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Evaluation of Hematological and Biochemical Effects of Kabuuti Herbal Cough Syrup: A Sub-acute Study in Wistar Rats

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ABSTRACT

Herbal medicines, including Kabuuti cough syrup, have gained popularity in treating various ailments, yet their safety and efficacy require scientific scrutiny. This study aimed to evaluate the hematological and biochemical effects of Kabuuti herbal cough syrup after 28 days of oral administration in Wistar rats. The herbal extracts, containing secondary metabolites like terpenes, alkaloids, steroids, and phenols, were administered at different doses to rats, with distilled water as a control. Hematological and biochemical parameters were assessed, showing no statistically significant changes in most parameters. However, there were decreases in mean corpuscular volume (MCV) levels and lymphocyte percentage, and increases in hemoglobin (HB) levels and gamma glutamyl transferase (GGT) levels observed in certain dosage groups. Despite these variations, the overall findings suggest that Kabuuti herbal cough syrup is safe for liver and kidney function in the short term, with no significant adverse effects observed.

Keywords: Kabuuti Cough Herbal Preparation; Haematological Effects; Biochemical Effects; Oral Administration; Wistar Rats

INTRODUCTION

The World Health Organization (WHO) defines herbal medicines (HM) as remedies containing crude "herbs, herbal materials, herbal preparations and finished herbal products that contain as active ingredients parts of plants, or other plant materials, or combinations[1, 2]. This definition covers three different categories, including authorized herbal medicinal products according to the European Union framework for medicines regulation, botanical or dietary herbal supplements used for medicinal purposes, as seen in the United States and almost all other countries, and single, individual preparations that are prepared and/or used by the consumers themselves or prescribed by a herbal practitioner, a physician, or a pharmacist[3].

Kabuuti cough extract, a herbal medicine in recent times, has been drawn to several beneficial effects on relief from cough, measles, whooping cough, tonsillitis, and flu. The syrup has become a popular form of cough remedy [4] and has been stocked in many pharmacies, drug shops, and supermarkets throughout Uganda, including Bushenyi district in Western Uganda [5]

The interest in the practical use of herbal substances and preparations for medicinal purposes is growing continuously worldwide, with not fewer than 80% of people worldwide relying on them for some part of primary healthcare [6]. Moreover, traditional herbal medicine 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) Approximately 65-80% of the population which lives in developing countries depends essentially on plants for primary health care [7].

Traditional medicines, including herbal medicines, have been, and continue to be, used in every country around the world in some capacity. In much of the developing world, 70-95% of the population relies on these traditional medicines for primary care [8]. Not only in developing countries but also in many developed countries, the use of herbal remedies is widely embraced, with complementary and alternative medicines (CAMs) now becoming mainstream in the UK, the rest of Europe, and North America [9]. For example, as many as 60 percent of those living in France, Germany, and the United Kingdom consume homeopathic or herbal products [10].

In developing countries, which represent the majority of the global human population, the use of herbal preparations for medicinal purposes has quite a different but very important significance: in such countries without adequate medical care by hospitals and/or local physicians and/or without an established health care system, traditional (herbal) medicines are usually the only available and affordable treatment. This applies, for

example, to some countries or regions in Latin America and Africa, including Uganda, where health care is not available to all population groups ([11]. Though the extent of traditional medicine use is not known in most settings in Sub-Saharan Africa, in those countries where herbal use has been assessed, high levels were reflected; for example, 90% of the population in Ethiopia used herbal remedies for their primary healthcare [12].

Previously, it was revealed that in Uganda there was one traditional healer for nearly 290 people compared to one Western-trained medical practitioner for every 10,000 people in the urban areas and 50,000 people in the rural areas respectively [13].

The Ugandan situation is not much different, and many communities, especially from the poor rural areas, still rely on herbal remedies. In addition, many Ugandans believe in the potency of herbal medicine, even when they can access modern medicine. In many cases, they would choose to combine both herbal and modern medicine, especially if they are afflicted with chronic ailments such as HIV/AIDS, hypertension, infertility, cancer, and diabetes [14]

Like most other African countries, Uganda has a large number of tribes, and each has its own peculiar customs and beliefs. The use of herbal medicine is equally different among the communities. Despite this, most of the medications have not been documented and scientifically evaluated to determine their efficacy, dosage, hematological and biochemical effects, and the alleged indications by the practitioners. These medications have been in use for centuries, and it is quite possible that they could possess some degree of potency, and can potentially be used in therapy for some of the current conditions [15]. Despite the high levels of usage and importance of traditional medicine, currently, there is little information regarding literature on the use of medicinal plants in Uganda, with only a few and far between authors having contributed. Moreover, relatively few herbal drugs have been evaluated scientifically to prove their safety, potential benefits, and effectiveness [16]. Additionally, there is still a lack of regulation, which leads to misuse of the medicines by unqualified practitioners and loss of credibility of the system, which can lead to toxicities and unknown drug effects, as observed in the case of cough herbal remedies. Therefore, the researcher is interested in evaluating the effects of Kabuuti cough product on hematological and biochemical parameters in Wistar rats after 28 days.

There is increased availability of Kabuuti herbal cough syrups, and it is readily used as over the counter. It is used in the treatment of many disease conditions such as respiratory infections, common cold, whooping cough, and many others [17]. Many people believe they are safe and effective, but the WHO (2003) requires evidence on the safety and effectiveness. Kabuuti has a mixture of plants which could have potential combined toxicity since it contains a variety of phytochemicals [18]. There is no documented information, to the best of our knowledge, on the toxic effects of Kabuuti herbal preparation on hematological and biochemical parameters of the liver and kidney, which may reflect on the short-term safety of this product. This study, therefore, seeks to evaluate the hematological and biochemical effects of the product in Wistar rats after 28-day oral administration.

METHODOLOGY

Study Design

An experimental study design was used for this research, conducted at Kampala International University-Western Campus at the Pharmacology Laboratory. The study adhered to OECD principles for Good Laboratory Practices [19].

Herbal Extract Materia

The kabuuti herbal cough syrup was selected due to its widespread use in treating various ailments. It is available over-the-counter in community pharmacies, clinics, and drug shops. In Bushenyi district, South Western Uganda, this extract is commonly used, yet no documented study on its biochemical and hematological parameters has been conducted to establish its safety on short and long-term use.

Extract Collection and Identification

The Kabuuti cough syrup product was obtained from an authorized worker at Nsimye Herbal Medicine Health Limited from the authorized distribution center. Samples were stored at the Pharmacology Department at Kampala International University for future reference.

Vaporization/Concentration and Storage

The collected sample was concentrated using the method described by Kelly (2016) with a vacuum concentrator. This process removed 99% of the water from the sample in a short time period, irrespective of its freezing or boiling point. The concentration was carried out at Mbarara University of Science and Technology Pharmacology Department.

Experimental Animal

Wistar rats, aged 7-8 weeks and weighing approximately 80-150g, were used. They were obtained from the Pharmacology Animal House at Mbarara University of Science and Technology. The animals were acclimatized for at least two weeks before the experiment and were maintained on standard feed and water. Animal procedures adhered to the Guidelines for the Humane Care

and Use of Laboratory Animals published by the National Institutes of Health, United States [20].

Clinical Observations, Feed Consumption, and Weekly Weight Monitoring

Animals were observed twice daily for clinical changes, including physical and behavioral changes. Weekly weight monitoring was conducted, and feed consumption was recorded.

Hematological Evaluation

Animals were fasted overnight, and blood samples were collected for hematological analysis, including white blood cell count, red blood cell count, hemoglobin concentration, and platelet count, among others. This evaluation was conducted at the Clinical Chemistry Laboratory at Kampala International University-Teaching Hospital.

Biochemical Evaluation

Blood samples were collected for biochemical analysis, including parameters such as total cholesterol, creatinine, liver enzymes, and protein levels. This evaluation was also conducted at the Clinical Chemistry Laboratory at Kampala International University-Teaching Hospital.

Quality Control

Quality control measures included the collection of herbal extracts from authorized sources, concentration using a vacuum concentrator, and the use of inbred rats to minimize genetic variations.

Data Analysis

Data were entered into MS Excel (version 2010) and analyzed using Graph Pad Prism. Statistical significance was determined using Analysis of Variance (ANOVA) followed by Tukey's post hoc test.

Ethical Considerations

Approval was obtained from the KIU/School of Pharmacy Research Committee, and permission was granted by the head of the Laboratory at Mbarara University of Science and Technology. International guidelines for handling laboratory animals were followed, including euthanasia conducted under anesthesia to minimize pain and suffering.

RESULTS

Percentage yield of kabuuti extracts

Percentage yield = (Weight of concentrated extract / Weight of kabuuti syrup) x 100
= (400.5 / 790) x 100

The percentage yield from 790g of kabuuti herbal cough syrup was 50.7%. However, the extract of kabuuti concentrate was paste-like, and not completely powdered.

Hematological parameters after 28 days following oral administration of Kabuuti cough herbal extracts

There was no statistical significance between the treatment groups and the control groups as analyzed by ANOVA ($p > 0.05$). However, there was a decrease in the levels of MCV in animals that received a dose of 250mg/kg compared to the control group (with a p-value of $p = 0.360$), animals that received a dose of 500mg/kg compared to the control group ($p = 0.515$), and a decrease in the levels of MCV in animals that received a dose of 1000mg/kg compared to those that received the control dose of distilled water ($p = 0.276$). Lymphocyte percentage for 250mg/kg had a p-value of 0.065 as compared to the control, and that of 1000mg/kg was ($p = 0.236$). WBC had a p-value of 0.394 for doses of 500mg/kg, and for doses of 1000mg/kg, the p-value was ($p = 0.655$). HB had a p-value of 0.581 for doses of 250mg/kg, and $p = 0.343$ for doses of 1000mg/kg. All animals in the control and treatment groups survived after the 28 days of the experiment.

Table 1: Hematological parameters after 28 days following oral administration of Kabuuti cough herbal extracts.

1 Haematological Parameters	N	Mean ±SEM on day 28			
		Control l ml/kg	250mg/kg	500mg/kg	l000mg/kg
WBC (10^3 /ul)	3	8.40±2.55	8.30±1.70	4.57±0.99	7.27±0.27
RBC (10^6 /ul)	3	4.89±0.94	7.10±0.53	5.56±0.66	5.43±0.58
HOB (g/dl)	3	11.03±1.07	13.50±1.15	10.63±1.59	10.13±1.43
HCT (%)	3	3.54±3.19	4.26±4.18	3.26±4.67	3.20±4.31
MCV (fl)	3	68.73±6.02	59.97±2.03	61.56±1.21	58.90±2.85
MCH (pg)	3	22.20±2.44	18.93±0.52	18.90±0.66	18.50±0.88
MCHC (g/dl)	3	31.73±0.55	31.70±0.40	30.76±0.49	31.53±0.37
PCT (%)	3	0.24±0.028	0.27±0.052	0.21±0.10	0.17±0.03
PLT (10^6 /ul)	3	289.00±28.02	359.33±78.82	280.66±143.55	228.66±52.18
LYMPH (%)	3	22.06±0.69	38.33±1.90	28.33±6.22	33.33±3.92
GRAN#	3	4.96±2.07	6.68±0.44	5.43±6.23	5.73±0.74

KEY: WBC: White blood cells, RBC: Red blood cells, HGB: Hemoglobin, HCT: Hematocrit, MCV: Mean corpuscular volume, MCH: Mean corpuscular hemoglobin, MCHC: Mean corpuscular hemoglobin concentration. PCT, PLT: Platelets, N: sample size, GRAN#: Granulocyte number, LYMPH (%): Lymphocyte percentage.

Biochemical parameters after 28 days following oral administration of kabuuti cough herbal syrup extract.

There was no statistical significance between the treatment groups and the control groups as analyzed by ANOVA ($p > 0.05$). However, there was an increase in the levels of GGT in animals that received doses of 500mg/kg and 1,000mg/kg of the extract compared to the control group. They had p-values of 0.605 and 0.402, respectively. ALB had a p-value of 0.245 compared to the control. CREAT J had a p-value of 0.184 for doses of 500mg/kg and urea had a p-value of 0.518 for 1,000mg/kg compared to the control.

Table 2: biochemical parameters after 28 days following oral administration of Kabuuti cough herbal syrup extract.

Parameter		250mg/kg	500mg/kg	l000mg/kg	Control lml/kg
ALT	3	306.56±20.73	285.00±74.43	440.40±24.04	309.50±65.29
AST	3	961.43±102.07	807.10±198.03	102.60±217.92	898.30±200.93
Urea	3	12.92±1.28	10.22±0.099	7.86±3.70	11.86±0.57
C.REATININE	3	6.66±1.28	13.73±3.26	5.66±3.51	3.86±3.23
TP	3	61.30±1.93	56.96±1.18	61.23±6.38	62.06±1.90
GGT	3	19.56±4.39	10.26±1.83	22.43±7.87	15.76±4.78
T-BIL	3	3.63±1.81	4.73±0.61	5.23±1.08	3.33±1.71
LB	3	26.66±0.93	26.90±0.47	19.56±6.08	28.73±1.22

Key: ALT: Alanine aminotransferase, AST: Aspartate aminotransferase, Urea, Creatinine, N: sample size, TP: total proteins, GGT: Gamma glutamyl transferase, T-BIL: Total bilirubin

DISCUSSION

Assessment of haematological parameters can be used to determine the extent of deleterious effects of foreign compounds, including plant extracts, on the blood. Haematological parameters such as red blood cell count (RBC), white blood cell count (WBC), platelet count, mean corpuscular volume (MCV), and mean corpuscular haemoglobin (MCH) are used to indicate the integrity of the hematopoietic tissue. Thus, changes in these parameters, specifically the amount and morphology of neutrophils, basophils, eosinophils, monocytes, lymphocytes, hematocrit, thrombocytes, MCV, and MCH, can be used to predict the protective or toxic effects of plant extract administration on the integrity of the hematopoietic tissue [21]. These laboratory investigations have been reported to be highly sensitive, accurate, and reliable, remaining the bedrock of ethical and rational research, disease diagnosis, prevention, and treatment (Okonkwo et al., 2004). The normal range of these parameters can be altered by ingestion of toxic plant extracts, including Kabuuti herbal extracts.

There was no statistically significant change in the haematological parameters at all doses after oral administration of Kabuuti herbal extract for 28 days. However, there was a decrease in the levels of MCV in animals that received a dose of 50 mg/kg compared to the control group (with a p-value of p=0.360), animals that received a dose of 500 mg/kg compared to the control group (p=0.515), and a decrease in the levels of MCV in animals that received a dose of 1000 mg/kg compared to those that received the control dose of distilled water (p=0.276). Lymphocyte percentage for 250 mg/kg had a p=0.065 compared to the control, and that of 1000 mg/kg was (p=0.236). WBC had p=0.394 for doses of 500 mg/kg, and for doses of 1000 mg/kg, the p-value was (p=0.655). HB had a p-value of 0.581 for doses of 250 mg/kg, p=0.343 for doses of 1000 mg/kg.

These changes, however, could be incidental or due to the presence of flavonoids, alkaloids, phenols, saponins, and gingerols since other parameters like HCT and MCHC did not show any significant change [22]. The study conducted by [7] showed that Zingiber officinale to Wistar rats caused a decrease in the diabetic-induced disturbances of some haematologic parameters in alloxan-induced diabetic rats, where RBC, WBC, and PCV had $p<0.05$.

The nonsignificant changes in urea and creatinine levels between the treatment groups and the control group imply that the aqueous extract of Kabuuti herbal extracts did not cause any serious damage to the kidney. However, there was an increase in the levels of GGT in animals that received 500 mg/kg and 1000 mg/kg doses of the extract compared to the control group. These had p-values of 0.605 and 0.402, respectively. ALB had

a p-value of 0.245 compared to the control. CREAT J=13.7g had p=0.184 for doses of 500 mg/kg and urea p=0.518 for 1000 mg/kg compared to the control and was high compared to the normal (Scr=0.7-0.9g).

According to Mansourian et al., [23] and Subramanya et al. [24], an increase in the levels of AST after administration of the herbal extract could probably be due to liver damage, cardiac infarction, or muscle injury. Because AST is not a specific marker for liver damage since it is found in other organs like the heart, kidney, and skeletal muscles. Instead, ALT is a more sensitive and specific marker for liver injury because it is localized in the hepatocytes, of which it did not show significant change compared to the control but was high. AST enzymes leak and increase in the bloodstream when these organs are damaged. And a decrease in the parameters could imply a Hepatoprotective effect. ALB reference value was 3.5-5.3g/dl and the test results after 500 mg/kg was 0.8g/dl with p-values of 0.40 which was decreased though statistically not significant. A decrease in albumin production can be due to chronic liver disease, such as cirrhosis and nephrotic syndrome, and these conditions occur after exposure to the extract for about 3 months and beyond.

CONCLUSION

There was no statistically significant effect on the hematological parameters after the oral administration of aqueous extract of Kabuuti for 28 days. There was an increase in the levels of GGT in animals that received 500 mg/kg and 1000 mg/kg doses of the extract compared to the control group. These had p-values of 0.605 and 0.402, respectively. ALB levels decreased with a p-value of 0.245 compared to the control. From the study results, we can conclude that the extract is safe on liver function since there was no statistical significance from the results indicative of liver function damage after a sub-acute study. Serum Creatinine and urea also increased in serum with values of 13.7g and 7.86g, respectively, and p-values of 0.184 and p=0.518, respectively, which were statistically not significant. Kabuuti herbal extracts are also safe on the kidneys since the biochemical parameters were not significant.

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