Journal of Advances in Medical and Pharmaceutical Sciences
4(4): 1-8, 2015, Article no.JAMPS.16877
ISSN: 2394-1111

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Acute and Sub-acute Toxicity of *HRT* 123 Polyherbal Formula

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Authors' contributions

This work was carried out in collaboration among all authors. Authors KDF and MOU designed the study and wrote the protocol. Authors KDF and JGG did the literature search, managed the animals, collected all data, performed the statistical analysis, and wrote the first draft of the manuscript. All authors read and approved the final manuscript.

Article Information

DOI: 10.9734/JAMPS/2015/16877
Editor(s):
(1) Divya Kesanakurti, Department of Cancer Biology and Pharmacology, University of Illinois College of Medicine, USA.
(2) Jinyong Peng, College of Pharmacy, Dalian Medical University, Dalian, China.
Reviewers:
(1) Tarek M Heikal, National Research Centre, Dokki, Egypt.
(2) Weiting Wang, Tianjin Institute of Pharmaceutical Research, Tianjin, China.
Complete Peer review History: http://sciencedomain.org/review-history/11587

Original Research Article

Received 17th February 2015
Accepted 8th April 2015
Published 28th September 2015

ABSTRACT

Plant based drugs (single or multi-component formulations) have been used in managing many health conditions worldwide. One such formulation is the herbal formula *HRT* 123 which is being used in managing conditions involving immunosuppression, HIV/AIDs and other infectious diseases of viral origin. This study screened for phytochemical constituents and evaluates acute and sub acute toxicity profile of aqueous *HRT* 123 extract. We evaluated acute toxicity of *HRT* 12 aqueous extract using the modified Arithmetic method of Karbar and sub-acute toxicity using the 14-day repeated dose oral toxicity testing procedure. For sub acute toxicity, thirty albino rats divided into five groups of six rats each received 0, 250, 500, 750, and 1000 mg/kg of extract respectively by oral route for 14 days. Animals were sacrificed and blood analyzed for haematological parameters, serum biomarkers and electrolytes while visceral organs (lungs, heart, liver, kidneys & spleen) were harvested for histopathological investigations. Phytochemical screening shows presence of alkaloids, saponins, tannins, flavonoids and carbohydrates. Acute toxicity shows oral LD_{50} to be more than 6000 mg/kg. Following sub acute administration, indices of liver (AST, ALT, total protein

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& albumin) and renal (sodium, potassium, chloride & creatinine) function and; haematological parameters were not significantly different in treated groups relative to controls. Liver weight displayed a dose dependent increase while the kidneys showed relative but non-significant decrease. These results are suggestive of the relative safety of *HRT* 123 but caution needs to be exercised with used of high doses and in the face of concomitant liver or kidney abnormality.

Keywords: Herbal tea; polyherbal formulation; repeated dose oral toxicity; serum biomarkers.

1. INTRODUCTION

Plant based drugs continue to play a central part in the management of numerous health conditions around the world particularly in resource constrained countries. Even so, the World Health organization estimates that almost 80% of the world's population rely solely on herbs for their health care needs [1]. The remaining 20% may also depend on drugs that are derived from plant sources in one way or the other. This extensive use is premised on the fact that herbal based drugs may be safer coupled with the prohibitive cost, unavailability, inconvenience or time consuming professional care associated with use of some conventional drugs [2]. As a result, a large and increasing number of patients use medicinal herbs or seek the advice of medical personnel regarding their use. This interest has led to increasing scientific scrutiny of the safety and therapeutic potentials of medicinal herbs with the aim of providing data to help patients and clients make wise and informed decisions about their use.

Herbal based products have often been prepared as single or multi-component products for use in a variety of ways: as powders and capsules, herb teas or tonics. Multi-component herbal preparations with more than one herb have continued to be produced across the world for use in the treatment of diverse medical conditions. While some are used as food supplements [3], others such as the polyherbal preparation *Diakyur* is used in the management of diabetes [4] and *Viracomb* is used for the management of conditions associated with immune suppression [5]. There are also polyherbal preparations that have been used as bitters or aperitifs [6,7]. Similarly other preparations are used for eclampsia in pregnancy [8] while *Gynocare* is used for management of gynaecological ailments [9]. Some of these preparations (such as the *African Herbal Formula*) have even appeared in literature with coded names [10].

The World Health Organization is positively predisposed to use of herbal medicines and polyherbal formulations to fill the gap especially in resource constrained countries. This is evidenced by elaborate guidelines which have been released in this regard [1,11] and concerted efforts to make member countries develop and build on same. However, because of their multi-component nature, assessment of the toxicity and toxicological profiles of these herbal products or their formulations have taken on an even greater level of importance.

This study sets out to evaluate and provide information on the acute and sub-acute toxicological profiles of *HRT 123,* a polyherbal product which has been used in management of conditions involving immunosuppression, HIV/AIDS and other viral related infections in the North central region of Nigeria.

2. MATERIALS AND METHODS

2.1 Preparation of *HRT 123* Extract

HRT 123 is presented as a coarse powder of 50 gm packets each containing 25 tea bags. It is made from plant parts/components from *Aframomum melegueta, Monodora myristica, Xylopia aethiopica, Gongronema latifolium, Allium sativum, Garcinia kola* and *Viscum album.* The coarse powder was finely divided with the aid of a rotary electric blending machine (National®, Model MX391N, Matsushita Electric Co. Japan), sieved (mesh size 850 µm) and then extracted by the hot maceration procedure. Five litres of boiling water was added to 500 gm of the powder (solvent to drug powder ratio of 10:1) and allowed to stand for 24 hours. The resulting mixture was sieved in three phases, through sieves of 800, 500 and 150 µm pore sizes respectively then filtered through Whatman Number 1 filter paper. The filtrate was evaporated to dryness in an oven (Memmert model 100-800, Germany) maintained at a temperature of 40℃. A dark green solid residue (yield of 10%) was obtained and kept in an airtight container in a refrigerator until needed for use.

2.2 Phytochemical Screening

Phytochemical analysis of the aqueous extract of *HRT 123* was carried out to determine the presence or absence of secondary metabolites constituting the bioactive constituents. This was done according to standard methods [12-14].

2.3 Experimental Animals

Sixty albino Wistar rats of either sex weighing 150 – 200 gm raised at the Animal House Unit, Department of Pharmacology, Faculty of Pharmaceutical Sciences of the University of Jos were used for the study. The animals were appropriately housed in a well ventilated room at controlled temperature (25±2℃) in a 12 hour dark-light cycle and had free, uninhibited access to feed and water throughout the duration of the study.

2.4 Acute Toxicity Study

Acute toxicity was carried out according to the modified Arithmetic method of Karbar as earlier described [15,16]. In this method, animals were randomly assigned to six treatment groups of five animals per group. Group one served as the control and received distilled water only. Groups two to six received 500, 1000, 2000, 4000 and 6000 mg/kg body weight of freshly reconstituted extract respectively. The animals were fasted overnight and received all treatments orally with the aid of an oral cannula. After administration, animals were allowed free access to food and water and observed for signs of toxicity. These signs included (but were not limited to) licking of the paws, salivation, aggressiveness, rubbing of nose on the floor or walls of the cage, food refusal, extreme lachrymation, stretching, lethargy, sleep, diarrhoea, convulsion, coma and mortality for 72 hours [17] Lethality was expressed as the LD_{50} which is dose that is lethal to 50% of the animals and is calculated as per the formula (Table 1).

2.5 Sub acute Toxicity Study

Protocol for sub acute toxicity study was modified from methods earlier described [3,18]. In this procedure, animals were divided into five groups of six rats per group. Group one was the control and received distilled water only. Groups two to five received 250, 500, 750 and 1000 mg/kg body weight of the extract respectively on a daily basis for 14 days. The extract was freshly reconstituted on all occasions and all treatments were administered orally with the aid of an oral cannula. The animals were observed for any manifest signs of toxicity or mortality over the period just as was done in acute toxicity study. Daily food and water intake; faeces and urine output and weekly body weights were monitored and recorded. The animals were sacrificed on day 15 under chloroform anaesthesia.

2.6 Analysis of Haematological Parameters and Serum Biomarkers

Blood was collected through cardiac puncture into plain bottles for serum biochemical analysis and into ethylenediaminetetraacetic acid (EDTA) bottles for haematological investigations on the haematology auto analyzer (Mindray BC 3200). Blood collected in plain sample bottles was allowed to clot. The blood was then centrifuged at 4000 rpm for 10 minutes, the serum collected and stored at -20℃ until needed for the investigations on the blood chemistry autoanalyzer (Cobas C311).

2.7 Histopathological Analysis

After dissecting the animals, some visceral organs (lungs, hearts, spleens, livers and kidneys) were weighed and fixed in formalsaline for seven days then processed according to standard histopathological methods. Processed tissues were embedded in paraffin and sections (5µm thickness) were cut out on a rotary microtom (Baird and Tatlock Limited, England). These were stained with haematoxylin and eosin dyes and assessed on Olympus XS2 – 107 BW microscope at X40 magnifications. Alterations of tissue architecture relative to the normal structure were noted and recorded.

2.8 Statistical Analysis

Results of food and water intake, faeces and urine output, body as well as organ weights were expressed as mean ± standard error of mean (SEM). Results of haematological and serum biochemical investigations were similarly expressed. All data was analyzed using one-way analysis of variance (ANOVA) and student's t-test followed by Turkey posthoc multiple comparison with the aid of SPSS statistical software. Differences in means between controls and treated groups were considered to be significant when $P < 0.05$.

3. RESULTS AND DISCUSSION

3.1 Phytochemical Screening

Phytochemical screening of the aqueous extract of *HRT 123* revealed the presence of alkaloids, saponins, tannins, flavonoids, carbohydrates and sterols in trace amounts as shown in Table 1.

Table 1. Phytochemical analysis of aqueous extract of *HRT 123*

Phytochemical group tested	Results
Alkaloids	++
Saponins	++
Tannins	+++
Flavonoids	++
Carbohydrates	+++
Anthraquinones	-
Steroids	+
Cardiac glycosides	-

+ = Present; - = Not present

3.2 Toxicity Studies

Toxicity studies are very important in scientific studies of herbal medicines and help in characterizing the safety profiles of many chemicals and substances. They may be of different types and employ various routes of administration. Acute toxicity testing determines the effects in a single animal species [19] and in many cases measures lethality which is expressed as the lethal Dose 50 (LD_{50}), the amount given at once that causes the death of 50% of a group of test animals. This study shows that the oral LD_{50} of *HRT 123* aqueous extract in albino wistar rats is more than 6000 mg/kg and none of the animals died following acute administration. The oral LD_{50} of more than 6000 mg/kg was calculated as in Table 2.

The Canadian Centre for Occupational Health and Safety (CCOHS); based on the Hodge and Steiner scale characterizes a test substance as extremely toxic when the LD_{50} is less than 1 mg/kg body weight, highly toxic at 1 – 50 mg/kg, moderately toxic at 50 – 500 mg/kg, slightly toxic at 500 – 5000 mg/kg, practically non-toxic at 5000 – 15000 mg/kg and relatively harmless when the LD_{50} is more than 15000 mg/kg [20]. Thus the results suggest that *HRT 123* extract is relatively safe given its oral LD_{50} of more than 6000 mg/kg. However some authors have suggested that acute toxicity data involving such a single dose administration of a test substance may often be of limited clinical application because cumulative toxic effects do occur even at very low doses. Therefore sub-acute (and chronic toxicity) studies are very necessary in evaluating the safety profile of phytomedicines especially in relation to predicting the hazard of long term, low-dose exposures [6] as is the case with *HRT 123* formula.

Sub-acute toxicity investigations revealed that food and water intake, urine and feaces output showed no significant changes in week one but by week two increasing doses of the extract led to significant but apparently non-dose dependent reductions in food and water input and; urine output. These observations were offset by the non-changes in body weight in weeks one and two (Tables 3 and 4).

Table 2. LD_{50} calculation

Group	N	Dose (mg/kg)	Dd	Md	Dd X Md
1	5	0	0	0	0
2	5	500	500	0	0
3	5	1000	500	0	0
4	5	2000	1000	0	0
5	5	4000	2000	0	0
6	5	6000	2000	0	0

LD_{50} is calculated as follows: $LD_{50} = LD_{100} - \frac{\Sigma Dd \times Md}{N}$

Where:

LD_{50} = dose that kills 50% of the animals in a group,
LD_{100} = dose that kills all the animals in a group
Dd = Dose difference, Md = Mean death
N = number of animals per group.
Substituting: $LD_{50} = > 6000 - \frac{0}{5}$

LD_{50} = > 6000. Thus the LD_{50} is more than 6000 mg/kg

Studies have shown that changes in body weight as well as in the weights of visceral organs may be a sensitive indicator of adverse affects of chemicals [21,22]. Significant changes are absent in recorded body weights as well as in the weight of visceral organs (Table 5). With respect to visceral organs, the significant increase in the weight of the liver and the lungs at the 750 mg/kg and 1000 mg/kg dose levels respectively may be viewed as compensatory effects. This may be so given that other parameters like indices of liver function (alanine amino transaminase enzyme, aspartate amino transaminase enzyme, albumin and total proteins) and; kidney function (sodium, potassium, chloride and creatinine) showed no significant changes in treated relative to control animals over all the dose levels (Table 6). Assessment of haematological parameters can be used to determine the toxic effects of xenobiotics including plant extracts on the blood constituents of an animal. Such analysis is relevant to risk evaluation because changes in the haematological system are highly predictive for human toxicity, when data are translated from animal studies [12]. In this study however, the haematological parameters remained largely unchanged in all the groups except for neutrophils that showed a relative progressive increase over the dose levels and only became significant at the 1000 mg/kg dose level (Table 7). As reported by other authors [23], increase in neutrophil counts are associated with acute insults to the body whether in the form of infection or not and drugs like corticosteroids, histamine and epinephrine are known to cause an increase in neutrophils count. It is likely that the extract produced an effect similar to any of the above drugs.

3.3 Histopathology Study

The visceral organs in the control group as well as the group treated with 250 mg/kg body weight of *HRT 123* extract following sub-acute administration appeared essentially normal without any adverse histological changes. However as dose of the extract increased from 500 to 750 to 1000 mg/kg, there were progressive histological changes in the kidneys which ranged from mild to moderate tubular damage to severe tubular necrosis at the 1000 mg/kg dose suggestive of progressive degenerations in the kidney with increase in dose. Steatosis / fatty damage was noticeable in the livers of animals treated with 1000 mg/kg bodyweight of the extract.

Table 3. Effect of sub acute administration of *HRT 123* extract on Food and water intake, body weight; faeces and urine output treated animals week 1

Treatment (mg/kg)	Food intake (gm)	Water intake (ml)	Urine output (ml)	Feacal output (gm)	Body weight (gm)
Control	91.14±22.25	135.00±19.09	19.00±5.00	68.38±14.09	203.33±6.87
Extract 250	91.43±36.52	116.00±22.34	23.00±7.00	67.47±15.05	245±22.73
Extract 500	80.71±16.77	102.14±24.76	16.00±5.00	60.86±12.47	204.17±9.75
Extract 750	85.14±37.19	90.43±39.06	8.00±4.00*	52.46±12.01	192.50±19.31
Extract 1000	76.57±44.09	108.86±39.72	12.00±5.00	45.71±13.65	175.83±23.52

Values represent mean ± SEM; N = number of animals per group = 6;
**significantly different from control at p<0.05*

Table 4. Effect of sub acute administration of *HRT 123* extract on Food consumption, water intake, body weight, faeces and urine production of treated animals week 2

Treatment (mg/kg)	Food intake (gm)	Water intake (ml)	Urine output (ml)	Feacal output (gm)	Body weight (gm)
Control	74.29±1.75	125.71±12.52	24.42±7.74	55.29±10.78	196.67±9.42
Extract 250	82.86±4.52*	120.43±13.19	31.43±9.50	56.00±6.99	237.00±30.59
Extract 500	72.29±10.08	114.00±26.39	24.86±5.00	52.14±12.78	199.00±5.83
Extract 750	70.71±3.19	91.14±6.36*	12.71±3.73*	39.29±10.50	167.50±19.31
Extract 1000	26.71±10.23*	56.00±29.77*	14.86±6.47	31.71±13.27	190.00±10.80

Values represent mean ± SEM; N = number of animals per group = 6;
**significantly different from control at p<0.05*

Table 5. Effect of sub acute administration of *HRT 123* extract on organ weights (gm) of treated animals

Treatment (mg/kg)	Liver weight (gm)	Kidney weight (gm)	Heart weight (gm)	Spleen weight (gm)	Lungs weight (gm)
Control	5.81±0.53	1.70±0.55	0.70±0.42	0.92±0.33	2.97±1.57
Extract 250	7.73±0.89	2.27±0.15	0.91±0.11	1.02±0.54	3.49±0.57
Extract 500	6.97±0.78	1.27±0.13	0.53±0.04	0.98±-0.16	2.44±0.01
Extract 750	10.51±0.06*	1.18±0.01	1.28±0.03	1.24±0.01	6.04±0.02*
Extract 1000	10.60±0.02*	1.19±0.01	1.31±0.01	1.30±0.01	6.04±0.03*

*Values represent mean ± SEM; N = number of animals per group = 6; *significantly different from control at p<0.05*

Table 6. Effect of sub acute administration of *HRT 123* extract on Serum biochemical parameters and electrolytes

Parameters	Treatment (mg/kg)				
	Control	Extract 250	Extract 500	Extract 750	Extract 1000
AST (IU/L)	100.50±24.97	79.60±14.25	85.25±13.42	85.50±9.88	71.00±9.00
ALT (IU/L)	40.83±17.20	35.00±8.60	31.25±4.92	31.17±4.06	37.00±1.00
Total protein (g/dl)	9.05±1.04	8.50±1.43	8.28±1.35	7.90±1.62	9.85±0.95
Total albumin (g/dl)	4.10±0.43	4.06±0.79	3.50±0.37	3.62±0.40	3.45±0.05
Chloride (mmol/l)	93.67±7.87	102.60±6.89	103.5±5.59	107.67±7.34	101.50±5.50
Potassium (mmol/l)	4.80±0.68	4.44±0.33	4.78±0.58	4.75±0.68	4.45±0.05
Sodium (mmol/l)	130.17±30.42	144.40±9.99	134.25±8.01	129.50±21.50	153.00±11.00
Creatinine (µmol/l)	164.00±37.71	135.80±42.37	151.25±20.52	118.83±31.16	120.00±2.00

Values represent mean ± SEM; N = number of animals per group = 6; There were no signifcant differences relative to control at < 0.05; AST = Aspartate Amino Transaminase enzyme; ALT = Alanine Amino Transaminase enzyme

Table 7. Effect of sub acute administration of *HRT 123* extract on haematological indices

Parameters	Treatment (mg/kg)				
	Control	Extract 250	Extract 500	Extract 750	Extract 1000
PCV (%)	40.17±4.81	38.80±6.55	40.25±3.56	42.00±1.83	40.00±0.00
RBC (x 10^{12})	4.22±0.35	4.42±1.03	4.35±1.09	4.57±0.29	4.00±0.20
WBC (x 10^{9})	5.23±2.36	6.62±0.91	6.70±2.81	5.58±2.31	5.80±1.30
Hb (g/dl)	12.55±2.00	12.86±2.11	13.43±1.18	13.90±0.53	13.15±0.15
Neutrophils (x 10^{9})	18.50±12.09	33.00±11.92	43.25±16.98	24.60±4.50	49.50±9.50*
Lymphocytes (x 10^{9})	80.17±11.96	66.20±12.51	55.50±17.33	74.40±4.54	48.00±7.00*
Monocytes	1.00±0.81	0.60±0.80	1.00±1.00	1.00±0.89	2.00±2.00
Eosinophils	0.33±0.47	0.20±0.40	0.25±0.43	0.00±0.00*	0.50±0.50
Basophils	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00

*Values represent mean ± SEM; N = number of animals per group = 6; *significantly different from control at p<0.05; PCV = Packed Cell Volume; RBC = Red Blood Cell; WBC = White Blood Cell; Hb = Haemoglobin*

This observation may be juxtaposed with the progressive even though non-significant decrease in kidney weight. A decrease in urine production and weight of the kidneys and deleterious histological changes as seen here point to the fact that utmost care must be taken with this product if it has to be used at high dose levels and even more so in patients who have underlying renal malfunction.

4. CONCLUSION

Findings from this study suggest that the aqueous extract of *HRT 123* is relatively safe following acute administration with oral LD_{50} of more than 6000 mg/kg. Furthermore, there were no deleterious toxic manifestations with respect to indices of liver and kidney function as well as the haematological system following sub acute administration. There were also no adverse histopathological manifestations on key organ systems.

CONSENT

It is not applicable.

ETHICAL APPROVAL

The authors declare that "Principles of laboratory animal care" (NIH publication No. 85-23, revised 1985) were followed. All experiments and procedures have been examined and approved by the Animal Care and Use Committee, Faculty of Pharmaceutical Sciences, University of Jos.

ACKNOWLEDGEMENTS

The authors thank Mr Christopher Nnebe for providing the sample of *HRT 123* used for the study and are grateful to the Head of Pharmacology Department, University of Jos in making the facilities of Pharmacology laboratory available for this study. The authors also wish to specially thank Mr Thomas P. Yakubu of the Pharmacognosy research laboratory, Mr Garba Gampyal of the Animal House Unit and Mr Sale Gotom of the Histology Unit, Department of Anatomy, University of Jos for technical assistance in the course of this study.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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Peer-review history:
The peer review history for this paper can be accessed here:
http://sciencedomain.org/review-history/11587