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Original Article

Evaluation of the effects of *Rosmarinus officinalis* (Rosemary) essential oil and its major compound (1,8-Cineole) on rat fertility and fetal skeleton morphology

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ABSTRACT

Rosemary essential oil (REO) is very promising as a therapeutic agent for various pathologies, especially as an antimicrobial. In spite of the growing interest in using REO and its major compound, it is necessary to perform toxicological studies to ensure that the chemical compounds found in the plant under study exert no adverse effects that would impair its use for therapeutic purposes. The purpose of this study is to determine whether rosemary essential oil and its major compound affect mouse fertility and fetal development during the organogenic period, which may trigger bone malformations. This study used pregnant females who received treatment orally (gavage) once a day from the 6th to the 15th day of gestation. They were divided according to the treatment received, and the groups studied included: Group I (negative control - Tween 80), Group II (1,8-cineole 134.55 mg.kg^{-1}), Group III (REO 300 mg.kg^{-1}), Group IV (REO 600 mg.kg^{-1}) and Group V (REO 1200 mg.kg^{-1}). The fetuses underwent the diaphanization technique and staining and were then individually assessed for the presence of bone changes. In females, signs of toxicity, birth rate, post-implantation losses, water and feed intake, and relative weight were assessed. The rats showed no signs of systemic toxicity, nor histopathological changes or alterations in the weight of analyzed organs that could infer any toxic effects. The administration of rosemary essential oil and its major compound 1,8-cineole did not cause differences in the reproductive rates studied in females. However, the treatments did interfere with fetal development, which was demonstrated through skeletal development delay in all groups studied. 1,8-cineole was the major compound of rosemary essential oil, as identified by chromatography. Groups IV, V and II showed a higher proportion of fetuses with skeletal abnormalities (78.31%, 100% and 95.06%, respectively). The alterations found in the fetuses of all studied groups suggest that exposure to these doses of the essential oil and 1,8-cineole should be avoided in pregnant women.

KEYWORDS: teratogenicity, embryotoxicity, rosemary, essential oil.

INTRODUCTION

Exposure of fetuses to certain chemical agents during gestation may increase the risk of adverse effects such as lethality, changes in behavior and general development, and bone malformations [1].

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The latter are characterized as permanent structural changes that negatively affect survival or health of the affected organism [2] and have been scientifically examined previously [3, 4, 5] to characterize new agents which may elicit such changes and to elucidate the mechanism of action of those already known.

Rosemary (*Rosmarinus officinalis* L.) is a small plant of the Lamiaceae family with a woody subarbustive habit. Native to the Mediterranean region, it is cultivated in almost all countries with temperate climates [6]. Methanolic extracts of *Rosmarinus officinalis* L. exert stimulatory effects on hepatic metabolism of estradiol and estrone, which is accompanied by a decrease in the uterotropic action of these estrogens [7]. Lemonica *et al*. [8] concluded that aqueous rosemary extract administered at a dosage of 26 $mg.day^{-1}$ may interfere with the implantation period; however, no effect on malformations and/or fetal abnormalities was observed.

The objective of the present study was to determine whether rosemary essential oil and its main constituent (1,8-cineole) would affect fertility of rats and fetal development during the organogenic period, which may be manifested as bone malformations, so as to evaluate the use of rosemary during pregnancy.

MATERIALS AND METHODS

Materials

Commercial rosemary essential oil purchased from Ferquima Indústria e Comércio Ltda (São Paulo, Brazil) with quality certification and containing 1,8-cineole (Sigma Technologies, Solon, OH, USA) was used. Qualitative and quantitative analysis of the oil was performed in the laboratory of the Chemistry Institute of the Federal University of Rio Grande do Sul (UFRGS), Porto Alegre, RS, Brazil, using gas chromatography with a mass detector and a split/splitless injector (GC/MS Shimadzu QP-5050A; Shimadzu, Tokoy, Japan).

Study animals

All procedures involving animals were approved by the Ethics Committee on Animal Use of the UFRGS (approval number 26988).

To evaluate the effects of rosemary essential oil and its main constituent (1,8-cineole) on rat fertility and fetal development, we used 17 male and 51 nulliparous female rats procured from the Center for Reproduction and Experimentation of Laboratory Animals (CREAL) of the UFRGS. Throughout the experimental period, the animals were kept in cages at constant humidity (50% ± 5%) and temperature (21 ± 2°C) and under a 12/12 h light/dark cycle and were provided with a commercial diet (Nuvital CR 1; Nuvital, Colombo, PR, Brazil) and water *ad libitum*.

Experimental procedures

Rats were allowed to mate during three cycles of five consecutive days with two days hiatus at a ratio of three females to one male. The females were placed in the males' cages for 2 h (6:00–8:00). The beginning of gestation (GD0) was confirmed by using a vaginal washing containing spermatozoa, and rosemary essential oil and 1,8-cineole were administered to the females orally (by gavage) using a volume of 1 mL/100 g body weight once per day from gestation day (GD) 6 to GD 15. The dosage of rosemary essential oil was chosen according to a previous *in vitro* study performed by Santin [9] on antimicrobial activity.

Group I – Negative control; rats received a vehicle treatment solution, Tween 80 (Sigma-Aldrich, St. Louis, MO, USA).

Group II – Main constituent (134.55 $mg.kg^{-1}$); rats treated with 1.8-cineole solution at a concentration of 44.85% (proportional to the compound's concentration in essential oil solution, i.e., 12%).

Group III – Dose 1 (300 $mg.kg^{-1}$); rats treated with rosemary essential oil (REO) 3% in emulsion containing Tween 3%.

Group IV – Dose 2 (600 $mg.kg^{-1}$); rats treated with rosemary essential oil (REO) 6% in emulsion containing Tween 3%.

Group V – Dose 3 (1,200 $mg.kg^{-1}$); rats treated with rosemary essential oil (REO) 12% in emulsion containing Tween 3%.

On a daily basis, body mass and feed and water consumption were recorded, and rats were examined for occurrence of hemorrhages, abortion, and possible systemic treatment effects.

Cesarean section and organ harvesting

On GD 21, cesarean section was performed, the uterus was removed and weighed before removing the fetuses, and the following characteristics were recorded: number of live and dead fetuses, respectively, weight, sex ratio, and external malformations. According to a previous study [8], birth rates were calculated as follows:

$$\frac{number\ of\ live\ fetuses}{total\ number\ of\ fetuses} \times 100$$

Post implantation losses were calculated as

$$\frac{(number\ of\ implantation\ sites - number\ of\ live\ fetuses) \times 100}{number\ of\ implantation\ sites}$$

The adult females' hearts, spleens, livers, and kidneys were collected and examined for macroscopic changes, and the mass of each organ relative to the individual's body mass was recorded. The organs were then fixed in buffered formalin solution and were examined in the Laboratory of Veterinary Pathology, Department of Pathology and Veterinary Clinic, Veterinary School, UFRGS.

Diaphanization and staining

The fetuses were subjected to a modified diaphanization technique [10] and were individually examined for bone alterations, based on an atlas of external bone anomalies in rats [11]. Fetuses previously fixed in 10% formalin were dehydrated for 24 h in 70% ethyl alcohol and in 96° GL ethyl alcohol, after which they were gutted and immersed in 30% sodium borate buffer solution for two days; buffer was exchanged every 24 h. For examination, the fetuses were kept in a 30% sodium borate solution containing 1 g/L trypsin at room temperature for the required period, and the solution was exchanged when it turned cloudy. For skeletal staining, potassium hydroxide solution (1.5%) was used with alizarin red dye for 24 h. After this, the skeletons were placed in solutions of glycerin and 1.5% potassium hydroxide at concentrations of 30% and 70% for 48 hours, each, and were then stored in glycerin for further evaluation [12].

Statistical analyses

Data distribution was assessed using a Kolmogorov-Smirnov test, and homoscedasticity was tested using Bartlett's test. Statistical analyses included a one-way analysis of variance and a chi-square test. Differences in quantitative variables were tested using a Bonferroni test, and qualitative variables were subjected to a chi-square test. All analyses were performed using Statistica 7.0 (Statsoft Inc, Tulsa, OK, USA). Statistical significance is reported at $P < 0.05$. Values are expressed as means ± standard error of the mean.

RESULTS

Chromatographic analysis of rosemary essential oil

The essential oil used in this study had been purchased from a commercial distributor; therefore, in order to confirm the main constituent and the oil's purity, chromatographic analysis was performed, which showed that 1,8-cineole was the predominant compound (44.85%; Table 1).

In the adult females, there was no evidence of hemorrhages, abortions, or differences between treatment group regarding water and feed consumption, body mass, or any other systemic parameter. Regarding relative organ weight, there was a significant increase in liver weight in the group V and in weights of the spleens and livers of the group II, compared to the group I (Table 2).

Administration of rosemary essential oil and its main constituent, 1,8-cineole, did not affect the adult females' reproductive indices such as the number of pups per litter, post-implantation losses, and birth rates. The three examined essential oil

Table 1. Major chemical composition of commercial essential oil of *Rosmarinus officinalis* L.

Constituents	Percentage (%)
1,8-cineole	44.85
Canphor	17.21
α-pinene	13.83
β-pinene	10.70

Table 2. Relative organ weight [(organ mass in relation to body mass) x 100] of rats exposed to Tween 80 (Group I), 300 mg.kg^{-1} of REO (Group III), 600 mg.kg^{-1} of REO (Group IV), 1200 mg.kg^{-1} of REO (Group V) and 134.55 mg.kg^{-1} of 1,8-cineole (Group II) from the 6th to the 15th day of gestation. Values are mean ± standard error.

Relative weight %	Groups				
	I (n = 12)	III (n = 10)	IV (n = 10)	V (n = 9)	II (n = 10)
Heart	0.24 ± 0.01	0.24 ± 0.01	0.24 ± 0.01	0.25 ± 0.01	0.25 ± 0.01
Spleen	0.22 ± 0.02	0.23 ± 0.01	0.26 ± 0.02	0.24 ± 0.01	0.29 ± 0.01*
Liver	3.71 ± 0.16	3.74 ± 0.10	3.97 ± 0.12	4.11 ± 0.12*	4.32 ± 0.15*
Left kidney	0.31 ± 0.01	0.26 ± 0.01	0.29 ± 0.02	0.28 ± 0.01	0.28 ± 0.02
Uterus	20.36 ± 1.14	24.00 ± 1.15	21.42 ± 2.07	22.24 ± 1.43	19.63 ± 2.00

*($p < 0.05$) in relation to the control group (Group I). ANOVA, followed by Bonferroni test.

dosages did not alter embryonic and fetal development with regard to fetal body mass on GD21, sex ratio, and external malformation rate (Table 3).

There were significant differences regarding the occurrence of anomalies in fetuses of females treated with essential oil and with 1,8-cineole. Table 4 shows bone alterations with regard to position, absence, shape (malformation), and calcification problems. Fetuses of females in groups IV, V, and II showed higher proportions of skeletal abnormalities, compared to those of group I rats. Fetuses of females in groups V and II showed higher rates of poor ossification of the skull, and those of females in groups III and IV showed a higher percentage of poorly ossified supraoccipital bones and irregular sternum bones, while group II fetuses showed a higher rate of poor ossification of thoracic limbs and femurs than group I fetuses.

DISCUSSION

The embryonic period referred to as post-implantation period is defined as the interval from complete implantation until the end of fetal organogenesis [13]. This developmental period comprises processes such as cell proliferation, differentiation, and migration and formation of early organs, and chemical substances that cause non-life-threatening injury to the fetus when administered to a pregnant female during this phase are referred to as teratogenic [14]. It is likely that metabolites of essential oils pass through the placenta due to the close (but not direct) contact between maternal and embryonic or fetal blood. Lipophilic substances can migrate by passive diffusion between these two circulation systems and reach equivalent levels in maternal and fetal blood. These substances are biotransformed to polar compounds and can accumulate in the fetus [15].

Table 3. Reproductive indices of rats exposed to Tween 80 (Group I), 300 mg.kg^{-1} of REO (Group III), 600 mg.kg^{-1} of REO (Group IV), 1200 mg.kg^{-1} of REO (Group V) and 134.55 mg.kg^{-1} of 1,8-cineole (Group II) from the 6th to the 15th day of gestation. Values are mean ± standard error.

Reproductive indices	Groups				
	I	III	IV	V	II
Number of rats	12	10	10	9	10
Number of fetuses	139	117	96	91	99
Body mass at birth (g)	4.61 ± 0.13	5.16 ± 0.18	4.85 ± 0.11	4.61 ± 0.14	4.93 ± 0.16
Sex ratio (male:female)	1.16:1	1.84:1	1.03:1	1.37:1	1.33:1
Number of puppies per litter	11.58 ± 0.8	11.70 ± 0.9	10.67 ± 1.0	11.38 ± 1.0	9.90 ± 1.3
Post-implantation losses (%)	3.92 ± 1.76	3.88 ± 1.31	4.87 ± 2.89	9.32 ± 3.41	1.11 ± 0.11
Birth rate (%)	100	100	100	100	100
External malformation rate (%)	0	1.71	2.02	2.10	1.01

The present study aimed to evaluate fertility and fetal development effects of three concentrations of rosemary essential oil (300, 600, and 1,200 mg.kg^{-1}) and 1,8-cineole (134.55 mg.kg^{-1}) administered to pregnant female rats during organogenesis. Organogenesis is highly sensitive to toxic substances [16] which may elicit adverse effects on skeletal development, termed anomalies [17, 18]. There was a high proportion of anomalies, predominantly in fetuses of groups V and II, which, however, were also evident in fetuses of females treated with low and intermediate oil concentrations, confirming its effect on bone formation [19]. Poor skull ossification in V and II group fetuses suggested delayed fetal development. In the sternum, there was a difference with regard to irregular sternites in III and IV group fetuses, compared to the control group, which also indicates delayed development [12]. Delays in bone development are associated with absence of ossification centers in bilateral structures, which is indicated by shape and/or size suggestive of an early stage of development [20].

The incidence of skeletal abnormalities and poor skull ossification increased with the treatment dosage, and the treatment seemed to adversely affect fetal development even at the lowest dosage. The observed changes were variations characterized by a delay in development [17, 18], whereas malformations, which are represented by partial or total absence of important bones, shortening, arching, asymmetry, fusions, clefts, or duplicity [20], were not observed. Lemonica *et al*. [8] found no significant effect on the skeleton of fetuses of rats treated with aqueous extract of *Rosmarinus officinalis* administered from day 6 to 15 of gestation. Our study shows, for the first time, that rosemary essential oil and its main constituent can cause delay in fetal bone development.

Reproductive indices of treated females showed no statistically significant differences to the control group; however, the rate of post-implantation losses showed an increasing trend in the groups that received rosemary essential oil, suggesting that higher dosages should be tested. A previous study [21] examined the mechanisms by which rosemary aqueous extract may influence embryo implantation, correlating its intake with possible alterations of embryo development. The extract was administered to Wistar rats orally at 260 and 1,040 mg.kg^{-1} from day 1 to day 4 of gestation, once per day. Treatment with 260 mg.kg^{-1} caused a significant increase in the number of anomalous embryos collected from the uterus on day 5, showing a toxic effect on fetuses; however, successfully implanted embryos showed normal development. Administration of 1,040 mg.kg^{-1} of

Table 4. Percentage of skeletal alterations in fetuses of rats exposed to Tween 80 (Group I), 300 mg.kg^{-1} of REO (Group III), 600 mg.kg^{-1} of REO (Group IV), 1200 mg.kg^{-1} of REO (Group V) and 134,55 mg.kg^{1} of 1,8-cineole (Group II) from the 6th to the 15th day of gestation.

	Groups				
	I	**III**	**IV**	**V**	**II**
Fetuses with skeletal abnormalities (%)	43.30	58.24	78.31*	100*	95.06*
Fetuses with skeletal abnormalities in (%)					
HEAD					
Poor ossification	33.10	18.68	4.82	86.60*	77.78*
Supraoccipital with incomplete ossification	0.00	3.30	4.82	0.00	0.00
Supraoccipital with poor ossification	0.00	21.98*	50.60*	0.00	4.94
Interparietal with poor ossification	0.00	0.00	0.00	0.00	1.23
Frontal with parietal fusion	0.00	0.00	0.00	3.09	0.00
Supraoccipital with parietal fusion	0.00	0.00	1.20	0.00	0.00
Juxtaposition of interparietals, parietals and frontals	7.90	12.09	0.00	4.12	0.00
Interparietal juxtaposition with parietal	0.00	2.20	1.20	0.00	0.00
STERNUM					
Unevenly positioned collarbone	0.00	2.20	0.00	0.00	0.00
Clavicle with poor ossification	0.00	5.49	0.00	0.00	4.94
Irregular sternebra	0.00	8.79*	33.73*	2.06	0.00
Sternebra with poor ossification	0.00	0.00	1.20	5.15	2.47
SPINE					
Cervical with poor ossification	0.00	2.20	0.00	0.00	0.00
Lumbar with poor ossification	0.00	3.30	0.00	0.00	0.00
THORACIC LIMBS					
Poor ossification	0.00	2.20	0.00	0.00	16.05*
PELVIC LIMS					
Femurs with incomplete ossification	0.00	1.10	0.00	0.00	0.00
Femurs with poor ossification	0.00	0.00	1.20	4.12	22.22*

*Significant difference ($P < 0.05$), Chi Square.

the extract showed no embryotoxic effect; however, on day 5, a 40% decrease in the number of blastocytes present in the uterus was observed. This suggests that treatment with higher dosages causes higher embryonic retention in the oviduct [21].

The main compound of rosemary essential oil identified by chromatographic analysis was 1,8-cineole, a monoterpene also identified in other studies on rosemary essential oil [22, 23] which, due to its pleasant aroma and taste, is frequently used in food, fragrances, and cosmetics; moreover,

it is also known and studied for its beneficial effects during respiratory tract infections [24].

Liver weights increased in females of the V and II groups, compared to those of the group I, which was also observed in previous studies [25, 26]. Absence of significant histological alterations in the studied organs and of signs of systemic toxicity such as apathy and diarrhea indicates that at the studied dosages and under our experimental conditions, rosemary essential oil and 1,8-cineole did not induce systemic toxicity in female rats [12]. No histopathological changes were observed, apart from hepatocellular swelling (i.e., liver enlargement characterized by hepatocellular hypertrophy) which is considered a common adaptive response of this organ to exposure to xenobiotics [27]. In a previous study, 1,8-cineole administered at 1,000 $mg.kg^{-1}$ caused an increase in relative and absolute mass of the liver of female rats [28]. In a different study on male rats treated for 28 days, similar results were observed [29]. Spleens of group II rats were heavier than those of controls; however, this was an isolated observation because clinical chemistry dosages were not used, and this change was not associated with histopathological effects. Previously, a reduction in absolute spleen mass in males treated with 1,000 $mg.kg^{-1}$ 1,8-cineole was observed [28].

The results of the present study suggest that *Rosmarinus officinalis* essential oil at dosages of 300, 600, and 1,200 $mg.kg^{-1}$ and its main constituent 1,8-cineole (134.55 $mg.kg^{-1}$) are non-toxic to pregnant rats when administered from GD 6 to 15; however, they interfere with fetal development as evidenced by the observed delayed skeletal development in fetuses of all treatment groups, suggesting that respective dosages should be avoided by rats during gestation.

CONCLUSIONS

Treatment with rosemary essential oil and its main constituent (1,8-cineole) did not affect fertility of rats treated from day 6 to 15 of gestation, and no signs of toxicity were observed in adult females.

Skeletal changes were observed in fetuses of all treatment groups, as evidenced by delayed skeletal development, suggesting that the dosages studied here should be avoided by females during gestation.

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CONFLICT OF INTEREST STATEMENT

The authors certify that they have no affiliations with or involvement in any organization or entity with any financial interest or non-financial interest in the subject matter or materials discussed in this manuscript.

ABBREVIATIONS

CREAL : Reproduction and Experimentation of Laboratory Animals
GD : Gestation day
REO : Rosemary essential oil
UFRGS : Federal University of Rio Grande do Sul

REFERENCES

1. Farrar, H. C. and Blumer, J. L. 1991, Annu. Rev. Pharmacol. Toxicol., 31, 525-547.
2. Chahoud, I., Buschmann, J., Clark, R., Druga, A., Falke, H., Faqi, A., Hansen, E., Heinrich-Hirsch, B., Hellwig, J., Lingk, W., Parkinson, M., Paumgartten, F. J. R., Pfeil, R., Platzek, T., Scialli, A. R., Seed, J., Stahlmann, R., Ulbrich, B., Wu, X., Yasuda, M., Younes, M. and Solecki, R. 1999a, Reprod. Toxicol., 13, 77-82.
3. Park, D., Jeon, J. H., Shin, S., Joo, S. S., Kang, D. H., Moon, S. H., Jang, M. J., Cho, Y. M., Kim, J. W., Ji, H. J., Ahn, B., Oh, K. W. and Kim, Y. B. 2009, Reprod. Toxicol., 27, 79-84.
4. Vianna, F. S. L., Kowalski, T. W., Fraga, L. R., Sanseverino, M. T. V. and Schuler-Faccini, L. 2017, Eur. J. Med. Genet., 60, 12-15.
5. Watson, A. T. D., Planchart, A., Mattingly, C. J., Winkler, C., Reif, D. M. and Kullman, S. W. 2017, Toxicol. Sci., 11, 1-12.
6. Hanson, J. R. 2016, Sci. Prog., 99, 83-91.
7. Zuh, B. T., Loder, D. P., Cai, M. X., Ho, C. T., Huang, M. T. and Conney, A. H. 1998, Carcino., 19, 1821-1827.
8. Lemonica, I. P., Damasceno, D. C. and Di-Stasi, L. C. 1996, Braz. J. Med. Bio. Res., 29, 223-227.

9. Santin, R. 2013, Antifungal potential and toxicity of essential oils from the Lamiaceae family. 104f. Doctoral Thesis in Veterinary Sciences, Federal University of Rio Grande do Sul, Veterinary Science, Porto Alegre, RS.
10. Taylor, W. R. and van Dyke, G. C. 1985, Cybiun, 9, 107-119.
11. Chahoud, I. 1996, Atlas of External and Skeletal Anomalies in Rats. Berlin, Institute of Toxicology and Embryopharmacology FU, Leipzig : Pr & C GmbH. CD Room.
12. Dantas, A. S. 2016, Study of reproductive toxicity and genotoxicity of the essential oil of Origanum majorana. 119f. Porto Alegre, RS. Thesis (Doctorate in Veterinary Sciences) – Veterinary Sciences, Federal University of Rio Grande do Sul.
13. Foote, R. H. and Carney, E. W. 2000, Reprod. Toxicol., 14, 477-493.
14. Bernardi, M. M. 2014, Exposição aos medicamentos durante o período perinatal in Farmacologia aplicada à medicina veterinária, H. S. Spinosa, S. L. Górniak and M. M. Bernardi, Guanabara Koogan, Rio de Janeiro, 824.
15. Burfield, T. 2000, Inter. J Aromath., 10, 14.
16. Oxendine, S. L., Cowden, J., Hinton, D. E. and Padilla, S. 2006, Neurotoxicol., 27, 840-845.
17. Chahoud I., Talsness, C. E., Walter, A. and Grote, K. 2015, Reprod. Toxicol., 58, 15-23.
18. Chahoud, I., Ligensa, A., Dietzel, L. and Faqi, A. S. 1999b, Reprod. Toxicol., 13, 375-381.
19. Mello, J. R. B., Langeloh, A., Habermehl, G., Krebs, H., Bastos, F. and Alvares, F. 2000, Ciência Rural, 411-415.
20. Lork, D. 1997. Evaluation of Skeleton in Methods in prenatal toxicology, evaluation of embryotoxic effects in experimental animals, D. Neubert, H. J. Nerker, T. E. Kawasigroc, Stuttgard, Georg Thieme Verlag, 474.
21. Damasco, D. C. and Lemonica, I. P. 1999, Rev. Bras. Toxicol., 12, 47-54.
22. Okoh, O. O., Sadimenko, A. P. and Afolayan, A. J. 2010, Food Chem., 120, 308-312.
23. Waller, S. B., Madrid, I. M., Silva, A. L., Castro, L. L. D., Cleff, M. B., Ferraz, V., Meireles, M. C., Zanette, R. and Mello, J. R. 2016, Mycopathol., 181, 857-863.
24. Juergens, U. R. 2014, Drug Res., 64, 638-646.
25. Debersac, P., Vernevaut, M. F., Amiot, M. J., Suschetet, M. and Siess, M. H. 2001, Food Chem. Toxicol., 39, 109-117.
26. Guerra, M. O., Borges, N. B. E., Bellei, P. M., Elias, M. A. F., Silveira, S. M. C. and Peters, V. M. 2009, Revista Interdisciplinar de Estudos Experimentais, 1, 8-13.
27. McGuire, E. J., DiFonzo, C. J., Martin, R. A. and Iglesia, F. A. 1986, Toxicol., 39, 149-163.
28. Caldas, G. R. F., Limeira, M. M. F., Araújo, A. V., Albuquerque, G. S., Silva-neto, J. C., Silva, T. G., Costa-silva, J. H., Menezes, I. R. A., Costa, J. G. M. and Wanderley, A. G. 2016, Food Chem. Toxicol., 96, 297-306.
29. Kristiansen, E. and Madsen, C. 1995, Toxicol. Lett., 80, 147-152.