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Chronic toxicological study of Ierobina® in rabbits

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ABSTRACT

Ierobina is a Brazilian phytopharmaceutical product used in the treatment of dyspepsia. Despite its widespread use in the country for over 75 years, only recently has its therapeutic efficacy been attested in animals; however, to date, no toxicological investigations have been carried out for this product. The present work seeks to evaluate the chronic toxicity of Ierobina administrated by gavage in rabbits, after product administration *per os* daily, at the doses of 2800 mg/Kg for 180 days. The product presented a low chronic toxicity; all observed alterations were reversible; and no animal died during the experiments. However, histological evaluations of the kidney, liver and other selected organs showed normal architecture, suggesting no morphological disturbances. Hence, considering the obtained results and the fact that Ierobina has been commercialized for decades in Brazil, with no reported cases of toxicity, the product appears to be safe for human use.

KEYWORDS: Ierobina, chronic toxicity, phytopharmaceutical product, rabbits

1. INTRODUCTION

Dyspepsia is a common term used to characterize abdominal pain centered in the epigastrium, sometimes combined with other gastrointestinal complaints. Historically, the word 'dyspepsia' has been used for a heterogeneous group of abdominal symptoms [1].

Specific symptoms include epigastric pain, epigastric burning, postprandial fullness, early satiation, bloating in the upper abdomen, nausea, and vomiting [2]. Patients with dyspepsia tend to witness a reduced health-related quality of life, as their symptoms, particularly abdominal pain and indigestion, cause emotional distress, problems with food and drink, and impaired vitality [3]. The pathophysiology of dyspepsia has been widely investigated over the past two decades; however, no definite single reason has been identified [1]. Gastrointestinal complaints rank among the most frequent reasons why people seek out medical advice. About 15-30% of adult patients suffer from a variety of functional dyspeptic conditions [4]. A study from the United Kingdom showed that 40% of the adults reported one or more symptoms of dyspepsia [5]. This disorder affected up to 29% of the population in a study of employees in the USA [6]. In a large population-based study from Europe, 21% of the subjects had experienced epigastric symptoms during the preceding 12-month period [7]. In the Korean population, the prevalence of uninvestigated dyspepsia was 11.7% [8]. As in other functional disorders, dyspeptic symptoms are more frequent in women (24.4%) than in men (16.6%) [7, 9].

Ierobina® is a registered trade name.

The therapy of functional gastrointestinal disorders is one of the domains of phytotherapeutic treatments [4]. This is partially due to the large availability of phytotherapeutics used to treat this illness, as well as the existence of a false perception that natural products are always safe [10, 11].

Often the adverse effects related to these products are verified, due to the lack of information concerning their safety [12]. Natural products possess bioactive components that can promote dangerous and lethal effects [13], which can occur through many mechanisms, including direct effect, allergic reactions, effects of contaminants, and interactions with other drugs or natural products. It is very difficult to determine the real frequency of the collateral effects of these herbs, due to a less extensive pharmaceutical surveillance of these products when compared to synthetic drugs [14]; therefore, the evaluation of the safety of these products is of utmost importance.

Ierobina is a phytotherapeutic product that has been commercialized in Brazil for many years, even without any proof of its effectiveness or safety. It was recently proven that the use of this product before the ingestion of a lipid rich diet induced a 40% increase in the activity of the lipoproteic lipase of the epididimal adipose tissue, an enzyme responsible for the capture of triglycerides, illustrating its pre-clinical effectiveness for dyspepsia [15]. In addition, Brazilian regulation determines that, for natural products to be commercialized, they must be submitted to pre-clinical toxicity tests [16]. Tagliati *et al*. [17] demonstrated that the administration *per os* of Ierobina to mice and rats presents no toxicological effects. The present study therefore aims to evaluate the chronic toxicity of Ierobina in rabbits after *per os* administration.

2. MATERIALS AND METHODS

2.1. Phytopharmaceutical product - Ierobina

Ierobina (control no. 8324) was furnished by Laboratório Belfar (Belo Horizonte, Brazil). It is a solution that is commercialized in 10 ml flacons containing the fluid extracts of *Solanum paniculatum* leaves (0.8 ml), *Remijia ferruginea* leaves (0.8 ml), *Erythraea centaurium* leaves (0.2 ml), and *Jacaranda caroba* aerial parts (0.2 ml).

2.2. Animals

Male and female albin New Zealand rabbits (6 male and 6 female, 800 g to 1000 g), provided by the Animal Shelter from the School of Pharmacy at Universidade Federal de Minas Gerais (UFMG), Belo Horizonte, Minas Gerais, Brazil, were used. The animals were allowed to acclimatize in the experimental room for 1 week before beginning the experiments. The animals were kept at a controlled temperature (23 ± 2°C) and under humidity conditions (50%-60%) in a 12-hour light/dark cycle (7:00-19:00). The experimental protocols have been approved by the Ethics Committee on Animal Experimentation (CETEA) from UFMG (protocol n^{o} 036/06).

2.3. Chronic toxicity studies

Ierobina was administered by gavage, daily, for 180 days at doses of 2800 mg/Kg (10 times the therapeutic dose in humans) in three male and three female rabbits. The control group (three male and three female rabbits) was treated with saline. Physiological responses and behavior were evaluated daily, while body weight changes and food and water consumption were recorded weekly [16] (OECD - guideline 452).

At the end of the period, the animals were fasted for 12 h and then euthanasiated. Blood samples were colleted in heparinized and dry non-heparinized centifuge tubes. Blood analysis (hematology and chemistry) were also performed.

2.4. Hematological and biochemical analyses

The heparinized samples were used to determine hematological parameters (total red blood cells, leukocyte and platelet counts, hematocrit, and hemoglobin). The non-heparinized blood was allowed to coagulate before being centrifuged. The serum was then separated and assayed for uric acid, albumin, total protein, glucose, blood urea nitrogen (BUN), aspartate transaminase (AST), alanine transaminase (ALT), alkaline phosphatases (Alk-P), cholesterol, and creatinine. Determinations were carried out using diagnostic kits (Analisa Diagnostica Ltda.). Assays were

performed in replicates (n = 8), and results are expressed as means ± S.E.M.

2.5. Histopathological studies

After collecting the blood samples, selected organs (heart, liver, pancreas, kidney, lungs, stomach, spleen, and testicles or ovaries) were carefully dissected out and weighed. Portions of these organs were fixed in 10% neutral formaline solution to undergo histopathological examinations. Tissues were processed by conventional technique. The paraffin embedded sections of 5 µm thickness were prepared and stained with hematoxylin and eosin for microscopic examination.

2.6. Statistical analysis

Statistical significance was determined by one way ANOVA test, followed by the Tukey test; $P<0.05$ was set as the criterion of significance.

3. RESULTS

3.1. Clinical and histopathological studies

No lethality was registered for doses of 2800 mg/Kg, during the 180 days of Ierobina administration. No evidence of differences in physiological responses and behavior, nor in food and water consumption, between the control and any of the treated groups could be observed in any of the time periods. Moreover, no significant difference in body weight gain and organ weights could be noted between the control group and the treated animals at any of the evaluated doses (Table 1).

Table 1. Body and organ weights (g) of rabbits treated with Ierobina.

	Control group (saline)	Ierobina 2800 mg/Kg
Body weight		
Male		
Initial	914.70 ± 100.16	889.06 ± 109.98
Final	3337.50 ± 423.23	3424.46 ± 444.37
Female		
Initial	877.43 ± 112.78	863.67 ± 98.37
Final	3028.55 ± 397.09	3111.24 ± 402.11
Organ weight		
Male		
Heart	6.25 ± 0.67	1.48 ± 0.19
Liver	59.33 ± 6.89	12.67 ± 1.84
Pancreas	1.90 ± 0.34	1.40 ± 0.57
Kidney	13.64 ± 1.57	3.66 ± 0.35
Lung	13.43 ± 0.49	2.41 ± 0.34
Testicles	6.8 ± 2.03	6.18 ± 1.06
Stomach	23.23 ± 2.04	2.56 ± 0.21
Spleen	1.02 ± 0.3	1.10 ± 0.17
Female		
Heart	6.62 ± 1.03	1.26 ± 0.26
Liver	66.8 ± 6.59	9.33 ± 0.89
Pancreas	1.80 ± 0.3	1.33 ± 0.65
Kidney	14.29 ± 1.34	3.19 ± 0.43
Lung	13.42 ± 0.13	2.29 ± 0.38
Ovaries	17.34 ± 1.88	2.70 ± 0.97
Stomach	29.44 ± 3.04	2.20 ± 0.35
Spleen	2.05 ± 0.72	1.26 ± 0.27

Data are expressed as mean ± S.D., $n = 3$. No statistical difference between control and Ierobina group ($P>0.05$).

3.2. Hematological and biochemical studies

The effect of chronic administration of Ierobina on hematological parameters is presented in Table 2. No significant changes were observed for the treated group as compared to the control, and all parameters remained within normal limits throughout the evaluated period.

The biochemical profiles of the treated and control animals are shown in Table 3. No statistical difference could be detected for the assayed parameters from the treated-animal group when compared to the control group ($P<0.05$).

4. DISCUSSION AND CONCLUSIONS

The current investigation showed that Ierobina, administered via *per os* for 180 days, using a 2800 mg/kg dose (10 times the therapeutic dose used to treat dyspepsia in humans), proved not to be toxic to rabbits. The physiological and behavioral result in the group treated with the test substance was no different than those observed in the control group.

Table 2. Differential white blood cell count of rabbits treated with Ierobina.

	Control	Ierobina
Male		
Global leukocytes	310 ± 0.7	5.10 ± 0.6
Red cells	6.14 ± 0.175	6.14 ± 0.175
Hemoglobin	12.75 ± 0.850	12.75 ± 0.850
Hematocrit	34.75 ± 9.70	36.35 ± 2.85
MCV	62.5 ± 1.5	59.0 ± 3.0
MCH	21.8 ± 1.0	20.70 ± 0.80
MCHC	34.75 ± 0.55	35.17 ± 0.493
Platelets	228.5 ± 33.5	563.50 ± 182.50
Female		
Global leucócitos	5.1 ± 0.6	3.35 ± 1.05
Global leukocytes	5.41 ± 0.67	5.39 ± 0.150
Red cells	15.78 ± 1.70	11.95 ± 0.450
Hemoglobin	26.85 ± 3.70	33.90 ± 1.400
Hematocrit	49.63 ± 1.41	63.00 ± 1.00
MCV	29.53 ± 4.63	22.10 ± 0.2
MCH	35.35 ± 0.15	35.15 ± 0.15
MCHC	211.50 ± 6.5	293.00 ± 66.00

Data are expressed as mean ± S.D., n = 3. No statistical difference between control and Ierobina group (P>0.05).

Table 3. Blood chemistry values of rabbits treated with Ierobina.

	Control	Ierobina
Male		
Uric acid (mg/dl)	0.34 ± 0.06	0.31 ± 0.80
Albumine (g/dl)	5.24 ± 0.49	5.34 ± 0.16
ALT (units/l)	261.37 ± 14.53	131.35 ± 10.45
AST (units/l)	85.95 ± 26.08	58.99 ± 15.78
Creatinine (mg/dl)	1.18 ± 0.06	0.95 ± 0.08
Alkaline phosfatase (units/l)	15.11 ± 5.08	14.19 ± 0.86
Gama GT (units/l)	13.69 ± 1.66	10.55 ± 0.37
Glucose (mg/dl)	94.33 ± 5.69	112.32 ± 13.69
Total proteins (g/dl)	6.54 ± 0.21	6.74 ± 0.37
Urea (mg/dl)	74.81 ± 14.98	79.30 ± 10.35
Female		
Uric acid (mg/dl)	0.26 ± 0.08	0.61 ± 0.315
Albumine (g/dl)	4.83 ± 0.37	5.21 ± 0.125
ALT (units/l)	139.53 ± 41.04	222.75 ± 59.15
AST (units/l)	74.8 ± 36.28	75.15 ± 27.05
Creatinine (mg/dl)	1.34 ± 0.29	1.45 ± 0.07
Alkaline phosfatase (units/l)	14.56 ± 5.11	13.82 ± 0.15
Gama GT (units/l)	13.29 ± 2.29	11.34 ± 1.91
Glucose (mg/dl)	72.84 ± 18.39	101.85 ± 0.05
Total proteins (g/dl)	6.97 ± 0.76	6.82 ± 0.50
Urea (mg/dl)	80.93 ± 22.99	87.14 ± 0.49

Data are expressed as mean ± S.D., n = 3. No statistical difference between control and Ierobina group (P>0.05).

No significant differences concerning water and ration consumption as well as the weight of the animals could be observed within the evaluated groups. Adequate consumption of water and ration with the necessary nutrients, due to their physicochemical properties [18], are important aspects to maintain homeostasis, considering that both can result in subnutrition or dehydration, which can consequently promote the loss of body weight [19, 20]. As body weight has been used as an indicator of adverse effects to drugs [21, 22], the results obtained suggest that Ierobina, after repeated administrations *per os* in higher doses than the normal therapeutic dose, proved to be safe when applied to the studied animals.

After the animal had been euthanasiated blood was taken for hematological and biochemical evaluation. The laboratory exams are extremely important to identify possible premature effects, such as hepatotoxicity or nephrotoxicity, which have been observed in patients under herb therapy [23, 24, 25]. Considering that the hematological and biochemical analysis of the present study presented no significant difference in the males or females, it can be concluded that the results represent evidence of the safety of Ierobina.

Other than the hematological and seric biochemical evaluations, macroscopic and microscopic tests of the liver, heart, pancreas, kidney, lungs, stomach,

spleen, and testicles or ovaries were also performed. The completion of these tests, mainly the histopathological tests, is essential in evaluating the safety of a product, in turn allowing for the verification of possible alterations [26, 27]. In the present study, the macroscopic and microscopic tests showed no alterations in the studied organs, confirming the results obtained in the evaluation of the blood parameters.

Therefore, in the present study, taking into consideration that no result was obtained showing any statistical difference in relation to the control group, this product's safety was confirmed in rodents [17]. Furthermore, as this product presents a clinical experience of 70 years in Brazil with no reported adverse effects, it can be concluded that the product presents an adequate safety for human beings. Nevertheless, clinical evaluation is warranted to confirm this hypothesis.

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