Research Article

28 Days Toxicity Study of the Hydroalcoholic Extract Rhizome of *Curcuma longa,* Flowers of *Cordia lutea,* Leaves of *Annona muricata,* Fruit of *Citrus aurantifolia,* Fruit of *Myrciaria floribunda* and Leaves of *Aspidosperma spruveanum* in Rats

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ABSTRACT

The association of the six plants in the form of hydroalcoholic extract, which has been shown to have therapeutic effects on people's health. To determine the safety of the association of the hydroalcoholic extract of the *Curcuma longa* rhizome; *Cordia lutea* flowers, *Annona muricata* leaves, *Citrus aurantifolia* fruit, *Myrciaria floribunda* fruit and *Aspidosperma spruveanum* leaves. The experimental design, 40 Holtzman rats (20 males - 20 females) were used; the guidelines or standards of the Organization for Economic Cooperation and Development were followed. **T**he study was performed in the Pharmacology department of Universidad Nacional Mayor de san Marcos for 28 days. The hydroalcoholic extract was administered orally for 28 days, observations, signs and weekly evolution of the body weight of the animals were made; at the end, a blood sample was taken for hematological and biochemical study; then they were sacrificed for anatomopathological study of liver, kidney; ANOVA was applied, considering p value <0.05 for significance. When the association was administered in the form of a hydroalcoholic extract, a homogeneous temporal evolution of body weight was observed. There was no significant variation in the levels of glycemia, urea, cholesterol, triglycerides, indirect bilirubin, transaminases, alkaline phosphatase and hemoglobin (p> 0.05); The findings show that the hydroalcoholic extract of this association of plants is not toxic in rats, when administered for a period of 28 days.

Keywords: Acute toxicity; Curcuma longa; Cordia, Annona, Soursop.

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INTRODUCTION

Plants are a rich source of metabolites useful for the preservation and health care [1], the extracts obtained from their various parts have medicinal properties available in various pharmaceutical formulations [2].

Traditional oriental medicine frequently uses the association of plants to treat chronic diseases, such as hyperlipidemia and obesity [3]. Ideally, the association of various active principles aims to generate synergism to increase the therapeutic effect and reduce adverse effects [4]; however, if the association has similar pharmacokinetic characteristics, it could generate toxicity during metabolism and elimination; The degree of toxicity produced depends on the plasma concentrations, the dose and the duration of therapy [5]; chronic diseases such as diabetes mellitus, cardiovascular disease, and cancer represent a serious health problem [1] that requires prolonged therapy, thereby increasing the risk of producing toxicity [6].

Curcuma longa, known as stick, goes to the Zingiberaceae family, is widely cultivated in Asia and Africa [7]; Curcumin is the main metabolite, it has anti-inflammatory and antioxidant properties, demonstrating efficacy in the prevention of cancer and in the suppression of tumor growth [8]; likewise, it contains essential oils, phenols, tannins, flavonoids, terpenes and alkaloids [9]; effective in treating infections [10]; Furthermore, it has been shown that Curcuma longa has been useful in the treatment of hyperlipidemias, increasing HDL, and reducing LDL and triglycerides [11]; Likewise, it is attributed a hepatic and renal protective effect on metal toxicity [7], and anticonvulsant effects attributed to the sesquiterpenes present [12].

Cordia lutea, known as flower of overo, is used to treat gastrointestinal, respiratory and dermatological disorders[13]; Like Cordia Verbenaceae, it has antitumor activity in vitro, as it reduces proliferation and viability, inducing apoptosis, and increasing the cytotoxic and antitumor effect [14]; its essential oils have anti-inflammatory effects, which is why it is recommended in acute inflammation [15].

Annona muricata, known as soursop, goes to the Annonaceae family, it is distributed in South America, Central America, West Africa and southeast Asia [1]; 220 components have been identified in the plant, mainly flavonoids, tannins and glycosides [16], acetogenins and alkaloids [1]; Some studies indicate antipyretic and sedative effects, useful in the treatment of respiratory diseases,

malaria, gastrointestinal disorders, alterations to the liver, heart and kidneys [1, 2, 16, 19]; Likewise, it has antiproliferative activity in human prostate cancer tumor cells (PC-3 cell) in in vitro studies [17]; and induce G1 cycle arrest and apoptosis in colon cancer cells [18]; they retain a chemoprotective effect in breast cancer induced by DMBA [19], a hypoglycemic effect for the treatment of diabetes mellitus, antioxidant activity in vivo and a protector of beta cells of the pancreas [20].

The genus Citrus (aurantifolia) is one of the greatest consumed and distributed fruits in the world. Several examples of the use of citrus fruits as therapeutic remedies including the use of orange, lime and lemon juices like remedies for the prevention of kidney stone; the use of citrus flavonoids as effective in vivo agents can modulate the metabolism of liver lipids; using orange juice to prevent inflammatory processes; the use of polyphenolics from calamondin and kumquat peel as effective antioxidant agents; the use of grapefruits as agents that can low the blood pressure and interfering with calcium channel blockers; the use of grapefruit as a snack and stomach tonic; the use of lemon juice with honey to relieve cough. There are some health benefits, like anti-cancer, anti-mutagenic, anti-allergic, and anti-aging activities of polyphenols [21].

Myrciaria floribunda, known like "camboim", "jabuticabinha", "murta", "duque", "goiabarana" and "araçazeiro" is native from Brazil[23]. The essential oil of M. floribunda has been reported rich in terpenoid compounds and biological activities like antimicrobial, anticholinesterase, antitumor and insecticidal. Just, a previous study with M. floribunda leaves reported that its ethyl acetate extract has phenolic compounds and flavonoid myricetin-3-galoctoside was identified. As flavonoids are known like anticancer and antioxidant agents, this drives us to assess the antiproliferative activity in tumor cell lines and determine the antioxidant capacity in the content of total phenolics, flavonoids and tannins of the extract of the leaves of M. floribunda, [22].

Representatives of the genus Aspidosperma are since Mexico to Argentina [24], and several species have traditionally used for malaria [25-28]. A recent review of the traditional use and antimalarial activity of Aspidosperma species revealed several scientific literature references on the use of 24 species to treat malaria / fevers, including Aspidosperma parviflorum, and 19 Aspidosperma species that have had their extracts and / or alkaloids evaluated for antimalarial activity in vitro and / or in vivo showing positive results. The ethanol extract of the bark of the trunk of Aspidosperma parviflorum provided ulein which reveals a high antimalarial activity in vitro and selectivity. Furthermore, the alkaloid fraction showed antimalarial activity similar to ulein. The high chemical diversity of alkaloids of Aspidosperma species and the small number of these alkaloids that have been analyzed to determine antimalarial activity, together with the traditional use of several species of this taxon for the treatment of malaria, also in other Meso and South American countries, deepen the research of plants of this genus of great interest in the search for natural medicines against malaria, [29].

MATERIALS AND METHODS

Preparation of aqueous ethanol extract of the association of the rhizome of *Curcuma longa*; *Cordia lutea* flowers, *Annona muricata* leaves, *Citrus aurantifolia* fruit, *Myrciaria floribunda* fruit and *Aspidosperma spruveanum* leaves

All plants were sun dried within the temperature range of 30–42 ° c for 5 days before being reduced in size to a coarse powder with an electric grinder. the coarse plant powder weighing 1000 g was extracted with 90% aqueous ethanol in three cycles using a Soxhlet extractor. the crude extract was filtered with Whatman no. 4 filter paper and the filtrate was concentrated in vacuo at 30 ° c to obtain 80 g of weight of the residue (8.7% w / w). the residue was stored in an airtight bottle, kept in a refrigerator at 4 ° c until use.

The toxicity evaluation at repetitive dose for 28 days by oral route was performed according to the standards of the organization for economic cooperation and development standard 407; 40 rats of the Holtzman line of 7 months, 20 males and 20 females, with an average weight of 350 ± 20 g and 275 ± 20 g respectively, were used, which were acquired at the national health institute (ins, lima-Peru) and installed, for air conditioning for 48 hours, in the Bioterium of the faculty of human medicine – unmsm, at an ambient temperature (22-26 ° c), humidity of 60-70% with 12-hour light / dark cycles they were given water and food ad libitum. four groups of ten rats (five males and five females) were randomly formed: group i: ssf 2 ml / kg; groups ii, iii, iv: hydroalcoholic extract of curcuma longa rhizome, cordia lutea flowers and Annona muricata leaves, citrus aurantifolia fruit, Myrciaria floribunda fruit and Aspidosperma Spruveanum leaves at a dose of 100 mg / kg, 200 mg / kg and 400 mg / kg respectively, which were administered orally once a day for 28 days, the evaluation method was double blind.

The evolution of body weight was evaluated at 0, 7, 14, 21 and 28 days; on day 28, the animals were subjected to general anesthesia (Halatal® 10 mg / kg) for the extraction of blood by intracardiac puncture and to carry out hematological and biochemical studies, as indicated in table 1; then, the animals were sacrificed with an overdose of pentobarbital (Halatal® 100 mg / kg) for the anatomopathological study.

The description of variables is expressed in means and standard deviation; the statistical comparison of groups, by means of one-way anova followed by a post-hoc turkey test, was considered significant with a $p < 0.05$ at the 95% confidence interval. the statistical program Spss version 17 was used.

During the study, the specifications proposed by the institutional committee for the care and use of animals

were followed, and the current regulations of the animal protection law (law 27265) were respected.

RESULTS AND DISCUSSION

The results show a tendency to increase the body weight of the rats that received the administration of the hydroalcoholic extract for 28 days, observing a homogeneous increase in both genders (figure 1) with the exception of the female rats of group ii (100 mg / kg) which reduced body weight during the last week.

The biochemical and hematological analysis at 28 days determined that there was no significant variation in the levels of glycemia, urea, cholesterol, triglycerides, indirect bilirubin, alanine aminotransferase (TGP), aspartate aminotransferase (TGO), alkaline phosphatase, hemoglobin, hematocrit, monocyte and lymphocyte count (p> 0.05); however, significant changes (p <0.05) were observed in group ii of female rats (100 mg / kg) as they presented decreased creatinine levels and male rats (200 mg / kg) presented reduction high-density lipoproteins (HDL); likewise, in group ii of male rats (100 mg / kg) there is evidence of an increase in total and direct bilirubin; likewise, leukocytosis is evidenced for group iv of male rats (table 1).

Table 1: Biochemical and hematological parameters when evaluating the acute toxicity of the rhizome association of *Curcuma longa; Cordia lutea* flowers, *Annona muricata* leaves, *Citrus aurantifolia* fruit, *Myrciaria floribunda* fruit and *Aspidosperma spruveanum* leaves when administered orally for 28 days in Holtzman rats.

Parameters	Males				Fémales			
	SSF 2ML/ KG	100 MG/KG	200 MG/KG	400 MG/KG	SSF 2ML/ KG	100 MG/KG	200 MG/KG	400 MG/KG
Glucose (MG/DL)	85,2 ± 12,2	87,8 ± 16,2	90,4 ± 9,8	94,4 ± 7,7	87,6 ± 7,7	92,6 ± 11,7	85,2 ± 12,2	95,8 ± 9,5
Urea (MG/DL)	21,2 ± 6,3	20,2 ± 4,9	18,6 ± 5,5	20,2 ± 8,2	19,0 ± 6,4	16,0 ± 4,5	20,0 ± 5,9	21,2 ± 6,3
Creatinin (MG/DL)	0,8 ± 0,1	0,6 ± 0,1	0,7 ± 0,2	0,6 ± 0,2	0,7 ± 0,1	$0,5 \pm 0,1^{B}$	0,7 ± 0,1	0,7 ± 0,1
Colesterol (MG/DL)	145,8 ± 37,1	193,4 ± 60,5	155,8 ± 32,4	172,2 ± 28,7	156,6 ± 21,9	168,8 ± 31,5	165,4 ± 20,1	174,6 ± 19,2
HDL (MG/DL)	58,6 ± 10,9	45,8 ± 12,1	$43,4 \pm 5,1^{B}$	51,2 ± 10,5	47,2 ± 6,9	43,8 ± 4,3	43,8 ± 4,3	49,4 ± 11,7
Triglycerides (MG/DL)	108,8 ± 20,2	136,0 ± 33,1	141,4 ± 44,3	152,6 ± 38,3	138,6 ± 23,1	166,6 ± 31,6	172,8 ± 44,4	133,0 ± 6,2
Bilirrubin Total (MG/DL)	1,01 ± 0,3	$2,19 \pm 0,5^{A}$	1,14 ± 0,7	1,39 ± 0,5	1,84 ± 0,8	1,21 ± 0,4	1,63 ± 0,3	1,06 ± 0,1
Bilirrubin Direct (MG/DL)	0,77 ± 0,2	$1,63 \pm 0,2^{A}$	0,79 ± 0,4	0,98 ± 0,3	1,30 ± 0,5	0,89 ± 0,2	1,09 ± 0,2	$0,75 \pm 0,1^{B}$
Bilirrubina Indirecta (MG/DL)	0,23 ± 0,1	0,56 ± 0,2	0,35 ± 0,3	0,42 ± 0,2	0,54 ± 0,3	0,32 ± 0,2	0,54 ± 0,1	0,31 ± 0,1
TGP (UI/L)	29,8 ± 9,7	33,6 ± 7,4	48,0 ± 21,6	27,8 ± 10,2	30,8 ± 12,5	31,2 ± 20,0	30,0 ± 10,2	28,8 ± 6,4
TGO (UI/L)	42,6 ± 11,6	47,6 ± 14,2	61,8 ± 37,3	42,4 ± 16,0	43,2 ± 16,4	34,6 ± 8,9	38,4 ± 12,5	40,2 ± 3,5
Alkaline Phosphatase (UI/L)	89,6 ± 16,7	85,2 ± 14,3	113,0 ± 42,2	101,4 ± 13,1	109,8 ± 18,7	117,0 ± 19,2	110,0 ± 44,0	92,0 ± 22,1
Hemoglobyn (G/DL)	11,9 ± 0,8	12,5 ± 0,8	11,7 ± 0,7	12,7 ± 0,8	11,7 ± 0,9	12,7 ± 1,1	12,1 ± 0,7	12,2 ± 1,8
Hematocrit (%)	35,0 ± 2,9	36,4 ± 4,6	37,4 ± 1,5	38,0 ± 3,1	34,2 ± 1,9	$39,2 \pm 4,3^{B}$	32,8 ± 4,8	35,6 ± 5,5
Leucocites (X 10^3/µL)	6,8 ± 1,7	7,5 ± 2,0	8,1 ± 1,3	$9,0 \pm 0,4^{B}$	6,7 ± 2,0	5,7 ± 1,2	6,0 ± 1,3	6,6 ± 0,9
Lymphocytes (%)	35,8 ± 6,3	35,8 ± 8,9	34,4 ± 74,4	39,4 ± 11,1	34,4 ± 7,9	32,2 ± 11,7	41,2 ± 7,8	37,2 ± 7,4
Monocites (%)	1,2 ± 1,3	1,2 ± 1,7	1,0 ± 1,7	0,2 ± 0,4	1,0 ± 0,7	1,8 ± 1,3	0,6 ± 0,8	1,4 ± 1,6

SSF: serum fi; Mean ± SD. ANOVA, Tukey test a Vs SSF Group (p <0.01) b Vs SSF Group (p <0.05)

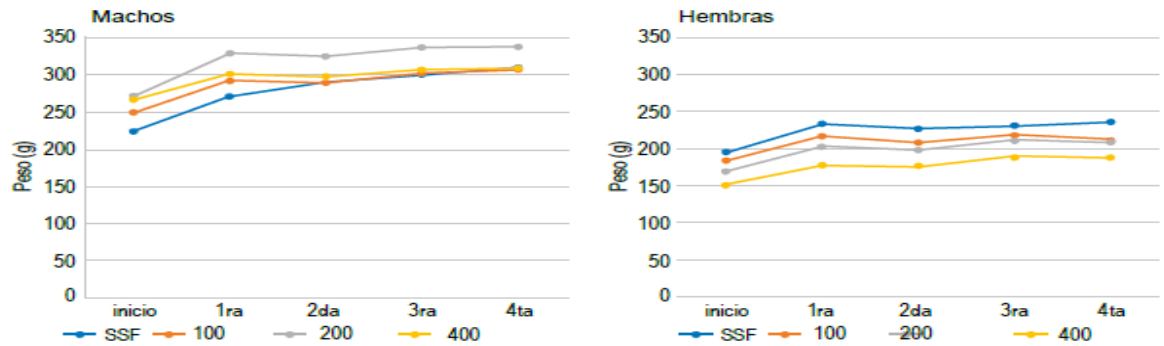
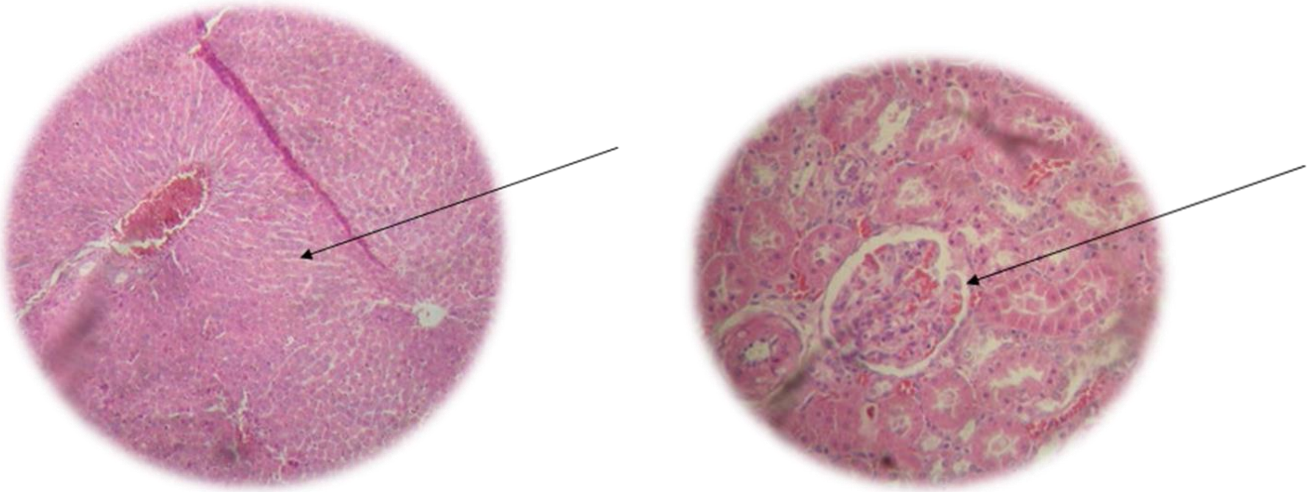


Figure 1: Variation of the body weight of the Holtzman rats that receive the oral administration of the hydroalcoholic association of the curcuma longa rhizome
Association; *Cordia lutea* flowers, *Annona muricata* leaves, *Citrus aurantifolia* fruit, *Myrciaria floribunda* fruit and *Aspidosperma spruveanum* leaves for 28 days.



Liver without Histological Alterations (40X). Kidney with Preserved Histological Structure (40X).

Figure 2: Histological manifestations in Holtzman rats that receive oral administration of the hydroalcoholic extract of the association of Cordia lutea, Annona muricata leaves, Citrus aurantifolia fruit, Myrciaria floribunda fruit and Aspidosperma Spruveanum leaves be administered orally for 28 days.

(The macroscopic and histopathological evaluations do not show signs of toxicity; liver and kidney assessment were performed (Figure 2), observing normal tissues)

DISCUSSION

Since ancient times, botanical medicine represents a therapeutic alternative, many agencies in the world have issued regulations related to the safety of herbal medicines, in order to protect and promote public health [22]; these drugs have shown various effects: anti-inflammatory, antineoplastic, hepatic protectors, in vitro and in vivo antioxidants, antimicrobial, sedative, hypnotic, among others [4]; prolonged therapies, necessary for the treatment of chronic diseases, increase the risk of nephrotoxicity and hepatoxicity [23], in addition, in some chronic diseases, such as the treatment of neoplasms, the prolonged use of standard drugs leads to the appearance of adverse effects severe, such as hair loss, anemia, gastrointestinal disorders, leukemia, resistance to chemotherapeutics [6]; the demand for these products has led to the need to evaluate not only efficacy, but also safety concerns [24].

The research has been carried out with the objective of determining the safety of the association of the hydroalcoholic extract of *Curcuma longa* rhizome; *Cordia lutea* flowers, *Annona muricata* leaves, *Citrus aurantifolia* fruit, *Myrciaria floribunda* fruit and *Aspidosperma spruveanum* leaves, administered orally to rats once a day for 28 days. observations indicate that there were no significant changes in body weight between the groups, nor were there any clinical changes (figure 1); modification of food intake is considered an indicator of toxicity [22].

It is also observed that the levels of transaminases do not present significant differences; it should be mentioned that the liver is the organ most affected by the acute or chronic presence of foreign substances, which are exposed directly after oral absorption through the first-pass liver phenomenon or later by metabolism [22]; the plants under study contain various metabolites, useful for the treatment of pathological conditions [1, 7, 10, 11, 13-20], the flavonoids present in the aqueous extract [9, 16] are reported as hepatoprotector [25]. furthermore, possibly at the dose levels used, the chemical components contributed by the three medicinal plants would have maintained an important balance, in such a way that it did not induce liver damage (figure 2).

Creatinine levels are considered as markers of renal impairment, they depend directly on body mass [22, 25]; it is evidenced that the female rats of group ii (100 mg / kg) reduced creatinine levels, and this coincides with the weight loss during the last week (table 1). the hematopoietic system is very sensitive to toxic substances [26]; this indicates the safety of the *Curcuma longa* rhizome association; *Cordia lutea* flowers, *Annona muricata* leaves, *Citrus aurantifolia* fruit, *Myrciaria floribunda* fruit and *Aspidosperma spruveanum* leaves as there were no hematological changes. the evaluation of pathological changes, by macroscopy and microscopy, are indicators of toxicity [27], in the macroscopic analysis of the tissues at all the doses tested, it did not produce changes in the vital organs of the treated animals; likewise, in the histopathological, there were no suggestive signs of toxicity; these results coincide with what was found in the hematological and biochemical parameters (table 1, figure 2).

CONCLUSION

It is concluded that the administration of the hydroalcoholic extract of the rhizome association of curcuma longa; cordia lutea flowers, Annona muricata leaves, citrus aurantifolia fruit, Myrciaria floribunda fruit and Aspidosperma Spruveanum rats leaves for 28 days, does not present acute and subacute toxicity, as evidenced by clinical, hematological, biochemical and histopathological

parameters; thus, demonstrating the safety of the atomized extract at the tested doses.

It is recommended to continue studies according to the standards of regulatory bodies, such as studies of chronic toxicity, reproductive toxicity, genotoxicity, endocrine changes, among others, in order to evaluate the total safety for use in humans.

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Competing Interests

Authors have declared that no competing interests exist**.**

Authors' Contributions

Both Authors contribute with the totally like: designed the study, performed the statistical analysis, wrote the protocol, and wrote the first draft of the manuscript. managed the analyses of the study. managed the literature searches and read and approved the final manuscript**.**

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