Pharmacognosy Magazine
ISSN: 0973-1296

PHCOG MAG.
An official Publication of Phcog.Net

PHCOG MAG. Research Article
Preclinical Toxicity Study of the phytomedicine – bee honey, propolis and *Mikana glomerata* extract in rodents

[1]Ponte, F. L. R; [2]Silva, A. A. R and [3*]Maia, M. B.S

[1]Departamento de Enfermagem, Centro de Ciências da Saúde da Universidade do Vale do Acaraú, Sobral, Ceará, Brasil; [2]Departamento de Ciências Biológicas, Campus Ministro Reis Velloso da Universidade Federal do Piauí; [3]Departamento de Fisiologia e Farmacologia, Centro de Ciências Biológicas da Universidade Federal de Pernambuco, Av. Prof. Moraes Rego, s/n. 50.670.901. Recife-PE, Brasil.

***[3*]Corresponding Author: Telephone :** 55-81- 2126 8530 FAX: 55-81- 2126 8976*

e-mail: mbsm@.ufpe.br

ABSTRACT - This study was designated to evaluate the preclinical toxicity of the phytomedicine - bee honey, propolis (1.8 %) and extract of *Mikana glomerata* Sprengel (3.7%), commonly used in Brazil in treatment of respiratory illnesses. In order to eavaluate the acute toxicity, groups of Swiss mice (n=10/group) received a single dose of the phytomedicine (7.5 to 35mL/kg; p.o.) or saline 0,9% (5ml/kg; p.o.). The acute toxicity data showed a low toxicity order for the phytomedicine. The absence or presence of toxicity by prolonged use of phytomedicine was also evaluated through biochemical and hematological analysis of Wistar rat blood samples using daily oral doses of 7.5 or 15 ml/Kg; during 90 days. The toxicity study did not show any treatment-related abnormalities in hematological (red blood cell, Hemoglobin, hematocrit, mean corpuscular hemoglobin, mean corpuscular volume, mean corpuscular hemoglobin concentration; leucocytes and platelets) or biochemical parameters (glucose, urea, cholesterol, HDL-cholesterol, triglycerides; alanine aminotranferase, aspartate aminotranferase, alkaline phosphatase and total proteins). No significant differences were found between the treated groups and the saline control group in regard to the rate of weight gain. The external apperance of several organs (heart, spleen, liver, kidney, suprarenal gland, stomach, lungs, testicles, ovaries and womb) were analysed, and any aparent and significant features or differences from control group were recorded. It was possible to verify significant differences ($p < 0.05$) in relation to the weight of the liver and kidney and spleen; which presented higher in the male animal treated with the phytomedicine. However, such alterations were not dependent dose and they just limited to the males. Therefore, through preclinical assay, it appears that no toxicological hazard (acute and sub-chronic) is related to the use of test phytomedicine.

KEYWORDS – Acute toxicity, sub-chronic toxicity, *Mikania glomerata*, Compositae.

INTRODUCTION

Increase in the demand of natural products has influenced the direction taken by many studies in pharmacology and toxicology. Since the largest part of the herbal remedies have no study concerning their safety, action for the legalization of norms of usage and registration of phytomedicines are absolutely necessary, so that indiscriminate use of specific herbals such as those containing pyrrolizidine alkaloids may be avoided. These alkaloids are hepatotoxic, and a single dose can be fatal (1).

Once the efficacy of a new phytomedicine is proven, an evaluation of its security in laboratory animals and through other experimental models should follow. Scientific reasons for the realization of toxicological studies are that many countries' legislation demands that a given phytomedicine must receive authorization for commercialization and prescription so as to constitute a medicine.

The duration of the pre-clinical toxicological studies depends on the application duration of the phytomedicine in human beings. The primary objective of toxicity evaluation studies is to assess the safety of the compound intended for clinical use by establishing acute, sub-chronic and chronic effect and reproductive toxicity (2, 3). In addition animal and cell testing for mutagenicity and carcinogenicity as well organ system toxicity are indispensables before human test can begin (4). These assays allow the detection of toxic effects, the understanding of toxic mechanisms and the definition of conditions in which these effects are produced. Thus, phytomedicines which are to be used only on determined occasions demand short studies,

while those used continuously have to be submitted also to clinical studies.

The global commerce of phytomedicines is estimated to amount to US$22 billion (5). Althought in Brazil, the sector is small yet (about US$ 500 million per annum), its potential is unlimited. Brazilian biodiversity is a treasure trove of unexplored chemical patrimony of medicines, food, cosmetics, fertilizers, pesticides, without counting an almost infinite number of molecules, enzymes and genes (6). In Brazil, the Secretary of Health and the National Agency of Sanitary Vigilance (ANVISA) were induced by the Sanitary Vigilance agencies of various States, preoccupied with the increase of consumption of phytomedicine products in relation to their efficacy and security, to elaborate – in the Regulation no. 116/96, dated 08/08/1996 – a technical guide of pre-clinical and clinical toxicological studies, complemented by general precepts for studies in therapeutic efficacy (7). This culminated in the Resolution no. 48/2004, which regulates at the moment the registration and commercialization of phytomedicines (8).

At the moment, phytotherapy exists principally on the informal market, representing a serious threat to public health, since the vegetal drugs are commercialized without any phytosanitary control in regard to their identity or purity. Better and more control in this pharmaceutical area is necessary, as the phytomedicines represent an alternative economically more viable to the population and also for reasons of a revival of historical knowledge (9). It is in this context that the present study proposes to contribute to the validation of bee honey with propolis and *Mikania glomerata* extract, a phytomedicine widely used by the brazillian population.

Mikania glomerata Sprengel (Compositae) is a sub-arbor climber of South American origins. It is widely used in popular medicine for respiratory illnesses, while showing additionally therapeutic proprieties as follows: tonic, purgative, febrifuge, as well as being used as appetite stimulant and in the treatment of influenza (10). It is usually administered in the form of syrup to alleviate asthma or coughing crises, as infusion for gargling and its tincture is applied by rubbing or in the form of compresses to areas affected by neuralgia, itchiness and rheumatic pain (11, 12). The coumarins present in large quantities in the extracts of its leaves are the chemical constituents which confers identity to the product, being responsible for the pharmacological effect for the bronchodilator and anti-asthmatic activities (12). The bronchodilator effect is a result of the relaxation of the smooth musculature of the respiratory system (13). Other studies show anti-inflammatory activity (11, 14, 15) and antiallergenic activity (11).

The aim of the present study was to evaluate preclinical toxicology data of the phytomedicine- bee honey, propolis and *M. glomerata* extract in rats and mice, in order to increase the confidence in extrapolating safety to humans, particularly with reference to its use as a herbal medicine.

MATERIAL AND METHODS

Drug

The phytomedicine - bee honey, propolis and extract of *M. glomerata* (Guaco) was kindly ceded by PRONATU Laboratories (São Paulo – Brazil).

Composition of the phytomedicine

Alcoholic Solution of própolis.....	*1.80% (p/v)*
Mikania glomerata S. (guaco) Extract.....	*3.70% (p/v)*
Bee Honey q.s.p.....	*100 ml*

The phytomedicine is a thick liquid of varying coloration from clear beige to brown in amber glass tubes of 100ml. The prescribed dose for human is equivalent to three tablespoon three time a day.

Animals

Swiss mice (25-30g) and Wistar rats (150-250 g) of either sex from the biothery of the Physiological and Pharmacological Department of the Biological Sciences Center of the Federal University of Pernambuco (UFPE) were kept under standard environmental conditions (25±2° C; 12/12 h light/dark cycle). They were housed in cages and fed with standard diet (Labina®) and water *ad libitum*. For experimentation, the animals were deprived of food overnight. All experiments were in accordance with the guidelines for Care and Use of Laboratory Animals.

Pre-clinical Toxicity

Acute toxicity

Groups of mouses (n=10 animals/group) were oraly treated with the test phytomedicine at doses of 7.5; 15; 25 and 35 ml/kg. The control group received saline solution 0.9% (5ml/kg; p.o).The percentage of death was observed attentively after 30, 60, 120 and 360 minutes and afterwards every 24 hours during 14 days, and the LD50 was calculated using probit method according to Miller & Tainter(16).The surviving animals were sacrificed through deep ether anesthesia and the

external apperance of death animals and apperance of visceras (heart, lungs, kidney, liver, stomach, ovaries, womb and testicles) were carefully noted.

Sub-chronic Toxicity

The rats of either sex were divided in 3 groups of 10. All animals were deprived of food and water 2 hours before, and 2 hours after each experiment. The phytoterapeutic (7.5 or 15 ml/kg) or saline solution 0.9% (5ml/kg; p.o.) were administered on the daily base for 90 days. The body weights were measured weekly, and the blood samples were taken by ocular puncture for biochemical and hematological analysis at the end of each experiment. Following the animals were sacrificed through deep ether anesthesia, followed by removal of organs (heart, spleen, liver, kidney, suprarenal gland, stomach, lungs, testicles, ovaries and womb) which were weighed and analyzed by macroscopic exams.

The blood was processed to determine the hematological parameters (red blood cell (RDC) hemoglobin (Hb), hematocrit (Ht), mean corpuscular hemoglobin (MCM), mean corpuscular volume (MCV), mean corpuscular hemoglobin concentration (MCHC); leucocytes and platelets) in a Coulter TKS analyzer of hematological cells. The blood film colored by the May-Grunwald-Giemsa method were used for the differential count of leukocytes (segmented, eosinophils, lynphocytes and monocytes). The biochemical parameters were determined in serum samples in concordance with the producer (Labtest Diagnostica – Brazil/SA) and the content of each constituent was measured by spectrophotometry: glucose (ortho-toluidine); urea (urease); cholesterol (Huang mod.); HDL-cholesterol (enzymatic method); triglycerides (Soloni mod.); alanine aminotranferase ALT (Frankel-Reitman); aspartate aminotranferase AST (Frankel-Reitman); alkaline phosphatase ALP (Bessey, Lowry mod.); total proteins (Biureto mod).

STATISTICAL ANALYSIS

The values were expressed as mean values ± standard error mean ($\bar{x}$ ± SEM). Results that presented probability of occurrence of nullity in 5% ($p<0.05$) or 1% ($p<0.01$) were considered statistically different. The variance analyses and the respective mean value comparative tests were realized following the GLM procedure of the Statistical Analysis System (SAS Institute, 1989).

RESULTS

Acute Toxicity

Table I summarises the data of acute toxicity of the phytomedicine: bee honey, propolis and *M. glomerata* extract (7.5; 15; 25 or 35 mg/kg; p.o.). As far as the clinical exams are concerned, no indices of toxicity were observed, neither any register of mortality at doses of 7.5 and 15 ml/kg, when compared to the control group. No mortality was also registered in the groups treated with the phytomedicine at the dose of 25 ml/kg. However, these animals became lethargic, returning to normal 8 hours after the beginning of the treatment. Mortality percentage in the group treated with the phytomedicine at the dose of 35 ml/kg was 70%. Ninety minutes after the administration of the dose the animals showed loss of motor coordination. Two animals of this group showed convulsions followed by death. The external apperance of organs (heart, liver, kidney, lungs, stomach, ovaries, testis, womb etc) of the surviving animals, 14 days after the administration of the phytomedicine (7.5; 15; 25; 35 ml/kg) did not show any alterations as compared to control group treated with saline 0.9%.

Subchronic toxicity of the phytomedicine: bee honey, propolis and M. glomerata extract

Subchronic administration effects on hematological and biochemical parameters in wistar rat

All animals survived until the end of 90-day period of treatment with the phytomedicine. Average feed and water consumption by male and female animal were similar to the control group (data not show). Results of sub-chronic toxicity study of the phytomedicine to establish their safety profiles are shown in table II and II.

Table I. Percentage of mortality in mice (males and females) of the groups treated with the phytomedicine.

	Honey, Propolis and *M.glomerata* Extract				Control Saline 0.9%
	7.5	15	25	35	5
	Ml/kg	ml/kg	ml/kg	ml/kg	ml/kg
N^0 Dead/Total animais	0/10	0/10	0/10	07/10	0/10
% Mortality	0	0	0	70	0

Table II. Hematological parameters in Wistar rats treated with the phytomedicine for 90 days.

Analises	Sex	Phytomedicine 15ml/kg	Phytomedicine 7.5ml/kg	Saline 5ml/kg
RBC [s] (mm^3)	M	5.7±0.1	5.6±0.1	5.7±0.1
	F	4.9±0.1	5.2±0.1	5.1±0.1
Hemoglobin [s] (g/ml)	M	17.7±0.9	17.4±0.9	17.4±0.9
	F	13.3±0.9	16.9±0.9	15.0±0.9
Hematocrit [s] (%)	M	53.4±1.1	52.4±1.1	52.8±1.3
	F	41.8±1.3	45.4±1.1	44.4±1.1
MCM (mm^3)	M	94.1±0.51	94.8±0,51	94.0±0.51
	F	94.6±0.51	94.6±0,51	93.8±0.51
MCV (mm^3)	M	93.0±1.1	93.2±1.1	93.4±1.2
	F	85.9±1.2	88.6±1.1	87.7±1.1
MCHC (%)	M	33.2±0.3	33,1±0,3	32.8±0.3
	F	31.8±0.3	32,6±0.3	33.1±0.3
Leucocytes (mm^3)	M	8340±0.5	7200±0.5	8725±0.6
	F	7875±0.6	8020±0.5	7640±0.5
Segmented (%)	M	35.0±4.8	36.8±4.8	27.8±5.2
	F	33,5±5,2	33.4±4.6	32.8±4.6
Eosinophilic (%)	M	2.0±0.46	3.0±0.55	2.4±0.55
	F	2.0±0.56	2.6±0.55	2.0±0.55
Lynphocytes (%)	M	61.8±4.7	60.0±4.7	68.5±5.2
	F	63.5±5.2	63.6±4.7	63.6±4.7
Monocytes (%)	M	3.2±0.4	2.6±0.4	3.3±0.5
	F	3.0±0.5	3.0±0.4	3.6±0.4
Platelets (mm^3)	M	276.0±30.5	268.0±30.5	243.8±34.1
	F	276.0±34.1	346.0±30.5	301.6±30.5

values for RDC should be multiplied by 10^6. Values for platelets should be multiplied by 10^3. [s]statistically significantly different between sex. $p < 0.01$ Males and females are represented by m and f respectively

Table III. Biochemical parameters in Wistar rats treated with the phytomedicine for 90 days (n=10/ group).

Biochemical	Sex	Treatment		
		Phytomedicine 15ml/kg	**Phytomedicine 7,5ml/kg**	**Saline 5ml/kg**
Glucose [s] (mg/dl)	M	62.9±6.8	49.7±6.8	50.6±7.6
	F	120.2±7.6	126,2±7,6	95,9±6,8
Urea [s](mg/dl)	M	30.5±2.1	32.2±2.1	28.6±2.3
	F	19.1±2.3	18.6±2.3	16.0±2.1
Cholesterol [s] (mg/dl)	M	70.8±7.9	77.4±7.9	90.7±8.9
	F	120.5±8.9	126.5±8.9	131.7±7.9
HDL [s] (mg/dl)	M	24.5±3.5	27.6±3.5	37.6±3.9
	F	53.2±3.9	52.9±3.9	53.6±3.5
Triglycerides [s] (mg/dl)	M	57.2±6.9	75.6±6.9	80.5±7.8
	F	87.6±7.7	108.3±7.8	92.4±6.9
AST [s] (U/L)	M	137.6±15.4	102.4±15.4	110.3±17.3
	F	55.0±17.3	67.0±17.3	47.2±15.4
ALT [s] (U/L)	M	115.2±10.0	99.4±10.0	106.0±11.2
	F	57.3±11.2	70.0±11.2	50.8±10.0
ALP [s] (U/L)	M	90.1±1.0	88.0±1.0	88.0±1.1
	F	116.0±1.1	128.0±1.1	116.0±1.0
Protein [s] (g/100ml)	M	5.4±0.5	6.2±0.5	6.0±0.6
	F	9.0±0.6	8.2±0.6	9.3±0.5

[s] *statistically significantly different between sex. $p < 0.01$*

Table IV. Mean values of weight square minimums (g) of Wistar rats organs subjected to treatment with the phytomedicine.

Organs (g)	Sex	*Treatment*		
		Phytomedicine 15ml/kg	**Phytomedicine 7.5ml/kg**	**Saline 5ml/kg**
Heart	F	0.77±0.06	0.74±0.06	0.80±0.06
	M	1.24±0.06	1.28±0.06	1.12±0.06
Spleen	F	0.50±0.06	0.62±0.05	0.58±0.05
	M	1.24±0.05 **	0.82±0.05	0.78±0.05
Liver	F	6.90±0.48	7.20±0.43	6.60±0.43
	M	10.34±0.43	12.10±0.43*	10.20±0.43
Kidney	F	0.71±0.04	0.78±0.03	0.71±0.03
	M	1.12±0.03	1.24±0.03 **	1.12±0.03
Suprarenal	F	0.020±0.004	0.023±0.004	0.018±0.004

Gland	M	0.048±0.004	0.052±0.004	0.045±0.004
	F	1.50±0.10	1.48±0.09	1.56±0.09
Stomach	M	1.70±0.09	1.90±0.09	1.74±0.09
	F	1.78±0.20	1.46±0.18	1.46±0.18
Lungs	M	2.92±0.18	2.78±0.18	2.70±0.18
Ov/Womb	F	0.72±0.10	0.70±0.09	0.70±0.09
Testicles	M	1.60±0.03	1.53±0.03	1.60±0.03

** statistically significantly different from control. $p < 0.05$*

*** statistically significantly different from control. $p < 0.01$*

No statistically significant differences were seen in the hematological and biochemical parameters of the groups treated with the phytomedicine (7.5 or 15ml/kg; p.o.) as compared to control group treated with saline 0.9%. Significant differences ($p<0.01$) were encountered relative to sex for RDC, Hb and Ht. In these parameters, values presented for males were superior to those of the females (table II). In regard to the biochemical analyses, the effect of sex was significant ($p<0.01$) in all parameters under study. The mean values obtained for females were superior to those obtained for males in the following parameters: glucose, cholesterol, HDL, triglycerides and alkaline phosphatase. In the remaining parameters the males obtained higher values than the females (table III).

Evaluation of chronic effect of honey with propolis and m. Glomerata extract on body weight gain and organs of wistar rats

Mean weight (g) of different organs and body weight (g) of the animals treated with the phytomedicine (7.5 or 15ml/kg; p.o.) are shown in table IV and figures 1a and 1b. Significant differences were found between the mean weights of the spleen (1.24±0.05), liver (12.1±0.43) and kidneys (1.24±0.03) of treated groups of male as compared to control group (Table IV).

As observed in the figures (1a and 1b), there were no statistically significant differences in the weight evolution for the male and female mice treated with the phytomedicine (7.5 or 15ml/kg; p.o.) as compared to control group.

DISCUSSION

The acute toxicity study registered a mortality index of 70% for the phytomedicine bee honey, propolis and *M. glomerata* at dose of 35ml/kg. Howevwr, It was not possible to calculate the LD50, since the administered volume could not surpass 1ml in the species under study (mice). The evaluation of the toxic effects showed that the animals treated with the phytomedicine (25 or 35ml/kg) became lethargic, returning to normal 8 hours after the treatment. Taking into account that these doses are equivalent to 39 and 55 times those used for the treatment of respiratory system affections (0.64 ml/kg or 24mg/kg of liquid extract of *M. glomerata*), according to information on the label of the product, it may be suggested that the product demonstrates a good margin of security in the animal species under study. Oliveira *et al.*, (17), determining acute toxicity in *M. glomerata* fluid extract (40ml/kg; p.o) in Wistar rats registered a mortality percentage of 40% in a period of observation identical to ours.

In general, the acute treatment with the phytomedicine did not show any index of toxicity as compared to the control group, from the beginning until the 14th day after the treatment for the surviving animals. Such observation indicates the absence of sequential toxicity of the product under study. Even though it was not possible to determine the DL_{50} of the studied phytomedicines, our results reveal a low order of toxicity of the same.

The chronic toxicity study allows the establishment of the existence or not of adverse effects, and later for the identification and characterization of the affected organ(s), defined by the cumulative effect of the administered substance after prolonged exposure.

The current study show that the chronic treatment with the phytomedicine at doses of 7.5ml/kg or 15ml/kg, which corresponding to 187mg/kg and 374mg/kg of *M. glomerata* fluid extract, respectively, was almost entirely without any toxic effects. These doses are equal to 11.7 and 23.4 times those used in chronic treatment of human beings (0.64ml/kg or 24mg/kg; p.o. of fluid extract). No abnormalities of behavior or body weight evolution were detected.

Figure 1a. Body weight evolution of Male rats treated for 12 weeks with the phytomedicine

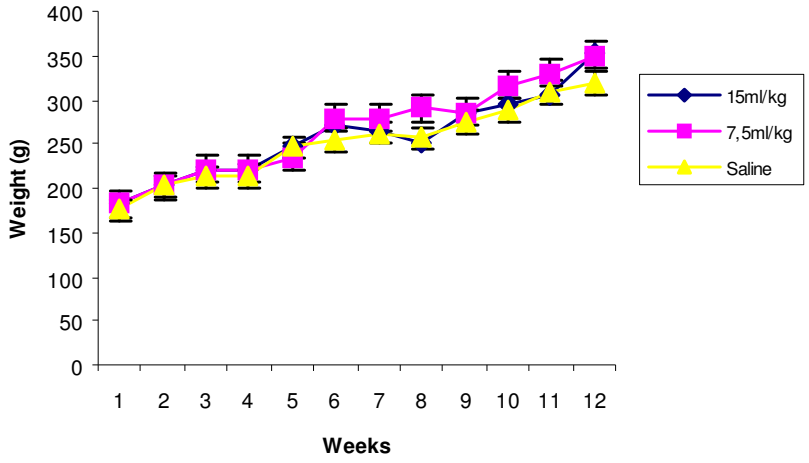
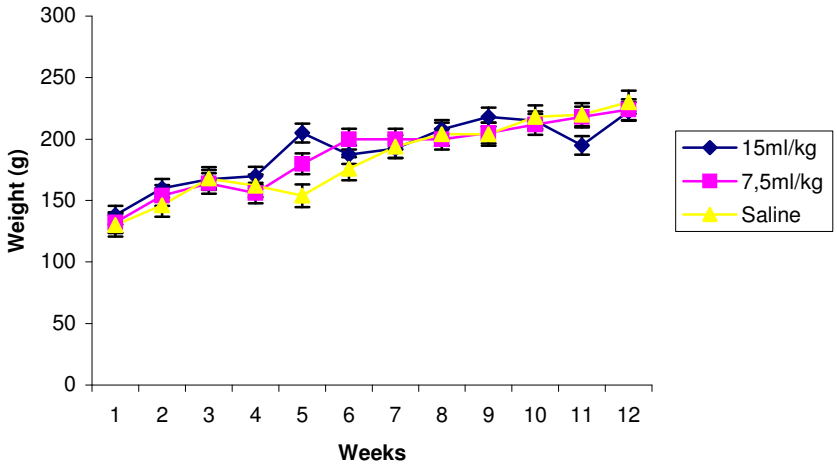


Figure 1b. Body weight evolution of Female rats treated with for 12 weeks withe the phytomedicine



Alterations in biochemical as well as hematological parameters may be considered toxicity indices of medicines as resultant of chronic use, as for example, the leukocytosis (antipirine, digitalises and terebinthine); hemolytic anemia (sulfones, isoniazid, dipyrone), hemorrhagic disturbances (aspirin) (18); increase of hepatic enzymes (aminoglycosides, acetaminophen, retrovir, zidovudine) (19). As far as the hematological and biochemical parameters are concerned, no index of significant alteration in relation to control appeared, after 90 days of treatment. All values found for these parameters are comparable to those existing in specialized literature (20, 21). A statistically significant difference ($p<0.01$) between sex was found for some hematological (Hc, Hb and Ht) and biochemical parameters. The verified differences are characterized as peculiar interspecies variations for each sex and do not reveal a pathological state. Other chronic toxicity studies also show these differences (21, 22).

The macroscopic analysis of many visceras such as lungs, liver, heart, kidneys and stomach did not reveal any alteration in relation to the control group. The statistic analysis revealed, however, a statistically significant difference ($p<0.01$) for the weight of the spleen of males treated with 15ml/kg; as well as of the kidney and liver of the males treated with 7.5ml/kg, when compared to control. However, such alterations were not dependent dose and they just limited to the males. Furthemore, the biochemical and hematological parameters related to indices of lesions/alterations of these organs, such as hepatic enzymes (AST, ALT and alkaline phosphatase), levels of urea (kidney) and red blood cells and platelets (spleen), did not differ from control. At the moment it is not possible to give any explication for these results. Additional studies, including histological analysis, should be undertaken in the future and may result in an explication for this observation.

In conclusion, the results obtained in this study give strong support that, in equivalent doses in human terms, the phytomedicines (7.5 or 15 ml/kg) did not present important toxicological (acute and chronic) effects in Swiss mice and Wistar rats of both sex.

REFERENCES

1. A.K. Jäger. Is tradition medicine better off 25 years alter? *J Ethnopharmacol*. **100:** 3 – 4 (2005).
2. G.C. CEVALLOS. Estudios de toxicologia preclínica para nuevos fármacos. *Arch Neurociências. México* **2:** 118-121 (1996).
3. H.S. Arun Kumar. Recent advances in assay methods and techniques preclinical safety studies on medical plants: Part II. *Pharmacognosy Magazine* **1:** 32-38 (2005).
4. R.B. Philp. Herbal Remedies: The good, the bad, and the ugly. *Journal of complementary and Integrative Medicine* **1:** 1-11 (2004).
5. R. Yunes, R.C. Pedrosa. Fármacos e fitoterápicos: a necessidade do desenvolvimento da indústria de fitoterápicos e fitofármacos no Brasil. *Quim. Nova* **24:** 147-152 (2001).
6. E.S. Garcia. Biodiversity, Biotechnology and Health. *Cad. Saúde Pública. Rio de Janeiro* **11:** 495-500 (1995).
7. BRASIL. Ministério da Saúde. Conselho Nacional de Saúde. Resolução nº 196/96. *Diário Oficial da União* 201, seção I (out/1996).
8. BRASIL. Ministério da Saúde. Agencia Nacional de Vigilância Sanitária. *Resolução-RDC nº48 de16/03/2004. Dispõe sobre o registro de medicamentos* fitoterápicos.
9. W. S. Bendazzoli. Fitomedicamentos: perspectivas de resgate de uma terapia histórica. *Mundo Saúde* **24:** 123-126 (2000).
10. A.L. Aboy, G.G. Ortega, P.R. Petrovick, A. Langeloh, V.L. Bassani. Desenvolvimento tecnológico de soluções extrativas de *Mikania glomerata* Sprengel (Asteraceae), Guaco. *Revista Brasileira de Ciências Farmacêuticas* **36:** 165-172 (2000).
11. I. Fierro, A.C. Borges da Silva, C.S. Lopes, R. Soares de Moura, C. Barja-Fidalgo. Studies on the anti-allergic activity of *Mikania glomerata*. *J. Ethnopharmacol*. **70:** 151-159 (2000).
12. L.K.A.M. Leal, A.A.G. Ferreira, G.A. Bezerra, F.J.A. Matos, G.S.B Viana. Antinoceptive, anti-inflamatory and bronchodilatator activities of Brazilian medicinal plants containing coumarin: a comparative study. *J. Ethnopharmacol*. **70:** 151-159 (2000).
13. C.S. Lopes, L.C.R.M. Carvalho, A.C. Pinto, J.M. Jansen, S. Alves Pereira, S. Moura. *Efeito do Guaco (Mikania glomerata) na Musculatura Lisa Respiratória*. (XII Reunião Anual da Federação da Sociedade de Biologia Experimental, Caxambu, 1997) p.283.
14. R. S. Moura, L.C.M. Carvalho, C.S. Lopes, M.A. Victor-de-Sousa. *Efeitos do Guaco (Mikania*

glomerata) sobre o edema podal em camundongos induzido pelo veneno da Bothrops jararaca. (Anais do XIV Simpósio de Plantas Medicinais do Brasil, Florianópolis, 1996) p.87.

15. M.G.M. Oliveira,; *et al*. Pharmacologic and toxicologic effects of two *Maytenus* species in laboratory animals. *J Ethnopharmacol* **34:** 29-41 (1991).
16. L.C. Miller, M.L. Tainter. Estimation of the ED_{50} and this error by means of logarithimic probit graph paper. *Proc. Soc Expl Biol*. **57:** 261-4 (1944).
17. F. Oliveira, S. Oga, G. Akisue, M.K. Akisue. Parâmetros Físicos e Químicos e Efeito Antiedema dos Extratos fluídos de Guaco (*Mikania glomerata* Sprengel) e de Guaco de Mato (*Mikania Laevigata* Schultz Bip. Ex Baker). *An Farm. Quim*. **25.(1-2):** 50-54 (1985).
18. A. Balcells. *La Clinica y el Laboratorio. Semiologia bioquimica, hematológica y* ***serológica***. ***Pruebas funcionales***. 12th ed. Espanha: Editora Marin. p. 573 (1990).
19. D.A. Vessey. Metabolism of xenobiotics by the human liver.In: Hepathology. A Textbook of Liver Disease. Eds: ***Zakin.D; Boyer. T.D***. Cap.9, 257-305 (1999).
20. B.M. Mitruka, H.M. Rawnstley. ***Clinical Biochemical and Hematological Reference Values in Normal Animals***. New York: Masson Publishing (1977).
21. E.R. Rodrigues, H.P. Pedrazzi, J.K. Bastos. Acute preclinical toxicity Study of Zanthoxylum naranjillo. *Extract. Phytherapy Research* **12:** 512-516 (1998).
22. M. Salazar, E. Martinez, E. Madrigal, L.E Ruiz, G.A. Chamorro. Subchonic toxicity in mice fed Spirulina maxima. *J. Ethnophamacol* **62:** 235-241 (1998).