen species (ROS) levels [1, 2]. The roots of *Glycyrrhiza glabra L. (G. glabra, Fabaceae)*, commonly known as 'licorice', possess biologically active components as triterpenes, saponins, flavonoids, isoflavonoids, chalcones, and glycyrrhizic acid, as previously reported for the extract [3, 4]. *G. glabra*-extracted flavonoids have been widely investigated for ability to inhibit lipid peroxidation [5] and for hydroxyl groups scavenging activity [6-9]. Isoflavon derivatives (*glabridin, hispaglabridin A, hispaglabridin B*), isolated from *G. glabra*, were shown to be effective in protecting liver mitochondrial function against oxidative stress changes [10] and *in vitro* models displayed considerable antioxidant/protective effect against the human lipoprotein oxidative system [4, 11]. *G. glabra* extracts possess anti-asthmatic, anti-inflammatory, anti-viral, anti-microbial, anti-oxidative, anti-cancer, immunomodulatory, hepatoprotective and cardio-protective properties [3]. Because of its excellent antioxidant activity, the licorice aqueous extract is used for *in vivo* treatment of peptic ulcer, hepatitis C, and pulmonary diseases [3] and also exhibited anti-helminthic and anti-microbial activity. The cytotoxicity of the methanol extracts of *G. glabra* roots have been studied against immortal human keratinocyte, liver carcinoma (HepG2) and lung adenocarcinoma (A549) [12]. *G. glabra* roots aqueous extracts could be used as potent tools for a further development of cytoprotective preparations with anti-infectious potential [13]. Some researchers emphasized the anti-asthmatic and antioxidant properties of the licorice root, as well as its effectiveness against pulmonary (PF) and hepatic fibrosis (HF) in rats [3, 14, 15]. Ram *et al.* [16] reported that the presence of glycyrrhizin, as a main component in the structure of *G. glabra* mitigates the severity of asthma inflammation in animal models. The bioflavonoids presence (*glabridin*), multiply increases the anti-inflammatory and antioxidant activity of the *G. glabra* extract [17, 18]. Ghorashi *et al.* [19] revealed that the aqueous *G. glabra* root extracts caused reduction of collagen deposition and reduced the PF cells formation. For this purpose, need of stable natural compounds with lower organ toxicity, excellent antioxidant-modulatory activity and therapeutic effect against acute toxicity was felt.

* To whom all correspondence should be sent:
E-mail: ykaramalakova@gmail.com

This study aims to investigate the antioxidant and oxidative stress protective effect of *G. glabra* root extract. For this reason, we studied: 1) the ascorbate and nitric oxide radical levels; 2) the ROS production as a real-time oxidative stress parameter; and 3) the antioxidant modulatory activity of *G. glabra* root extract against Bleomycin-induced acute toxicity in IRC mice.

## MATERIALS AND METHODS

### *Plant extract and chemicals*

The air-dried *G. glabra* roots were made into a coarse powder and after dissolution in 2 L of distilled water were subjected to a hot maceration process, with continuous stirring for 48 h. The aqueous extract was filtered through muslin cloth and the filtrate was concentrated by evaporation on a bath and then lyophilized. The extract was made and provided from INMAS, India as a reference. Dimethyl sulfoxide (DMSO), N-*tert*-butyl-alpha-phenylnitrone (PBN), 2-(4-carboxyphenyl)-4,4,5,5-tetra-methylimidazoline-1oxyl-3-oxide (carboxy-PTIO.K), bleomycin sulfate (C55H84N17O21S3, EP 9041-93-4) and PBS were purchased from Sigma Chemical Co, St. Louis, USA.

### *Animals*

24 male ICR mice, aged 6–8 weeks, weighing 43-48±2.0 g were obtained from the Medical Faculty, Trakia University, Stara Zagora, Bulgaria. The animal procedures were in accordance with Directive 2010/63/EU on the protection of animals used for experimental and other scientific work (131/ 6000-0333/ 09.12.2016). Animals were housed in polypropylene cages at 18–23±2$^0$C and under a light/dark period of 12/12h daily. They were fed on a standard commercial feed, after 6 days of acclimatization on humidity 55% and free access to tap water. The lyophilized extract was dissolved in distilled $H_2O$ and preserved at 4$^0$C until use. The animals were weighed every morning using a digital scale, and changes were recorded.

### *Bleomycin administration*

Mice were divided into four groups (n=6) for a period of 14 days and administration of the drugs was through intraperitoneal (i.p.) injection. The groups were:

A) Control group (CG) (standard diet, tap fresh water);

B) Bleomycin sulfate administration (BLM) (0.069 U/ml; 0.321 U/kg body weight in saline (250μl) was given i.p. finishing on day 14);

C) *G. glabra* extract (80 mg/ml; 0.208 mg/kg body weight in distilled $H_2O$ (250 μl) was given once daily i.p. continued on schedule till the 14. day);

D) *G. glabra* extract (80 mg/ml; 0.208 mg/kg body weight) + bleomycin (0.34 U/kg) (extract was injected once daily, 2 h prior to antibiotic, continued on schedule till the 14. day).

On day 14, mice were euthanized and their livers, lungs and spleens were removed in cold PBS solution and studied by direct and spin-trapping EPR spectroscopy.

### *Electron Paramagnetic Resonance (EPR) measurements*

The measurements were performed on an X-Band, Emx$^{micro}$ spectrometer (Bruker) with settings: center field 3505 G; sweep width 10-30 G; microwave power 12.70-12.83 mW; receiver gain 1 × $10^4$ -1 × $10^6$; mod. amplitude 5.00 G; 1-5 scans. Spectral processing was performed using Bruker WIN-EPR and SimFonia software.

*EPR in vivo evaluation of ROS productions:* The ROS production was studied according to Shi *et al.* [20]. Briefly, about 0.1 g of tissue samples were homogenized after addition of 1.0 ml of 50 mM solution of the spin-trapping agent PBN/ DMSO solution.

*EPR in vivo evaluation of ascorbate ($Asc^{\cdot}$) radicals:* The $Asc^{\cdot}$ levels in tissue homogenates were studied according to [21] with slight modifications. Tissue samples were weighed and homogenized in DMSO (10% w/v) and centrifuged at 4000 g, at 4$^0$C for 10 min. Supernatants were collected and $Asc^{\cdot}$ radicals were measured immediately in cold.

*EPR in vivo evaluation of nitric (NO•) radicals:* Tissue NO• radicals were studied according to methods [22, 23] adapted for EPR estimation of the spin-adduct formed between Carboxy-Ptio.K and generated radicals.

### *Statistical analysis*

EPR spectral processing was performed using Bruker Win-EPR and SimFonia Software. Statistical analysis was performed with Statistica 8.0, Stasoft, Inc., one-way ANOVA, Student's t-test to determine significant differences between data groups. The results were expressed as means ± standard error (SE). A value of $p< 0.05$ was considered statistically significant.

## RESULTS AND DISCUSSION

Bleomycin (BLM) is used as an antibiotic possessed acute oxidative stress in animals. In the organs, BLM is rapidly metabolized by a complex of extracellular matrix deposition and oxidative changes [24], resulting in the generation of toxic products, which determine acute/general toxicity. Moreover, acute conditions reduce antioxidant capacity [9, 24]. Many authors have reported of active biomolecules from plant antioxidants against drug-possessed toxicity, as effective ROS inhibitors [25]. Kim *et al.* [18] reported that licorice (*G. glabra*) could prevent inflammatory processes, due to the presence of flavonoid structure - glabrin which modulates acute oxidative stress caused by tissue injuries. Over the last 15 years, the activity of aqueous/ alcoholic root herbal extracts and their components as ROS scavengers on acute toxicity have been studied and their protective effects have been investigated [26]. In this research, we showed that the application of *G. glabra* root extract, pointedly inhibited tissue free radical formation in acute model and reduced oxidative stress levels.

### *G. glabra root extract regulates body weight gain at acute toxicity*

14 days after the start, the average weight gain of mice receiving BLM significantly decreased ($p<0.05$), compared to the CG. In contrast, administration of *G. glabra* extract and *G. glabra* + BLM significantly increased weight gain ($p<0.05$). No significant differences were observed regarding daily food consumption. In accordance with our results, Ghorashi *et al.* [19] found that animals receiving licorice extract + BLM showed a significant increase in weight, compared to the BLM group.

### *G. glabra root extract regulates ROS production and Asc· radicals at acute toxicity*

Thermodynamically, ascorbic acid is the end product of a series of oxidized free radicals. This means that all oxidizing species which have a higher redox potential could be reduced and as a result, generate ascorbate radical [27]. Asc· radical has a relatively long half-life which makes it extremely convenient for direct detection by EPR spectroscopy, both *in vitro* and *in vivo* systems [21]. This property makes it the best non-toxic endogenous marker, proving toxic ROS in biological systems [28].

To investigate the effects of *G. glabra* root extract on acute toxicity, we measured ROS production (Fig. 1) in liver, lung and spleen from all tested groups. ROS products in liver (0.3249±0.08 *vs.* 0.4889 ±0.001, $p<0.00$), lung (0.559±0.02 *vs.* 0.759 ±0.001, $p<0.01$) and spleen (0.211±0.07 *vs.* 0.433 ±0.02, $p<0.00$) of *G. glabra* treated mice were statistically significantly lower, compared to CG. However, ROS production in the three organs significantly increased ($p< 0.05$) in BLM acute model, compared to CG. The combination with *G. glabra* root extract correspondingly reduced the increased tissue lipid peroxidation.

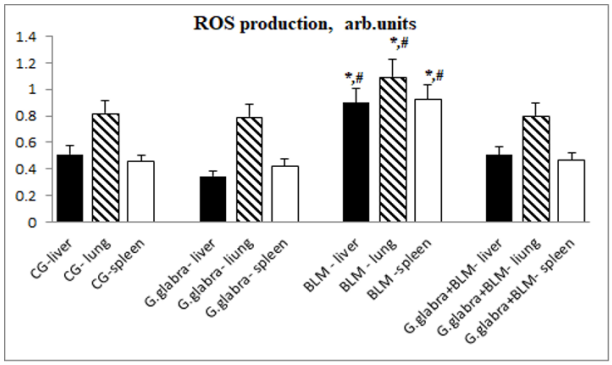


**Figure 1.** The ROS products in organ homogenates. *G. glabra root* extract normalizes lipid products accumulation in acute models. The experiments were repeated three times. * $p < 0.04$ *vs.* the CG group; #$p < 0.05$ vs. the *G. glabra* + BLM group ($n = 6$).

Based on the measured hyperfine splitting constants, the radicals trapped by PBN spin-adduct (consisting of six spectral lines) was identified as oxygen-centered lipid radicals (LO·) [29]. In this aspect, Sen *et al.* [29] found that treatment with licorice root extract containing glycyrrhizin, retain normal lipid peroxidation levels and oxidative stress parameters.

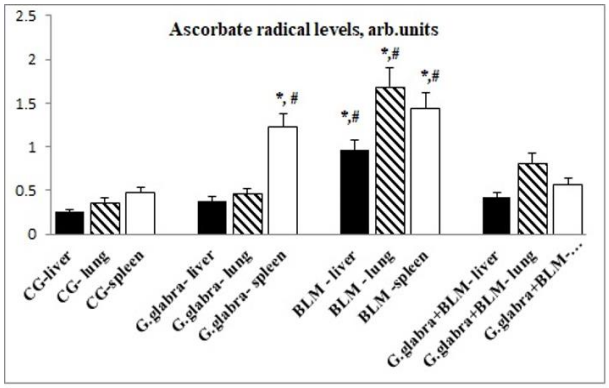


**Figure 2.** *In vivo* Asc• levels in organ homogenates. Results were calculated by double integration of the corresponding EPR spectrum immediately registered in plasma (expressed in arbitrary units/ *arbt. units*). The experiments were repeated three times. *$p < 0.05$ *vs.* the CG group; #$p < 0.05$ *vs.* the *G. glabra*+BLM group ($n = 6$).

Asc· levels in the tissues homogenates of *G. glabra* root extract mice, CG and in BLM combination are shown on Fig. 2. Asc• levels in liver and lung of *G. glabra* treated group were close to the CG ($p$=0.00). Interestingly, Asc• radicals in *G. glabra*-treated spleens were statistically significantly increased (almost three-fold), compared to the CG (1.226 ±0.048 *vs*. 0.472 ±0.014, p <0.002). In addition, the BLM-induced free ascorbate increase, recovered after treatment with plant extract ($p$<0.003).

Modern investigation showed that glycyrrhizic acid, *G. glabra* component, relieves drug-induced hepatic oxidative changes and pulmonary injuries in animals [30, 31]. Moreover, glycyrrhizin, a component of licorice root, reduces inflammatory cytokines (TNF-α, IL-1β) and as strong antioxidant and anti-inflammatory agent [32] could reduce acute oxidative stress in organs. However, reduced ROS products after *G. glabra* administration alone or in *G. glabra* + BLM combination, suggest that ascorbate and other endogenous antioxidant molecules were involved in the neutralization of lipid peroxidation. Our studies ascertained that the *G. glabra* treatment practically did not cause lipid peroxidation processes, but regulates those caused by acute oxidative stress. In addition, statistically higher ascorbic acid levels in spleen registered after *G. glabra* treatment were probably due to residual oxidative processes in the organ. Bonnet and Walsh [33], reported that *G. glabra* roots contain an antioxidant - licocalchone-c, inhibitor of oxidative stresses and inflammation processes.

### *G. glabra root extract regulates the in vivo nitric (NO•) radicals imbalance at acute toxicity*

*In vivo* nitric oxide (NO•) is a free radical formed from its precursor, L-arginine [34] and was identified as an endogenous oxidative marker maintaining respiratory homeostasis [35-39]. To confirm the efficacy of *G. glabra* extract in neutralizing acute toxicity generated by BLM, NO• levels in liver, lung, spleen tissues (Fig. 3) were evaluated.

In *G. glabra* extract tested group insignificantly lower NO• levels (in all organs) were measured compared to the CG. The data demonstrated the BLM toxic effects, and showed an almost three-fold NO• radicals increase in lungs (16.55 ± 0.93 *vs* 8.557 ± 0.9 a.u., p<0.001), relative to the controls.

Fig. 3

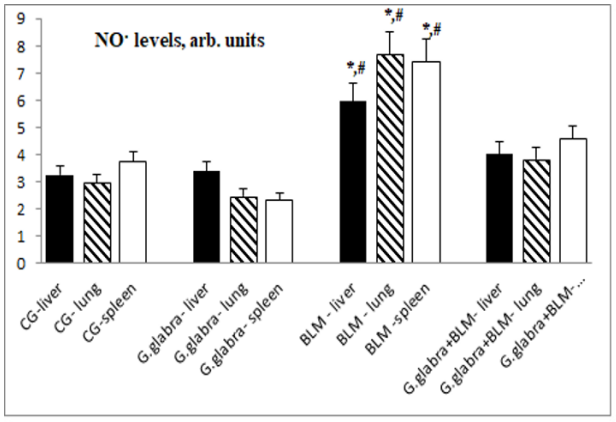


**Figure 3.** *In vivo* NO• free-radical formation in organs. Results were calculated by double integration of the corresponding EPR spectrum immediately registered in plasma (expressed in arbitrary units). The experiments were repeated three times. *p < 0.05 *vs.* the CG group; #p < 0.05 *vs.* the *G. glabra*+BLM group (n = 6).

However, *G. glabra* extract administration modulated the BLM acute toxicity in *G. glabra* + BLM- treated animals (9.331 ± 0.7 *vs* 16.55 ± 0.93 a.u., p<0.05). The antioxidant protective effect of the *G. glabra* extract is confirmed by the absence of residual oxidation processes, resulting in the scavenging effect against the accumulation of non-toxic NO levels, key factor to overcoming of BLM-induced acute oxidative stress.

## CONCLUSION

Based on the present *in vivo* EPR spectroscopy investigations, we consider that *G. glabra* root extract exhibits antioxidant behavior and reduces lipid peroxidation and nitric oxide scavenging. Further experiments on the chemical content and the different biological properties of the plant extract can be added to the treatment of acute respiratory diseases associated with oxidative stress.

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***Conflict of Interest****: The authors declare that they have no conflict of interest.*

## REFERENCES

1. R. Arora, A. S. Dhaker, M. Adhikari, J. Sharma, R. Chawla, D. Gupta, A. Zheleva, Y. Karamalakova, R. Kumar, R. K. Sharma, A. Sharma, S. Sultana, R. P. Tripathi, V. Gadjeva, *Z. Naturforsch. C*, **66** (2011).
2. R. Arora, Herbal Radiomodulators: Applications in Medicine, Homeland Defence and Space. *CABI* Publishing, Wallingford, Oxon, UK, 2008, p. 332.
3. M. N. Asl, H. Hosseinzadeh, *Phytother. Res*., **22** (2008).

4. Z. Karami, H. Mirzaei, Z. Emam-Djomeh, A. R. Sadeghi Mahoonak, M. Khomeiri, *Internat. Food Res. J.*, **20** (2013).
5. A. S. Yadav, D. Bhatnagar, *Biofactors*, **29** (2007).
6. A. K. Ratty, N. P. Das, *Biochem. Med. Metab. Biol*., **39** (1988).
7. B. Yang, A. Kotani, K. Arai, F. Kusu, *Anal. Sci*., **17** (2001).
8. Y.Karamalakova, J. Sharma, G. Nikolova, S. Stanev, R. Arora, V. Gadjeva, A. Zheleva, *Biotechn. & Biotechnolog. Eq.*, **27,** 3861 (2013).
9. L. Dlamini, C. Tata, M. Djuidje, M. Ikhile, G. Nikolova, Y. Karamalakova, V. Gadjeva, A. Zheleva, P. Njobeh, D. Ndinteh, *J. of Ethnopharmacol.*, **231,** 429 (2019).
10. H. Haraguchi, N. Yoshida, H. Ishikawa, Y. Tamura, K. Mizutani, T. Kinoshita, *J. Pharm. Pharmacol*., **52** (2000).
11. N. P. Visavadiya, B. Soni, N. Dalwadi, *Int. J. Food Sci. Nutr*., **60,** Suppl. 2 (2009).
12. N. Basar, O. A. Oridupa, K. J. Ritchie, L. Nahar, N. M. Osman, A. Stafford, H. Kushiev, A. Kan, S. D. Sarker, *Phytother. Res*., **29** (2015).
13. N. Wittschier, G. Faller, A. Hensel, *J. Ethnopharmacol*., **125** (2009).
14. Y. W. Chin, H. A. Jung, Y. Liu, B. N. Su, J. A. Castoro, W. J. Keller, … A. D. Kinghorn, *J. Agricult. and Food Chem.*, **55** (2007).
15. Y. L. Lin, Y. C. Hsu, Y. T. Chiu, Y. T. Huang, *Phytoth. Res.*, **22** (2008).
16. A. Ram, U. Mabalirajan, M. Das, I. Bhattacharya, A. K. Dinda, S. V. Gangal, B. Ghosh, *Internat. Immunopharmacol*., **6** (2006).
17. J. S. Kang, Y. D. Yoon, I. J. Cho, M. H. Han, C. W. Lee, S. K. Park, H. M. Kim, *J. Pharmacol. Experimental Therapeutics*, **312** (2005).
18. H. S. Kim, K. S. Suh, A. Ko, D. Sul, D. Choi, S. K. Lee, W. W. Jung, *Intern. J. Molecular Med.*, **31**(1) (2013).
19. M. Ghorashi, M. Ali Rezaee, M. J. Rezaie, M. Mohammadi, A. Jalili, M. R. Rahmani, *Food and Agricult. Immunol.*, **28**, 1 (2017).
20. H. H. Shi, Y. X. Sui, X. R. Wang, Y. Luo, L. L. Jia, *Comp. Biochem. Physiol. C, Toxicol. Pharmacol*., **140** (1) (2005).
21. G. R. Buettner, B. A. Jurkiewicz, *Free Radic. Bio. Med*., **14** (1993).
22. K. Yokoyama, K. Hashiba, H. Wakabayashi, K. Hashimoto, K. Satoh, T. Kurihara, N. Motohashi, H. Sakagami, *Anticancer Res*., **24** (2004).
23. T. Yoshioka, N. Iwamoto, K. Ito, *J. Am. Soc. Nephrol*., **7** (1996).
24. F. Vatansever, W. C. M. A. de Melo, P. Avci, D. Vecchio, M. Sadasivam, A. Gupta, M. R. Hamblin, *FEMS Microbiol. Rev*., **37** (2013).
25. M. S. Brewer, *Compreh. Rev. in Food Sci. and Food Saf.*, **10**, 4 (2011).
26. S. Kumar, A. K. Pandey, *The Scientific World J*., **16**, ID 162750, (2013).
27. A. Zheleva, Monograph, Academic Press, Trakia University, 2012.
28. I. Spasojevic, M. Mojovich, A. Ignatovic, G. Bacic, *J. Serb. Chem. Soc*., **76** (5) (2011).
29. T. Miyazawa, T. Chiba, T. Kaneda, *Agric. Biol. Chem*., **49** (10) (1985).
30. S. Sen, M. Royt, A. S. Chakraborti, *J. Pharmacy and Pharmacol*., **63** (2011).
31. L. Gao, H. Tang, H. He, J. Liu, J. Mao, H. Ji, H. Lin, T. Wu, *Front. Pharmacol*., **6** (2015).
32. J. K. Kim, S. M. Oh, H. S. Kwon, Y. S. Oh, S. S. Lim, H. K. Shin, *Biochem. Biophysical Res. Communic*., **345**(3) (2006).
33. C. S. Bonnet, D. A. Walsh, *Rheumatology*, **44** (2005).
34. S. Noguchi, K. Yatera, K. Y. Wang, K. Oda, K. Akata, K. Yamasaki, T. Kawanami, H. Ishimoto, Y. Toyohira, H. Shimokawa, N. Yanagihara, M. Tsutsui, H. Mukae, *Respir. Res.*, **5**, 15 (2014).
35. H. Sugiura, M. Ichinose, *Nitric Oxide*, **25** (2011).
36. V. L. Kinnula, C. L. Fattman, R. J. Tan, T. D. Oury, *Am. J. Respir. Crit. Care Med*., **172** (2005).
37. I. Georgiev, I. Kanelov, T. Mircheva, V. Ivanov, S. Dimitrova, Y. Iliev, J. Nikolov, L. Lazarov, A. Roussenov, *Revue Méd. Vét.*, **160**, 335 (2009).
38. Y. Karamalakova, G. Nikolova, M. Adhikari, S. Stoev, P. Agarwal, V. Gadjeva, Z. Zhelev, *Comp. Clin. Path.*, **27,** 1487 (2018).
39. G. Nikolova, Y. Karamalakova, N. Kovacheva, S. Stanev, A. Zheleva, V. Gadjeva, *Reg. Toxicol. and Pharmacol.*, **81,** 1 (2016).