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Toxicological Evaluation of the Hydro-alcohol Extract of the Dry Leaves of *Peumus boldus* and Boldine in Rats

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The hydro-alcohol extract of the dry leaves of *Peumus boldus* and boldine, showed abortive and teratogenic action and changes in the blood levels of bilirubin, cholesterol, glucose, alanine aminotransferase (ALT), aspartate aminotransferase (AST) and urea in rats. The long term administration of the extract and boldine did not cause histological modification during a period of 90 days.

Keywords: Peumus boldus; toxicology; hydro-alcohol extract; boldine.

INTRODUCTION

Peumus boldus Mol (Monimiaceae) is an evergreen shrub or small tree that grows in Central Chile (Giron *et al.*, 1991). It has been used in South America against liver diseases and gallstones (Cederbaum *et al.*, 1992; Magistretti, 1980) and as a vermifuge. Bourgoin and Verne (1872) extracted from the leaves of *Boldo* an alkaloid which came to be known as boldine (Fig. 1). Hansel (1991) demonstrated the presence of ascaridole, eucalyptol and p-cymol, and 0.25% to 0.5% of an alkaloid mixture. It must be noted that the volatile oil in the leaves contains about 40% ascaridole, a rather toxic component. Since no chronic toxicity testing has been carried out, Hansel (1991) has recommended that prolonged use of the herb or any consumption by pregnant women should be avoided. Moreno *et al.* (1991) working with boldine, was able to induce weakly cytoplasmic 'petite' mutation in haploid yeast cells. However, Tavares and Takahashi (1994) demonstrated that boldine did not induce a statistically significant increase in the frequency of chromosome aberrations and sister-chromatic exchanges (SCEs) in either test system. The purpose of the present study was to determine its toxicity in pregnant rats and to examine the histology of the heart, liver and kidneys. In addition, changes in blood cholesterol, bilirubin, creatinine, glucose transaminases (AST, ALT) and urea were studied.

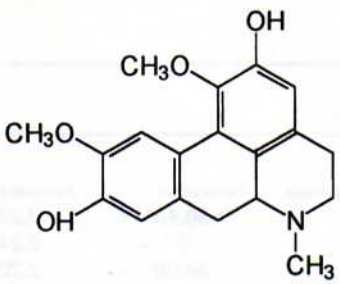

Figure 1. Chemical structure of boldine.

MATERIAL AND METHODS

Animals. Adult Wistar rats (160–220 g) were kept under standard laboratory conditions of temperature (23° ± 1°C), relative humidity of approximately 60% and 12/12 h light/dark cycle. The animals received a commercial rat diet (Purina®) and tap water *ad libitum*.

Plant material. The dried leaves of *Boldo* were obtained at a local store in the State of Pernambuco. The plant material was identified botanically and a voucher specimen is deposited in the Herbarium of the Antibiotics Department of the UFPE under the registry number: 6248 IA-UFPE. The dried leaves of *Boldo* were extracted with 92.8 EtOH (2 L) yielding 108 g of dry crude extract. boldine was from Sigma Chemical Co.

Phytochemical screening. Preliminary screening of the extract showed the presence of steroids, triterpenoids, mono and sesquiterpenes, flavonoids, alkaloids and reducing sugars, and an absence of coumarins, iridoids, cardiac glycosides, saponins and hydrolysable tannins (Wagner and Bladt, 1996).

The study of pregnant rats. Female Wistar rats were mated with males of previously confirmed fertility (one male for three females). Vaginal smears were examined each morning for the presence of sperm on day 1 of

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Table 1. Groups treated with hydro-alcohol extract of the dry leaves of *P. boldus*

Group	Dose (mg/kg)	Day of pregnancy	Number of rats
1	800	1–5	20
2	500	1–5	20
3	Control	1–5	20
4	800	7–12	20
5	500	7–12	20
6	Control	7–12	20

Table 2. Groups treated with boldine

Group	Dose (mg/kg)	Day of Pregnancy	Number of rats
1	800	1–5	20
2	500	1–5	20
3	Control	1–5	20
4	800	7–12	20
5	500	7–12	20
6	Control	7–12	20

pregnancy (Almeida *et al.*, 1988). Inseminated animals were isolated in cages and divided into 12 groups, each group with 20 animals, which were treated with the extract of *Boldo* and boldine according to Tables 1 and 2.

Each group treated had its corresponding control group. The extract and boldine was administered orally at 500 and 800 mg/kg. The control group received an equivalent amount of saline 0.9% (orally) 0.1 mL/100 g. Each animal was weighed and killed by cervical displacement on day 19 of gestation. A laparotomy was performed and the uterus and ovaries were removed. Resorptions (embryotoxicity/fetotoxicity) were counted and viable implants were examined. The number of live/dead fetuses, viability, growth and deformity of new borns and maternal weight gain were recorded.

Long term administration effects. Male Wistar rats were studied. All were submitted to administration of 50 and 200 mg/kg, orally, 20 animals for each dose, for 90 days. The blood level of bilirubin, cholesterol, creatinine, glucose, AST, ALT and urea was measured at 0, 30, 60 and 90 days. The method used for each was colorimetric, using the Labtest® diagnostic system. At termination each animal was killed by cervical displacement 24 h after the last treatment, then the liver, heart and kidneys were removed for histological study (Casarett and Doull, 1975).

Table 5. Fetal malformation percentage in the viable implants with extract

Fetal observation	Dose 800 mg/kg (day 1–5)	Dose 800 mg/kg (day 7–12)
Absence of paw—inferior %	–	3.58
Absence of the external ear %	–	3.58
Absence of tail %	1.53	–

Statistical evaluation. The results were analysed using Student's *t*-test and chi-square test for statistical analysis (Siegel, 1975).

RESULTS

Teratogenic and abortive activity in pregnant rats

As observed in Tables 3 and 4, the extract and boldine in a dose of 800 mg/kg administered from day 1 to day 5 and from day 7 to day 12 led to low fetal toxicity. Furthermore malformations of the implant and viable fetus were observed in the animals killed on day 19 of pregnancy (Tables 5, 6) A weight decrease of the malformed fetus of about ±47% in relation to the viable fetus also occurred. A dose of 500 mg/kg did not produce any fetotoxicity.

Table 3. Incidence of resorption in the extract and control groups

Group	Dose	Number of implants	Resorptions	Total number of corpora lutea	Resorption index	Average fetal body weight
1	800 day 1–5	160	30	160	38–75[a]	2.289 ± 0.202
2	500 day 1–5	171	00	171	00	2.310 ± 0.199
3	800 day 7–12	163	43	163	36.38[a]	2.260 ± 0.210
4	500 day 7–12	165	00	165	00	2.277 ± 0.200
5	Control day 1–5	166	00	166	00	3.225 ± 0.256
6	Control day 7–12	177	00	177	00	3.774 ± 0.221

Values are mean ± SEM for 20 animals.
[a] $p < 0.5$ vs control group (chi-square test).

Table 4. Incidence of resorption in the boldine and control groups

Group	Dose (mg/kg)	Number of implants	Resorptions	Total number of corpora lutea	Resorption index	Average total body weight
1	800 day 1–5	166	34	166	30.48[a]	2.267 ± 0.199
2	500 day 1–5	164	00	164	00	2.248 ± 0.189
3	800 day 7–12	165	43	165	36.06[a]	2.235 ± 0.181
4	500 day 7–12	166	00	166	00	2.247 ± 0.185
5	Control day 1–5	176	00	176	00	3.342 ± 0.231
6	Control day 7–12	167	00	167	00	3.191 ± 0.261

Values are mean ± SEM for 20 animals.
[a] $p < 0.5$ vs control group (chi-square test).

Table 6. Fetal malformation percentage in the viable implants with boldine

Fetal observation	Dose 800 mg/kg day 1–5	Dose 800 mg/kg day 7–12
Absence of paw—inferior %	–	3.58
Absence of the external ear %	–	3.58
Absence of tail %	1.53	–

Histological studies

In a dose of 500 and 800 mg/kg, no overt signs of toxicity were observed in the heart and kidneys. On the other hand, the liver showed alterations of low intensity. However, steatosis was only observed in two animals not associated with sinusoidal congestion or leukocyte infiltration, at a dose of 800 mg/kg. In Tables 7–9, alterations in the levels of ALT, AST, cholesterol, glucose, bilirubin and urea were observed. A dose of 500 mg/kg did not show any significant modification during a period of 90 days.

DISCUSSION

The popular use of *Boldo* as a hepatic protector, cholagogue/choleretic and antiinflammatory agent was reported by Cederbaum *et al.* (1992), Lanhers *et al.* (1991) and Magistretti (1980). Oral administration of crude extract upto 3 g/kg did not demonstrate any acute toxic effect in laboratory animals (Magistretti, 1980). However, *in vitro* studies of genotoxicity of eucaryotic

Table 7. Blood chemistry profile before, during, and after treatment with the extract

	Before	After 30 days	After 60 days	After 90 days
Cholesterol	136.33	229.16[a]	178.96[a]	109.86[a]
AST	79.83	139.33[a]	128.83[a]	51.33[a]
ALT	42.83	85.50[a]	103.66[a]	53.83
Bilirubin				
Total	1.20	0.65[a]	0.40[a]	0.34[a]
Direct	0.66	0.38	0.30	0.17
Indirect	0.54	0.26	0.10	0.17
Glucose	134.66	89.63[a]	86.16[a]	80.83[a]
Creatinine	0.82	0.71	0.65	0.64
Urea	45.96	37.38[a]	35.90[a]	35.48[a]

Values are mean ± SEM for 20 animals.
[a] $p < 0.05$ vs control group (Student's *t*-test).

Table 8. Blood chemistry profile before, during and after treatment with doldine

	Before	After 30 days	After 60 days	After 90 days
Cholesterol	131.33	226.16[a]	170.96[a]	100.86[a]
AST	70.83	149.33[a]	138.83[a]	51.33[a]
ALT	41.83	84.50[a]	101.66[a]	52.83
Bilirubin				
Total	1.20	0.65[a]	0.40[a]	0.34[a]
Direct	0.65	0.37	0.29	0.18
Indirect	0.55	0.28	0.11	0.16
Glucose	124.66	90.63[a]	85.16[a]	79.83[a]
Creatinine	0.82	0.70	0.66	0.63
Urea	45.96	37.38[a]	35.90[a]	35.48[a]

Values are mean ± SEM for 20 animals.
[a] $p < 0.05$ vs control group (Student's *t*-test).

Table 9. Blood chemistry profile of control group

	Before	After 30 days	After 60 days	After 90 days
Cholesterol	136.33	135.16	136.96	134.86
AST	79.83	79.33	78.83	77.33
ALT	42.83	85.50	103.66	53.83
Bilirubin				
Total	1.20	1.21	1.20	1.19
Direct	0.66	0.68	0.67	0.63
Indirect	0.54	0.53	0.53	0.56
Glucose	134.66	133.03	134.01	132.23
Creatinine	0.82	0.81	0.82	0.84
Urea	45.96	44.18	45.90	45.08

and prokaryotic cells demonstrated some alteration. Research done so far seems to confirm the folklorie usage of *Boldo* for liver and gallstone treatment (Magistretti, 1980; Speisky and Cassels, 1994; Valenzuela *et al.*, 1991). However, there is little knowledge regarding its toxicity in long term treatment. Hansel (1991) alerts against the use in pregnant women and Guinaudeau *et al.*, (1975) quotes the presence about 16 different components of unknown toxicity. The study of pregnant rats treated orally with 800 mg/kg of crude extract of *Boldo* and boldine demonstrated anatomical alterations in the fetus. We also observed incidents of blastocystotoxic-antizygotic action and a few cases of abortive activity in both groups treated. This observation indicates that *Boldo* acts at the beginning of egg division and also during implantation. The physiopathological mechanism of this effect is our major area of interest for future studies.

In the second part of the experiment, it was noticed that after 30 days of daily oral administration of crude extract and Boldine, there was a significant increase of cholesterol, AST, ALT in the blood level, and a reduction in the level of total bilirubin, urea and glucose. There was no significant alteration in the level of creatinine in the blood during the 90 days the animals were under treatment with the crude extract and boldine. It was also observed that there was a small histological alteration at the liver site and one of these alterations, steatosis, is rare and was only observed in two animals, certainly not associated with sinusoidal congestion, central-lobular distribution, or leukocyte infiltration, and also, a moderate focal infiltration in two animals. There were no histological alterations detected in heart or kidney tissues. Finally, we would suggest that as a result of biochemical and histological alterations observed in this study, moderation and care should be used in the administration of *Boldo*, especially on long term use and during the first trimester of pregnancy, mainly because of the lack of knowledge of the mechanisms of action which produce the different compounds which are present in the leaves of *Boldo*.

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