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Phytochemical Screening and Acute Toxicity of Kabuuti Herbal Cough Syrup in Wistar Rats

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ABSTRACT

Kabuuti herbal cough syrup is widely used remedy that is used for the relief of cough and it's available on over the counter in pharmacies and drug shops in Uganda since its non prescription drug. It contains the following ingredients according to the package label; *Z. officinale*, *E. globulus*, *Citrus medica* and sorbitol as from the package label. This study aim to assess the phytochemical screening and acute toxicity of Kabuuti herbal cough syrup in wistar rats. The toxicity profile of the Kabuuti extract was studied in wistar rats. The acute toxicity study to determine LD50 was done using Lorke's method which proceeded in two phases I and II. Phytochemical screening was also done to find out secondary metabolites that were present. The effect of the extract on hematological parameters and biochemical parameters of the liver and the kidney were observed. The phytochemical screening showed the presence of phenols, flavonoid and steroids. The LD_{50} of the extract was greater than 5000 mg/kg. The doses administered in phase I showed sedation, urination and decreased activity as signs of toxicity and behavior changes. In phase II the dose of 1600 mg/kg produced shivering, 2900 mg/kg caused shivering and sedation and 5000 mg/kg caused shivering and hypnosis. No mortality was registered. The result equally revealed that the extract caused significant decrease in white blood cell count compared to the control group and the treatment group with the dose of 1600 mg/kg (P=0.004). Significant decrease in lymphocytes was noticed in 1000 mg/kg and 1600 mg/kg as compared to control (P=0.002). Kabuuti herbal cough extract may not be very safe at a wide range of doses due to its large LD_{50}. It should be taken with much caution.

Keywords: LD_{50} (Acute toxicity), acute, alanine aminotransferase, aspartate aminotransferase and creatinine.

INTRODUCTION

Herbal medicine has become a popular form of healthcare [1] and has been practiced in many countries for centuries [2]. With an estimate of about 25% of all modem medicines directly or indirectly derived from plants sources [3]. The use of herbal medicinal products and supplements was said to have increased tremendously over the past three decades with not less than 80% of people worldwide relying on them for some part of primary healthcare [4]. Traditional herbal medicines was getting significant attention in global health debates. In China, traditional herbal medicine played a prominent role in the strategy to contain and treat severe acute respiratory syndrome (SARS) [5]. About 65-80% of the population which lives in developing countries depends essentially on plants for primary health care [6]. In some Asian and African countries, up to 80% of the population relies on traditional medicine for their primary health care needs [7]. Considering the continent of Africa, traditional (or ancestral) African medicine seems to be much more prevalent compared to conventional, Western medicine. And in West Africa, for instance, it was estimated that between 70-80% of the population rely on traditional medicine [8]. More than 60% of Uganda's population depends on traditional medicine because it is accessible, affordable and culturally familiar. An estimated traditional health practitioner for every 200-400 Ugandans (compared to 1 western- trained doctor per 20,000) [9]. Herbal medicine has long been used to manage a range of common conditions, including malaria, cough, digestive and respiratory problems, toothaches, skin diseases, and childbirth complications [10]. Previously in 2003, it was revealed that in Uganda there was one traditional healer for nearly 290 people compared to one Western-trained medical practitioner for every 10,000people in the urban areas and 50,000people in the rural areas respectively [11]. Despite the high levels of usage and importance of traditional medicine, so far, relatively few herbal drugs have been evaluated scientifically to prove their safety, potential benefits and effectiveness and currently, and the major pharmaceutical companies have demonstrated renewed interest in investigating higher plants as sources for new lead structures and also for the development of standardized phytotherapeutic agents with proved efficacy, safety and quality [12, 13]. Nevertheless, there is still lack ofregulation which leads to misuse ofthe medicines by unqualified practitioners and loss ofcredibility ofthe

system and this can lead to toxicities and unknown drug effects which has been a similar case to cough herbal remedies.

Kabuuti herbal cough syrup is widely used remedy that is used for the relief of cough and its available on over the counter in pharmacies and drug shops in Uganda since its nonprescription drug [14]. It contains the following ingredients according to the package label; *Z. officinale, E. globulus, Citrus medica* and sorbitol as from the package label.

According to a study, daily oral administration of EOE at doses of 396,792 and 1188 mg/kg b.wt showed the LD50 of EOE was 3811.5 mg/kgwhich indicated that EOE had no side effects on rats. Recently, experimental findings showed that sorbitol definitely produce hepatic glycogen when fed to fasted rats over a period of days. Glycogen formation also occurs when sorbitol is given intraperitonally to fasted rats [15]. There is limited information on acute toxicity of combined administration of *Citrus medica*, sorbitol, *E. globulus* and *Z. officinale* extract (Kabuuti cough syrup). Therefore the acute toxicity of these different ingredients which is the LD50 of Kabuuti herbal cough syrup was determined in this study. The purpose of this study was to determine the phytochemicals present, acute toxicity, hematological and biochemical parameters of Kabuuti herbal cough syrup in Wistar rats.

Materials and Methods

Study design

The study was an experimental design done at Mbarara University of science and Technology Extraction and phytochemical screening was conducted at MUST pharmaceutical analysis lab and acute toxicity.

Herbal extract material

The selection of Kabuuti cough syrup has been based on its wide use by many people to treat a number ofailments. The syrup is available over the counter in community pharmacies, clinics and shops for home based commodities.

Extract collection and identification

The Kabuuti cough syrup product was collected from the authorized distributor in Mbarara town. The collected samples were concentrated using a vacuum concentrator. In vacuum concentration, the sample was dried by converting liquid to vapour. Vacuum concentrators removed 99% of the water in a relatively short period of time and accommodated the sample regardless of its freezing or boiling point. Centrifugal force, heat and vacuum to remove moisture from a sample. In a vacuum concentrator, samples were held in a rotor that spins at 1700 RPM creating a centrifugal force. The centrifugal force was to prevent liquids from bumping out of the tube when a vacuum is applied. Heat was applied indirectly through the walls of the vacuum chamber.The gel concentrate was weighed using an analytical scale and packed in clean labeled bottles.

Phytochemical screening methods

Phytochemical examinations were carried out for all the extracts as per the standard methods from Trease and Evans [16].

Experimental animals

Male wistar rats 7 weeks old, were used for this experiment. They were got from pharmacology animal house at Kampala International University-Western Campus. A standard cage was used to house the rats. Standard feeds and water ad libitum were used. The animals were randomly selected, marked to permit individual identification, and kept in their cages for at least 5 days prior to dosing to allow for acclimatization to the laboratory conditions.

Acute toxicity study

It was done using Lorke's method [17] because it uses fewer animals that is 12 animals.

Clinical observations

All animals were observed daily for morbidity and mortality. The observations included changes in skin, fur, eyes, mucus membrane, and autonomic activity like lacrimation, piloerection, pupil size and unusual breathing pattern were observed. Tremors, salivation, rolling, chronic convulsions, loss of righting reflex, coma and death were being observed. Ocular examination was conducted on all animals prior to the initiation of experiments and during day prior to euthanasia. All animals were weighed at the beginning and at the end of the study.

Hematological evaluation

WBC count, WBC differential counts including; neutrophils (NO), lymphocytes (LY), monocytes (MO), oesonophil (EO) and basophils (BA) were evaluated. Red blood cells (RBC) total count, haematocrit (HCT), haemoglobin (Hb), mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH), mean corpuscular haemoglobin concentration (MCHC) and platelet count (PCH) were evaluated. These were carried out according to a routine method described by Docie, [18].

Biochemical evaluation

Serum biochemical parameters which included urea, creatinine, alanine aminotransferase (ALT) and aspartate aminotransferase (AST) were analyzed by using a semi-automatic biochemical analyzer.

Quality Control

The herbal extract was bought from authorized distributor of the Kabuuti herbal cough syrup. The extract was concentrated using a vacuums concentrator to avoid destruction or loss of some phytochemicals. In bred rats were used to minimize the genetic variations and the animals wererandomly assigned to control and treatment groups. There was double entry of results to ensure quality of data. A trained laboratory technician assisted in the drug administration and extraction.

Data analysis

Data was collected and analyzed using Microsoft Excel 2010 and SPSS version 16 to obtain descriptive statistics of mean and standard error of the mean.Data was then analyzed using Graph Pad Prism - 6 software to compute for multiple comparisons of mean using the Tukey's test.

Ethical considerations

Ethical approval was sought from the IREC (Institutional research and ethics committee) of Kampala International University-Western Campus and a sign clearance from Mbarara University of Science and Technology Research and Ethics Committee (MUST-REC). International guidelines for the handling of laboratory animals was followed and the animals were sacrificed under general anesthesia.

Limitations and delimitations

There was lack of good state of the art laboratories in Bushenyi District and at Kampala international university pharmacology laboratory which do not have a vacuum concentrator the sample.This limitation was overcome by transporting the sample to Mbarara University of Science and Technology laboratory for vacuum concentration.

RESULTS

Table 1. Acute toxicity of Kabuuti herbal cough extract (LD50)

PHASE	DOSE	No of rats	Death after 24 hours.	Death after 14 days.
1	10 mg/Kg	3	0/3	0/3
1	100 mg/ Kg	3	0/3	0/3
1	1000 mg/Kg	3	0/3	0/3
2	1600 mg/Kg	1	Oil	Oil
2	2900 mg/Kg	1	Oil	Oil
2	5000 mg/Kg	1	Oil	Oil
Control	1 ml of distilled water	1	Oil	Oil

Table 2: Phytochemical screening of Kabuuti herbal cough syrup

Serial no.	Phytochemical Constituent	Test	Result
1	Saponins	Foam test	+
2	Phenols	Ferric chloride test	+
3	Tannins	Gelatin test	-
4	Alkaloids	Dragendoffs test	-
5	Flavonoids	Alkaline reagent test	++
6	Steroids		++

+ indicates presence of constituent and – indicates absent

Table 3: Effects of Kabuuti herbal cough extract on hematological parameters of Wistar rats

Parameters	NO.	Dose(mg/Kg)			1ml of distilled water	P. values
		1600	2900	5000		
WBC(109/I)	1	4.01*	17.14	10.08	13.15	
LYMP(l09/1)	1	2.34*	10.74	7.38	11.15	
GRAN(109/I)	1	0.91	4.37*	1.37	1.26	
RBC(1012/I)	1	3.38*	6.72	7.05	7.33	
HGB(g/dl)	1	6.30*	13.50	14.90	13.90	
MON(109/I)	1	0.75	2.03	1.33	0.74	
HCT%	1	19.84*	37.08	40.67	40.66	
MCV(fl)	1	59.00	55.00	58.00	55.00	
MCH(pg)	1	18.60	20.20	21.20	18.90	
MCHC(G/dl)	1	31.80	36.50	36.70	34.10	
PLT(109/I)	1	209.00	413.00	36.00*	466	
PCT%	1	0.15	0.26	0.02	0.34	
MPV(fl)	1	7.20	6.30*	7.2		
LYMP%	1	58.5*	62.6	73.2	84.8	
MON%	1	18.8*	11.9	13.1	5.6	
GRAN%	1	22.7	25.5	13.6	9.6	

DISCUSSION

The phytochemicals screening showed the present of phenols, flavonoids and steroids. The extract lacked alkaloids and saponins. The LD50 of the crude herbal extract of Kabuuti cough syrup on wistar rats using Lorke's method. The LD50 was greater than 5000 mg/kg since no rat died at the dose of 5000 mg/kg on short term use. The signs of toxicity observed included sedation, urination, defecation, decreased activity, itching. The effect of the crude herbal extract of Kabuuti cough syrup on the hematological parameters of the Wistar rats after 14 days of a single oral administration showed statistically significant decrease in platelets when compared with the control group (14 days). Decrease in platelets (thrombocytopenia) may mean poor clotting patterns caused by the extract fecting the platelets directly of the bone marrow [19].Increase in platelets (thrombocytosis) may result in clotting disorders like disseminated r disease [20]. This study showed a significant decrease in white blood cells compared to the control group and the treatment group dose of 1600 mg/kg. (P=0.004).A decrease in white blood cells is referred to as leukopenia. Decreased WBCs may be seen in viral infection, aplastic anemia [21]. A significant decrease in lymphocytes was noticed in 1000 mg/kg and 1600 mg/kg as compared to control. (P=0.002).The decrease can be caused by acute infection, burns, trauma etc. this does not mean that the extract caused the decrease, it may be the rats were infected in the process. There is no evidence of the constituents in the extract to cause this decrease. There was significant decrease in red blood cells in 1600 mg/kg as compared to the control (P=0.0001) and significant decrease in hemoglobin of 1600 mg/kg as compared to control. (P=0.002). A decrease in red blood cells can be seen in various types of anemias and as a result it also caused a decrease in hemoglobin [22]. There's no published evidence of the constituents of Kabuuti to cause a decrease in red blood cells and hemoglobin. An elevated monocyte count is referred to as monocytocis it may be observed in the recovery phase of some infections, sub-acute bacterial endocarditis, TB, cirrhosis [23]. This study showed a significant increase for monocytes as observed in 1600mg/kg when compared to control (P=0.007). The effect of the crude herbal extract of Kabuuti on biochemical parameters on the liver and kidney of the wistar rats after 14 days of single oral administration showed significant increase in creatinine in all the extract groups as compared to control. Creatinine has been found to be a fairly reliable indicator of kidney function. Elevated creatinine levels signifies impaired kidney function. As kidneys become impaired for any reason the creatinine level in blood will rise due to poor clearance of creatinine by the kidneys [6]. ALT is an intracellular enzyme present in the liver tissue. It is considered a more specific marker for liver disease compared to AST [24]. A significant decrease in ALT in 100mg/kg and 5000mg/kg as compared to control (P=0.003) was observed. A significant decrease in ALT shows a normal liver function. There was a significant increase in AST in 1600, 2900 and 5000mg/kg as compared to control (P=0.008, 0.013 and 0.036 respectively).Increase in AST may mean liver damage, decrease is not of any toxicological significance. Decrease in AST may be due to hepatoprotective effects of the extract [25]. AST is not specific to the liver unlike ALT and thus the decrease may be incidental [26].

CONCLUSION

This study showed that the LD50 of Kabuuti cough herbal syrup was greater than 5000 mg/kg. The phytochemical screening showed that flavonoids, phenols and steroids were present. 1600 mg/kg decreased RBC and HGB. 1600mg/kg, 2900mg/kg and 5000 mg/kg increased creatinine. They have an effect on the kidney.Therefore higher doses in phase 2 should be used with care since they caused increase m monocytes, decrease in white blood cells and lymphocytes.

RECOMMENDATIONS

The hepato-protective properties of the extract should be further researched due to decreased ALT levels at doses 5000 mg/kg. The anti-oxidant properties of the plant on vital organs should be researched by determining biomarker enzymes in the organs.

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