ORIGINAL ARTICLE

PHYTOCHEMICALS AND TOXICOLOGICAL EFFECTS OF HERBAL DRINK (HD) ON LIVER AND KIDNEY IN WISTAR RATS

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ABSTRACT

Herbal medicine use is on the increase worldwide and many studies have associated their use with hepatotoxicity and nephrotoxicity. Herbal drink (HD) is a commercial herbal supplement mainly used in Uganda. In this study, we evaluated the phytochemical composition, and acute and sub-chronic toxicological effects of HD on hepatorenal integrity in Wistar rats. Modified Lorke's method was employed in the acute toxicity study. For the sub-chronic toxicity study, a total of sixteen (N= 16) Wistar rats were randomly allotted to four groups of four rats each (n=4). Normal control group, NC (normal dose equivalent of distilled water), Group T1 (half normal dose of HD), Group T2 (normally recommended dose equivalent of HD), and Group T3 (twice normal recommended dose of HD). All treatments were done by intragastric gavage for a period of twenty-eight days. Phytochemical analysis of HD showed the presence of alkaloids, glycosides, tannins, phenolic compounds, flavonoids, saponins, and carbohydrates. The animals were able to tolerate a dose of up to 5000 mg/Kg. It was demonstrated that continuous twenty-eight-day treatment with HD does not significantly ($p>0.0$ alter kidney and liver function parameters in Wistar rats. This observation concurs with the histopathological findings of the liver and kidney tissues which showed no remarkable histomorphological alterations. Regular intake of HD does not have any significant toxic effects on the liver and kidney in Wistar rats.

Keywords: Herbal drink, Phytochemicals, Toxicity, Liver, Kidney

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INTRODUCTION

The use of Herbal drugs is on the rise in many different areas in the world (1, 2), for supplementation or replacement of conventional medical drugs, and are said to be a major cause of liver injury (3). The conventional use of herbal remedies for the treatment of different diseases across the world dates back to time immemorial (4, 5). Plants produce secondary metabolites with therapeutic potential to treat both chronic and acute diseases, especially for preventive therapy (6).

Herbal plants are used for the healing and prevention of different ailments and form the foundation for discovery and synthesis of novel therapeutic drugs (5, 7). About 80% of the world population including in East Africa still depends on herbal medicine (3, 8, 9). In Uganda, four out of five people mainly seek medical services from traditional medicine practitioners and earlier studies have shown that there is atleast one traditional healer per village (9, 10).

Traditional herbal medicines are mainly preferred because of their established therapeutic value especially for prevention (10). Several other reasons include: ineffectiveness or dissatisfaction with conventional drugs, need to boost the immune system, desire to have control over health care decisions, accessibility and cheap as compared to the biomedical medicine, compatible with the users' values, and lastly herbal remedies are considered of natural origin and hence innocuous with no adverse effects since the users do not consider herbal remedies as drugs (10).

Herbal drugs though obtained from plants, have active ingredients that are still potent chemicals and therefore, they can have adverse effects and interactions with drugs, with each other, food or alcohol (11). Herbal drugs can induce toxicity due to their complex nature and variability since they are obtained mainly from plants and processed through different methods and steps which may include harvesting, grinding, drying and boiling (12). A given plant contains many variable products due to differences in the time of harvesting, plant part, drying and extraction methods. These conditions are likely to cause differences in batches and variability of the final product (13). Other factors likely to cause toxicity include; chances of misidentification of the plants, phytochemical interactions, product contamination, decomposition and adulteration (14).

Recent studies from Uganda and Nigeria have separately highlighted the relationship between the utilization of herbal drugs and liver diseases (3). In a group of 365 patients in Nigeria, the consumption of herbal remedies was reported in 46% of patients with liver disease (3). In Uganda, the consumption of herbal drugs was associated with significant liver fibrosis in both human immune virus (HIV) infected and HIV-uninfected patients (3). The liver and kidney are prone to toxic effects of xenobiotics (drugs) (15, 16). Liver diseases account for over two million deaths

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annually globally, mainly through acute hepatitis, liver cirrhosis and liver cancer (3). Drug induced liver injury (DILI) includes any liver malfunction caused by herbs or any other xenobiotics, after a considerable exclusion of other etiologies (3). Amongst patients with DILI, herbal drugs account for the second most common precursor (17). Patients suffering from chronic liver disease (CLD) are more susceptible to herbal induced liver injury (HILI) upon using herbal drugs (16). Organ damage is either triggered through intrinsic or idiosyncratic reaction (16, 18). Idiosyncratic liver damage largely is dose independent and presents in unpredictable way, while intrinsic liver damage is the opposite (13). This explains the complexity of the pathogenesis of idiosyncratic HILI and idiosyncratic DILI (15, 16, 18).

HD is a herbal supplement said to be a mixture of pure water, Aloe vera extract (10%), lemon juice (10%), green tea (5%), cactus and citric acid (acidulant) as indicated on the label by the manufacturer. Aloe vera consists of many compounds like, dithranol, aloin, chrysarobin and aloe emodin. Intake of excessive Aloe vera was associated with clinical symptoms such as abdominal pain, fever, dizziness, and increase in plasma aminotransferases in patients:- kidney, electrolyte imbalance, diarrhea, conventional drug interaction, bleeding, toxic hepatitis and liver damage (19, 20). Some species of Aloe vera plants are composed of many alkaloids which can trigger or inhibit the liver enzymes systems such as cytochrome P450 (21). In recent past, many cases of the Aloe vera induced hepatotoxicity have been reported. The toxicological effects of many herbal products are not well explained (22). This research therefore seeks to evaluate the toxicological effects of HD on liver and kidney in Wistar rats.

MATERIALS AND METHODS

Determination of phytochemical composition

Qualitative determination by standard procedures as described by Harborne (22).

Ethical considerations

The Kampala International University Animal Research Ethics Committee provided ethical approval (KIU-2021-55) prior to this study.

Acute toxicity study was determined by modified Lorke's method

This method has two phases which are phases 1 and 2 respectively.

In phase 1, nine (N=9) Wistar rats were divided into three (3) groups of three (n=3) Wistar rats each. Each group was administered different doses (10, 100 and 1000 mg/kg) of HD respectively. The Wistar rats were observed for twenty-four (24) hours to monitor their behavior as well as if any mortality occurs. In

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Phase 2, three (N=3) animals, which were randomly allotted to three groups of one (n=1) Wistar rat each. The animals were administered higher doses (1600, 2900 and 5000 mg/kg) of HD respectively and then observed for twenty-four (24) hours for any behavioral changes as well as mortality.

Sub-chronic study

A total of sixteen (N= 16) mature Wistar rats weighing 180-200g were obtained from the animal house of Kampala international University western campus and were randomly allotted to four groups of four rats each (n = 4). The animals were housed in wooden metabolic cages at good laboratory conditions and had access to food and water ad libitum and did not interact with other animals which were not part of the study.

HD was administered orally at doses that were calculated with reference to the recommended dose (by the manufacturer of HD, one bottle of 280ml per day) for human considering the average human body weight (70kg) to the average weight of a mature Wistar rat of about 180 - 200g (the rats were weighed at the beginning of the study). Normal control, NC (0.8ml of distilled water per kg of body weight), Treatment groups, treated with doses, T1 (0.4 ml), T2 (0.8ml) and T3 (1.6 ml) of HD per kilogram of body weight of the animals.

After a study period of twenty-eight days, all the experimental animals were anaesthetized by intraperitoneal administration of xylazine and ketamine (2 mg + 80 mg respectively) and sacrificed before obtaining the blood samples by cardiac puncture and liver and kidney tissues. All animal remains were collected and taken for incineration.

Biochemical analysis

Serum biochemical analyses, alanine aminotransferase (ALT), Aspartate aminotransferase (AST), Alkaline phosphatase (ALP), albumin concentration, Total bilirubin concentration, Direct bilirubin concentration were carried out to assess the changes in liver function. Serum creatinine and blood urea nitrogen (BUN) were also determined as kidney function biomarkers by standard spectrophotometric methods according to the Cypress diagnostic kits instructions.

Histology examination

After 28 days of treatment in various groups, the animals were sacrificed and dissected to isolate the liver and kidney of each animal. After isolation, the organs were fixed in 10% formalin for two days in polypropylene bottles. After two days, the liver and kidney tissues

were removed from the polypropylene. The pieces of liver and kidney were further processed for dehydration with isopropyl alcohol of increasing strength (70%, 80% and 90%) for 12 h each. The final dehydration was carried out using absolute alcohol with about three changes for 12 h each. The clearing of the tissues was done by using chloroform with two changes for 20 to 30 min each. The liver and kidney tissues were further subjected to paraffin infiltration in automatic tissue processing unit. They were washed with running water to remove formalin completely. For the removal of water, the alcohol of increasing grades was used and finally alcohol was removed by using chloroform and then chloroform was removed by paraffin infiltration. Hematoxylin-eosin was used to stain the sections before photo-microscopic assessment using a photo-microscope.

Statistical analysis and presentation of data

Statistical analyses were performed using Graph Pad Prism 5 software (Graph Pad Software, San Diego, CA). All parameters' results were expressed as mean ± standard error of mean (SEM) and all the statistical comparisons were made by means of the one-way ANOVA test, followed by Turkey's test post hoc analysis, a P value <0.05 was considered significant. The data was presented in tabular and graphical form.

RESULTS

Qualitative phytochemical composition of herbal drink (HD)

HD contains alkaloids, glycosides, tannins and phenolic compounds, flavonoids, saponins, and carbohydrates, according to a phytochemical qualitative and quantitative examination. Aloe emodin and anthraquinones tested negative, proving that they weren't present in the herbal mixture (Table 1).

Serum liver function enzymes and biomarkers after treatment with HD for 28 days.

The results of liver function enzymes are presented in Table 2. The study findings showed that there was no significant difference ($p>0.05$) in AST and ALT activities in all the treatment groups compared to the NC. ALP activities for the treatment groups T1 and T2 are significantly ($p<0.05$) lower than that of the NC. The ALP activity for treatment group T3 shows no significant difference ($p >0.05$) to that of the NC.

The results of liver function markers (Table 3), the albumin concentration for treatment groups T2 and T3 are significantly higher ($p<0.05$)

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than that of the NC. The Albumin concentration for the T1 shows no significant difference with the NC (P=0.1606). There is no significant difference between the total bilirubin concentrations of the NC and T1 and T2 (P > 0.05). However, total bilirubin concentration for T3 is significantly higher than NC (P= 0.0118). Direct bilirubin for the treatment groups were all significantly lower (p<0.05) than that of the NC.

Serum biomarkers of kidney function after treatment with HD for 28 days

The results of kidney function biomarkers are presented in table 4. Serum creatinine concentration for T1, T2 and T3 showed no significant difference with the NC (p > 0.05). BUN concentration for T1 showed no significant difference with that of the NC (P=0.8938) while that of T2 and T3 are significantly lower than that of the NC (p<0.05).

Histology of liver and kidney tissues after treatment with HD for 28 days

The results of liver histology examination (Figure 1), the liver tissues showed mild hepatocyte degeneration. However, such changes were considered environment-related and not related to HD since similar effects also occurred in the NC group. Histopathological examination on the kidney tissue (Figure 2) showed no remarkable histomorphology alterations.

DISCUSSION

Phytochemical components of herbal drink, HD

The results obtained from this study (Table 1) showed that the HD contains high amount of phenolic and flavonoid compounds. In an earlier study, Pei et al. (23), reported that antioxidant properties, reactive oxygen species scavenging and cell function modulation of flavonoids could account for the large part of their pharmacological activity. Flavonoids, anthraquinones and tannins are powerful antioxidants against free radicals and are described as free-radical scavengers (24). This activity is attributed to their hydrogen-donating ability. Indeed, the phenolic groups of flavonoids serve as a source of a readily available hydrogen atoms such that the subsequent radicals produced can be delocalized over the flavonoid structure (25). Carbohydrates have also been reported to have immunomodulatory acidity while anthraquinones and glycosides are powerful purgative and anti-microbial agents.

In a recent study, it was reported that green tea consumption did not adversely alter renal

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function as assessed by serum creatinine concentration, serum urea concentration and glomerular filtration rate (26). In this present study, treatment of Wistar rats with HD did not have any adverse toxic effects on the liver and kidney function which is in consonance with what was earlier reported.

Effect of HD on liver function biomarkers

Determination of changes in liver function biomarkers is essential in assessing liver function. ALT is a more specific enzyme to the liver and is released from the hepatocyte into the bloodstream when the cells are damaged. Therefore, high ALT activity may indicate liver injury (Pavlik et al., 2019). Findings of the present study (Table 2) showed that the mean ALT activities in the treatment groups and NC showed no significant difference ($P>0.05$). This observation concurs with the histological findings of the liver tissues (Figure 1) which show mild hepatocyte degeneration. However, such changes were considered environment-related and not related to HD since similar effects occurred in the NC group. The liver function biomarkers, Albumin, total bilirubin and direct bilirubin concentrations of the treatment groups show no significant difference from those of the NC (Table 3), these shows that HD does not have any toxic effects on hepatocytes and does not significantly alter liver function.

Effect of HD on kidney function biomarkers.

The kidneys are essential organs to human health, they filter toxic metabolic waste production from circulation (Barnett and Cummings, 2018). Several biochemical markers are available for the assessment of renal function (26). Creatinine is usually formed from creatine's non-enzymatic breakdown in the muscle, the rate depending on the muscle mass. This creatinine, in turn, is excreted from the body via urine by the kidney. If renal clearance is impaired, serum creatinine levels increase above normal. Elevation in serum creatinine levels is usually associated with kidney injury. This makes creatinine an essential biomarker of kidney function (15).

Measurement of serum creatinine and BUN levels provide an indication of renal function, elevated serum levels suggest impaired renal filtration (26). Green tea and its polyphenolic constituents have been shown to induce multiple beneficial effects (20). Several studies have investigated the effect of epigallocatechin-3-gallate (EGCG) the major component of green tea on kidney function

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using animal models of nephrotoxicity and induced renal damage (20). Some concerns have been raised regarding reported hepatotoxicity in some subjects (15, 16). However, many studies have shown that hepatotoxicity is mild, rare and is only associated with high EGCG intake (26).

In a study conducted by Bassuony et al. (27) a natural juice mixture containing lemon decreased serum creatinine and blood urea nitrogen in both normal and high fat diet Wistar rats. The mixture further increased total protein and globulin. These results concur with what has been observed in the present study.

In our study, there is no significant difference between serum creatinine and BUN levels of treatment groups generally treated with HD and the NC as shown in the results in table 4, implying that renal filtration was unaffected. These results are in consonance with those reported in two earlier separate studies by Essex et al., (26) and Nalimu et al. (28).

CONCLUSION

Regular oral intake of HD does not have any significant toxic effects on liver and kidney in Wistar rats.

Recommendations

From the findings of this study, people should continue to consume HD since it has no toxic hepato-renal effects.

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Table 1: Qualitative phytochemical composition of herbal drink (HD)

Components	**Observation**
Alkaloids	Positive (++)
Flavonoids	Positive (+++)
Glycosides	Positive (+++)
Anthraquinones	Negative

Saponins	Positive (+++)
Tannins	Positive (++)
Emodin	Negative

+ traces present ++ moderate amount present +++ adequate amount present

Table 2: Serum liver function enzymes in Wistar rats after treatment with herbal drink for 28 days

GP	ALT	AST	ALP
NC	8.944±0.515[a]	33.94±0.65[a]	164±11.97[a]
T1	7.972±0.514[a]	37.30±0.66[a]	116.80±6.89[b]
T2	9.133±0.311[a]	34.56±1.93[a]	123.0±5.2[b]
T3	8.750±0.337[a]	36.17±0.63[a]	178.0±5.9[a]

Serum ALT, AST and ALP activities. NC: normal control, Treatment groups: T1, T2 and T3. When compared to the normal control NC, mean values labeled with letter ***a*** show no statistically significant difference (P>0.05), mean values with letter ***b*** show statistically significant difference (P<0.05).

Table 3: Serum liver function markers in Wistar rats after treatment with herbal drink for 28 days

Group	Total Bilirubin	Direct Bilirubin	Albumin
NC	0.5687±0.3355[a]	0.302±0.024[a]	0.8628±0.016[a]
T1	0.624±0.034[a]	0.07333±0.00290[b]	0.7854±0.016[a]
T2	0.678±0.023[a]	0.0695±0.0077[b]	1.359±0.031[b]
T3	0.7343±0.0134[b]	0.04857±0.00884[b]	1.038±0.017[b]

Serum albumin concentration (mg/dl), Total bilirubin concentration (mg/dl) and Direct bilirubin concentration (mg/dl). NC: normal control, Treatment groups: T1, T2 and T3. When compared to the normal control NC, mean values labeled with letter ***a*** show no statistically significant difference (P>0.05), mean values with letter ***b*** show statistically significant difference (P<0.05).

Table 4: Serum Kidney function biomarkers

Group	Serum creatinine	Blood urea nitrogen (BUN)

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NC	4.60±0.30[a]	2.32±0.18[a]
T1	4.40±0.20[a]	2.19±0.06[ad]
T2	4.00±0.30[a]	1.32±0.09[b]
T3	4.90±0.10[a]	1.48±0.15[b]

Serum creatinine concentration (mg/dl) Blood urea nitrogen, BUN concentration (mg/dl). NC: normal control, Treatment groups: T1, T2 and T3. When compared to the normal control NC, mean values labeled with letter ***a*** show no statistically significant difference (P>0.05), mean values with letter ***b*** show statistically significant difference (P<0.05).

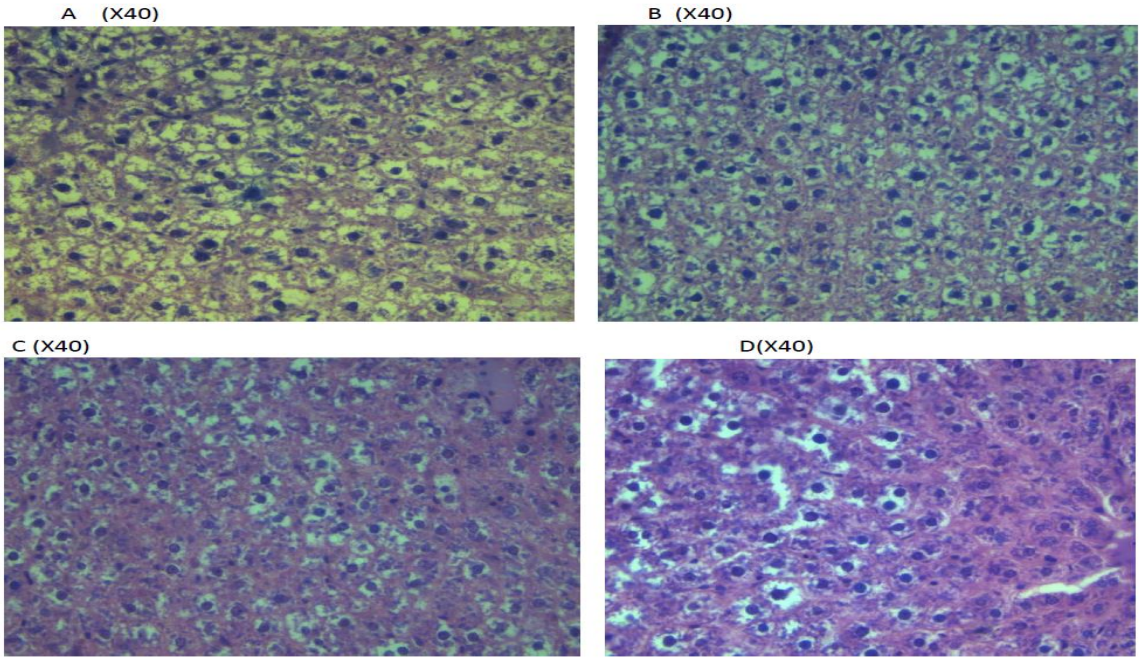

Figure 1: Histological micrographs for the liver tissues, H&E stain: (A) Normal control, NC, (B) treatment group T1, (C) treatment T2, and (D) treatment group T3

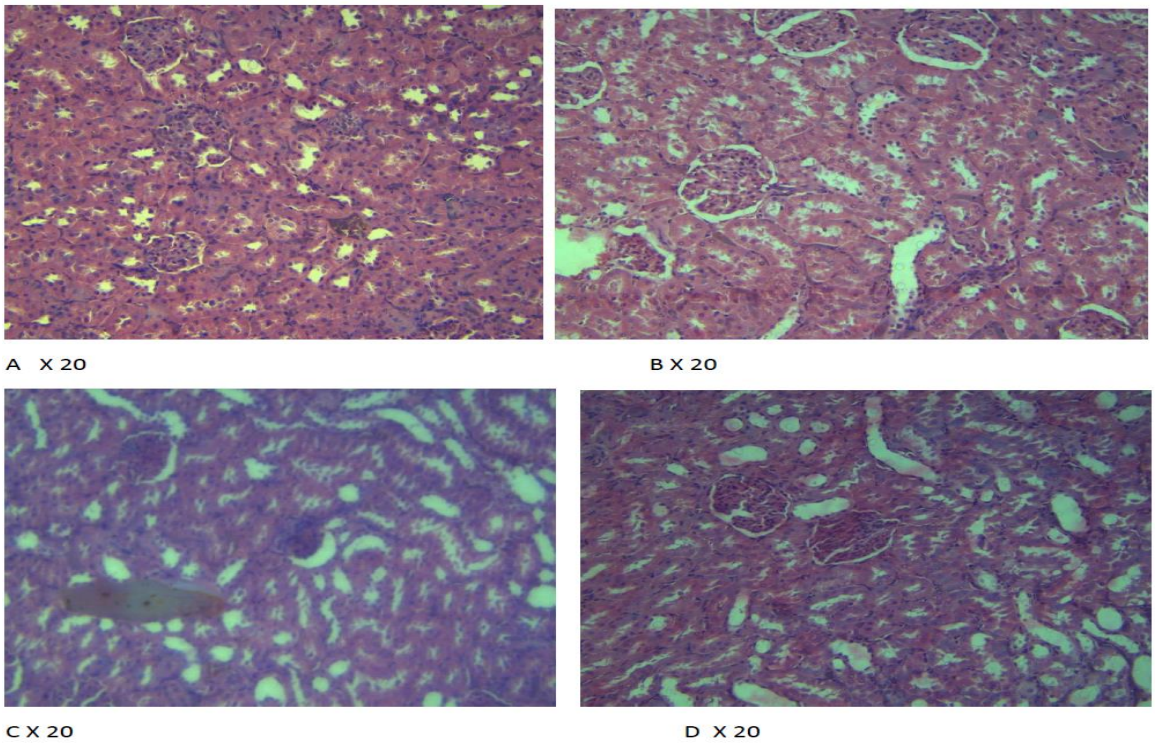

Figure 2: Histological micrographs for the kidney tissues, H&E stain: (A) Normal control, NC (B) treatment group T1 (C) treatment T2 and (D) treatment group T3