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ACUTE AND SUBACUTE TOXICITY STUDIES OF BUKUMOL® POLYHERBAL FORMULATION BY ORAL ADMINISTRATION IN RODENTS; ASSESSMENT OF LIVER AND KIDNEY FUNTIONS

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ABSTRACT

The potential toxicity of Bukumol® Polyherbal Formulation (PHF) in Wistar rats was assessed through the Acute and Subacute Toxicity Tests. The rats were sacrificed, haematological parameters, plasma biochemical parameters and histopathological examination were carried out. Acute toxicity study showed that the oral LD_{50} value of the PHF was greater than 5000 mg/kg. The subacute toxicity study of the PHF at doses 1000, 1500 and 2000mg/kg did not produce any observable symptoms of toxicity and no significant variation in body weight, food, and water consumption or mortality in all treated rats. However, the administration of the PHF to rats at 2000mg/kg showed a significant decrease in Mean corpuscular hemoglobin concentration (MCHC). The result showed that subacute treatments with the PHF at dose 1500 mg/kg and 2000mg/kg significantly elevated Total protein (T.P) and Glutamic-oxaloacetic transaminase (GOT) respectively. Histological studies showed that at the dose of 2000 mg/kg, there were histopathological changes in the liver and kidney.

KEYWORDS: Bukumol® Polyherbalformulation(Phf), Toxicity, Haematological, Biochemical, Histological.

INTRODUCTION

Medicinal plants have been utilized for an extensive period in order to address various ailments and health conditions. Instead of solely relying on singular plant extracts, these plants are typically combined to form PHFs that encompass a myriad of complex ingredients. These formulations, known as polyherbal formulations (PHF), are composed of portions of plants or unpurified plant extracts that encompass multiple constituents. It is widely acknowledged that these constituents within PHF work together, resulting in a synergistic effect.[1]

The public's recent growing interest in herbal remedies has been attributed to several factors, including: (i) varying claims about the efficacy or effectiveness of botanical medicines, (ii) consumers' inclination towards natural therapies and growing curiosity about alternative medicines, (iii) the view that herbal products are better than manufactured products, (iv) dissatisfaction with the results obtained from mainstream pharmaceutical products (orthodox medicine) and the belief that herbal medicines can prove effective in treating a number of diseases where conventional treatments and drugs have failed, are considered ineffective or inadequate, (v) the exorbitant cost and side effects of most modern medicine, (vi) advances in the field of quality, effectiveness and safety of medicinal plants thanks to scientific and technological advances, (vii) patients' perception that their physicians have not made an accurate diagnosis, thereby fostering the belief that herbal medicine offers an alternative and (viii) shift towards self-medication.[2]

Moreover, it has been noted that in most countries with elevated levels of PHF adoption, these therapeutic commodities are introduced into the marketplace without undergoing obligatory standardized safety or toxicological assessment. Many of these countries also lack effective machinery to regulate manufacturing practices and quality standards. Additionally, these PHF are persistently accessible to consumers without a prescription regimen grounded in verifiable evidence, thus making it exceedingly difficult to identify or acknowledge the potential hazards associated with an inferior product.[3]

Nephrotoxicity, a condition that can manifest as a renal ailment or dysfunction, is frequently brought about by drugs, chemicals, industrial substances, or environmental toxic agents. This detrimental condition can result in profound impairment in most of the kidney's crucial functions, ultimately leading to systemic toxicity due to a diminished ability to excrete bodily

waste, an inability to maintain the balance of bodily fluids and electrolytes, and a reduction in the synthesis of essential hormones.[4]

Many reactive intermediate species can produce oxidative stress, which can be equally detrimental to the cell. When protective defenses are overwhelmed by excess toxicant assault, the effects of reactive intermediate species lead to deregulation of cell signaling pathways and dysfunction of biomolecules, leading to failure of target organelles and eventual hepatocyte and supporting cells death. Research aimed at elucidating the molecular mechanism of the pathogenesis of chemical-induced liver diseases is fundamental for preventing or devising new modalities of treatment for liver injury by chemicals.[5]

Although it has been acknowledged that a myriad of genetic factors influences the susceptibility of specific individuals to chemical-induced liver injury, environmental factors, lifestyle choices and underlying pathological conditions also have important roles in the pathogenesis of chemical liver injury. Hepatic injury can be classified into hepatocellular, cholestatic and mixed depending on the pathophysiological mechanisms of the insult and the vital liver function affected. These different classes also show different patterns of abnormalities on liver function tests.[6]

The WHO emphasizes the need for rigorous scientific research to evaluate the safety, efficacy, and quality of traditional medicines, including PHFs. A major hindrance in the incorporation of herbal medicine in modern medical practice is the lack of scientific and clinical data that proves the safety and effectiveness of herbal medicine. It is also important to establish the active components of the various herbal extracts used.[7]

Bukumol® PHF consists of two (2) different medicinal plants including *Ananas comosus* and *Carica papaya.* It is used primarily to treat typhoid in Nigeria. The pharmacological activities include antioxidant activities, anti-inflammatory activities, boost immunity, aid digestion *et al.* Due to multiplicity of usage and tendency for prolonged use of this product, a study is required to investigate the possible acute and sub -acute toxicity effects on kidney and liver.[1]

This study was designed to evaluate the 30-day exposure of the product in Wistar rats by determining its liver and kidney function parameters as well as its histoarchitecture. It is hoped that the data and findings gathered during this study would provide a justification for

the consumption of these herbal preparations and protect public health against the various adverse effects that may arise from their consumption.

MATERIALS AND METHODS

Materials

Animal cages, weighing balance, crucibles, water bath, aspirator, feeding bowls, hand gloves, syringes (1ml, 2 ml, 5 ml) and needles, Ethylene diamine tetra acetic acid (EDTA) tubes, oral gavage, beakers, test tubes, cotton wool, clean glass tubes and test tube racks, dissecting board, dissecting kit, Deep Freezer (Haler Thermocool), Disposable Micropipette tips, Micropipette [Huawei] (0.5 – 50microlitre and 100 – 1000microlitre), Multi-channel micropipette [Huawei] and Automated hematological analyzer.

Chemical reagents

Distilled Water, Diethyl ether, Normal Saline Solution, EDTA, Polyherbal Formulation.

Polyherbal mixture

Bukumol® polyherbal formulation (composition *C. papaya*, *A. comosus* and honey) 75cl bottles were obtained from IBK Herbal Healthcare Limited, located at No. 66, Abadina Quarters, University of Ibadan, Ibadan, Nigeria in June 2023. Bukumol®is a brown colour homogenous liquid.

Preparation of stock solution and calculation of dose

Two (2) crucibles were washed using distilled water and evaporated to dryness. 5ml of the Polyherbal Formulation was measured using a measuring cylinder, the 5ml of the formulation was poured to each of the crucibles. They were then placed in an oven with a temperature of 124°F until it was evaporated to dryness. The weight of the residue was gotten by subtracting the crucible containing the residue from the weight of the crucible which was measured prior to the evaporation to dryness. The average of the residue was gotten and multiplied by 100 mg/ml to get the stock concentration.

Calculation of dose

The dose to be administered to the treatment group was calculated using the formula:

Dose = weight (kg) x dose (mg/ml) / stock concentration (mg/ml)**.**

ACKNOWLEGMENT

The authors acknowledged all individuals and organizations for their kind support.

Ethical approval

Clearance was obtained from the Bingham University Ethics Review Committee and the protocols followed the Guidelines for Laboratory Procedures laid down by the Bingham University Ethics Review Committee.

Experimental animals

The animals (Wistar albino rats of both genders; 150-220g) were obtained from National Veterinary Research Institute, Vom, Jos, Plateau State, Nigeria and kept at the Department of Pharmacology and Toxicology Animal House of the Faculty of Pharmaceutical Sciences, Bingham University, Karu, Nigeria. Before initiating these experiments, the animals were maintained under standard environmental conditions and fed with standard Pfizer-branded rodent feed (Livestock Feed, Nigeria Ltd) and given access to water ad libitum. All animals were kept at room temperature in cross-ventilated rooms, without illumination at night to achieve the 12h light/ 12h dark period. The animals were acclimatized to the laboratory condition for 14days prior to the experiment, during which they were given access to food and water ad libitum.

Acute toxicity study

The single-dose acute oral toxicity study was evaluated following the recommendations by OECD Guidelines (425).[8] Acute toxicity studies were carried out on Wistar rats weighing 160-165g each one, using a single dose, which was administered orally. Nine (9) wistar rats were randomly grouped into 3 groups, were designed for the study of acute toxicity via the oral route. Each group, 3 wistar rats, received respectively, a single oral dose of 2000 and 5000mg/kg body weight of PHF. After being fasted for 24 hours, 2000mg/kg of the formulation was administered to the first group and observed for any signs of toxicity for 24hours. The following day, the second and third group received 5000mg/kg of the PHF and observed for any gross changes for 14days according to OECD 425 guideline 2008. The general behavior of rats and signs of toxicity were observed continuously for 1h after the oral treatment and then intermittently for 4h and thereafter over a period of 24h. The rats were further observed once a day up to 14 days for behavioral changes and signs of toxicity and/or mortality.

Sub-acute toxicity study

For the study of subacute toxicity, four experimental groups were set up. A total of 20 adult Wistar rats of both genders were weighed and randomly allotted to 4 groups of 5 animals

each and treated. The doses (1000, 1500, 2000 mg/kg) were administered daily using oral gavage for 28days of the test period (10). The doses for the subacute toxicity test were established using the LD_{50} (20, 30 and 40%). Rats in different groups were observed closely for any behavioral changes, feeding, and drinking habits, as well as body weight and general morphological changes. At the end of the study period, all animals were fasted overnight, the animals were euthanized under diethyl ether (Sigma, USA) anesthesia and sacrificed. Blood samples were collected from the abdominal aorta under anesthesia with ether in two types of tubes: one with EDTA and the other without additives. The anticoagulated blood (tube with EDTA) was analyzed immediately for hematological parameters. The second tube was centrifuged at 3000 rpm at 4° C for 10 min to obtain the serum for biochemical analysis. Additionally, liver and kidneys were dissected and wet sections from these organs were examined histopathologically.[9]

Haematological parameters

The hematological parameters white blood cells (WBC), red blood cell (RBC), haemoglobin (HGB), pack cell volume (PCV), platelet (PLT), neutrophil (N), lymphocyte (L), macrophage (M), esinoph6ils (E), mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH) and mean corpuscular hemoglobin concentration (MCHC) were evaluated.[10]

Biochemical parameters

Serum biochemical parameters including total bilirubin (T-BIL), direct bilirubin (D-BIL), alkaline phosphatase (ALP), glutamic pyruvic transaminase (GPT), glutamic-oxaloacetic transaminase (GOT), urea, total protein (TP) and albumin (ALB) were also analysed.[10]

Statistical analysis

Statistical analysis was performed using IBM SPSS Statistics Version 20 software. Data obtained were expressed as Mean ± SD. The student's-test was conducted to determine significant differences and p values for significant difference between the mean of control and test groups was considered at $p \leq 0.05$.

RESULT

Table 1: Acute Toxic Effect of Bukumol® Polyherbal Formulation (Phf) in Rats.

OBSERVATION	CONTROL	TEST 1	TEST 2
	Normal Saline	2000 mg/kg	5000 mg/kg
Number of Death	0/3	0/3	0/3

No mortality was recorded in both control and experimental groups; the acute toxicity was found to be greater than 5000 mg/kg as indicated in table 1 above.

Table 2: Sub-Acute Toxic Effect of Bukumol® Polyherbal Formulation (Phf) In Rats.

S/N	GROUP	TREATMENT	DOSE
1.	CONTROL	Normal Saline	2ml
2.	20% LD50	PHF	1000 mg/kg
3.	30% LD50	PHF	1500 mg/kg
4.	40% LD50	PHF	2000 mg/kg

Table 2 indicates the sub-acute toxic effects of Bukumol ® polyherbal formulation (PHF). PHF at the outlined subacute doses (20% LD_{50} – 40% LD_{50}) (1000mg/kg- 2000mg/kg) did not produce any obvious symptoms of toxicity or mortality among the experimental groups.

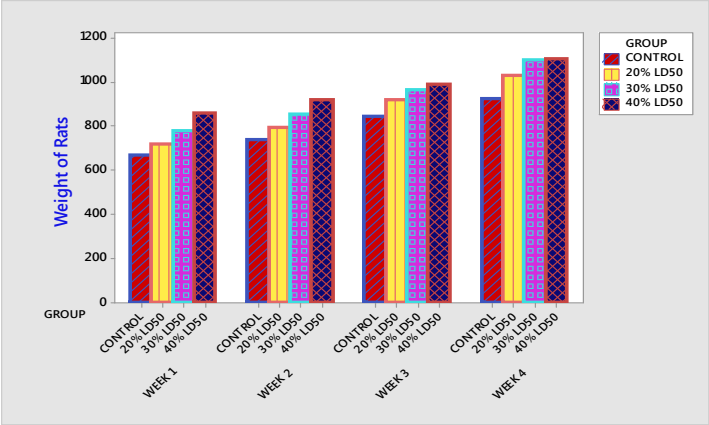

Figure 1: Effect of Bukumol® Polyherbal Formulation (Phf) on Body Weights of Rats.

There was no significant difference in the weight of experimental groups compared to the control group. All animals exhibited normal changes in weight without remarkable increase in body weight at the sub-acute doses of 1000, 1500 and 2000 mg/kg of the PHF comparable to the control group as indicated in Figure 1.

Table 3: Effect of Bukumol® Polyherbal Formulation (Phf) on Haematological Parameters In Rats.

Parameter	Control	20% LD50 1000mg/kg	30% LD50 1500mg/kg	40% LD50 2000mg/kg
WBC	10.20 ± 4.06	11.63 ± 2.70	11.53 ± 4.38	20.30 ± 5.96
RBC	7.83 ± 0.46	8.00 ± 1.04	8.37 ± 0.84	8.33 ± 1.03
HGB	16.30 ± 1.57	15.50 ± 1.68	16.17 ± 1.45	16.57 ± 1.62
PCV	46.00 ± 5.00	46.00 ± 4.58	49.33 ± 5.51	49.33 ± 3.06
PLT	692.30 ± 118.40	568.70 ± 56.10	653.70 ± 159.00	789.00 ± 150.20
N	14.00 ± 4.00	14.33 ± 4.51	15.00 ± 5.29	11.67 ± 5.03

L	77.67 ± 6.43	79.67 ± 4.04	78.00 ± 5.29	81.00 ± 2.65
M	6.00 ± 2.65	2.67 ± 0.58	5.33 ± 0.58	5.00 ± 1.73
E	2.33 ± 0.58	3.33 ± 1.16	1.67 ± 0.58	2.33 ± 1.53
MCV	58.33 ± 3.21	57.67 ± 2.08	59.00 ± 1.00	62.00 ± 5.57
MCH	20.73 ± 0.87	19.43 ± 0.81	19.40 ± 0.35	20.73 ± 2.11
MCHC	35.50 ± 0.66	33.63 ± 1.69	33.53 ± 1.48	32.97 ± 0.99*

Haematological Parameters: ***WBC*** *(White Blood Cell);* ***RBC*** *(Red Blood Cell);* ***HGB*** *(Hemoglobin);* ***PCV*** *(Pack Cell Volume);* ***PLT*** *(Platelet);* ***N*** *(Neutrophil);* ***L*** *(Lymphocyte);* ***M*** *(Monocyte);* ***E*** *(Eosinophil);* ***MCV*** *(Mean Corpuscular Volume);* ***MCH*** *(Mean Cell Hemoglobin);* ***MCHC*** *(Mean Corpuscular Hemoglobin Concentration).*

The data represents the Mean ± SEM for each group of animals, n = 6 (number of animals per group).

Table 3 illustrates the effect of PHF on the haematological parameters of rats after oral administration of PHF. The analysis of these hematological parameters, which included white blood cell count (WBC), red blood cell count (RBC), hemoglobin (HGB), packed call volume (PCV), platelet count (PLT), neutrophils (N), lymphocytes (L), monocytes (M), eosinophils (E), mean corpuscular volume (MCV), and mean corpuscular hemoglobin (MCHC) did not show any significant difference between control and experimental groups, although there was an observed relative decrease in MCHC ($p< 0.05$) among the treated groups at the dose of 2000 mg/kg of the PHF.

Table 4: Effect of Bukumol® Polyherbal Formulation (Phf) on The Biochemical Parameters of Rats.

Parameter	Control	20% LD50 1000mg/kg	30%LD50 1500mg/kg	40%LD50 2000mg/kg
T.BIL (µmol/L)	11.77 ± 2.27	10.23 ± 0.91	11.30 ± 2.51	13.93 ± 0.35
D.BIL (µmol/L)	3.33 ± 0.55	2.73 ± 0.55	3.27 ± 0.61	3.43 ± 1.00
ALP (UI)	129.20 ± 19.7	112.77 ± 11.48	114.07 ± 15.70	115.60 ± 23.00
GPT (UI)	171.20 ± 66.00	98.23 ± 8.64	103.10 ± 20.80	104.20 ± 26.70
GOT (UI)	79.60 ± 1.25	91.93 ± 2.15	95.17 ± 5.66	96.87 ± 12.06*
UREA (mmol/L)	11.27 ± 1.29	10.30 ± 0.78	11.97 ± 0.95	11.80 ± 1.31
CREATININE (mmol/L)	84.77 ± 13.18	88.10 ± 7.54	76.07 ± 4.43	101.37 ± 10.42
T.P (mmol/L)	58.70 ± 2.35	63.23 ± 6.16	67.93 ± 2.35*	65.13 ± 3.01
ALB (mmol/L)	33.07 ± 1.91	33.73 ± 2.36	34.17 ± 1.05	34.07 ± 2.15

Biochemical parameters: ***T.BIL*** ***(****Total Bilirubin);* ***D.BIL*** ***(****Direct Bilirubin);* ***ALP*** *(Alkaline Phosphatase);* ***GOT*** *(Glutamic-Oxaloacetic Transaminase);* ***GPT*** *(Glutamic Pyruvic Transaminase);* ***T.P*** *(Total Protein);* ***ALB*** *(Albumin).*

The data represents the Mean ± SD for each group of animals, n = 6 (number of animals per group).

Total Bilirubin (T.BIL), Direct Bilirubin (D.BIL), Alkaline Phosphatase (ALP), Glutamic Pyruvic Transaminase (GPT), Glutamic-Oxaloacetic Transaminase (GOT), urea, creatinine, Total protein (T.P), and albumin (ALB) of PHF after 28days oral administration are indicated in table 4 above. The animals treated with 1500 mg/kg of the PHF developed a significant increase in T.P. ($P < 0.05$) while those treated with 2000 mg/kg of the PHF showed a significant increase of GOT ($P < 0.05$).

HISTOPATOLOGICAL STUDY: EFFECT OF BUKUMOL® POLYHERBAL FORMULATION (PHF) ON SOME VISCERAL ORGANS

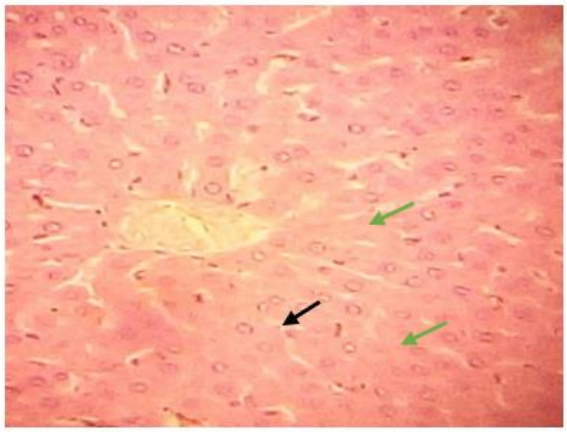

FIGURE 2: ***Liver section of the normal control animals showed normal central vein (black arrow), and radiating chords of hepatocytes (green arrow). (H&E. X 400).***

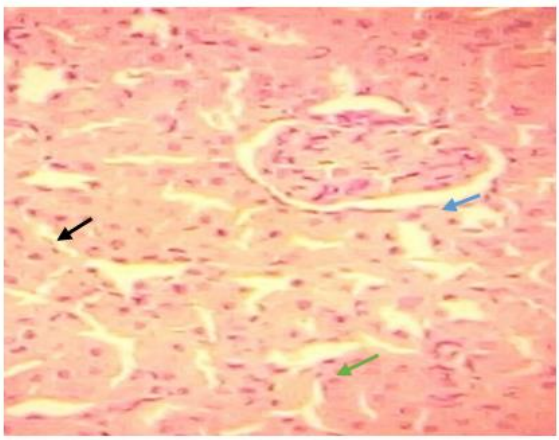

FIGURE 3: ***Kidney section of the normal control animals showed normal renal tubule (black arrow), podocyte (green arrow), and glomeruli tuft of capillaries (blue arrow). (H&E. X 400).***

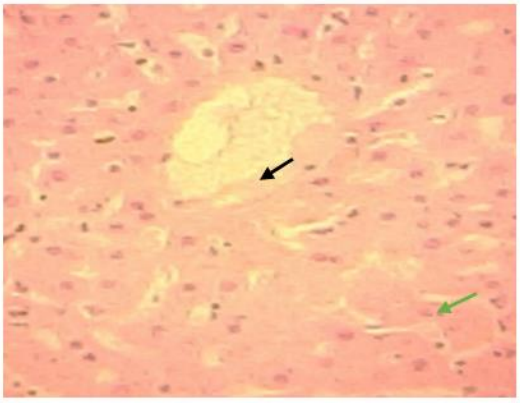

FIGURE 4: ***Liver section of animals administered with 20% extract showed normal central vein (black arrow), and radiating chords of hepatocytes (green arrow). (H&E. X 400).***

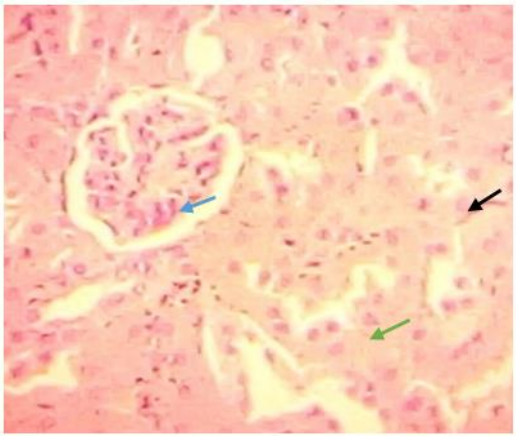

FIGURE 5: ***Kidney section of animals administered with 20% extract showed regular renal tubule (black arrow), podocyte (green arrow), and glomeruli tuft of capillaries (blue arrow). (H&E. X 400).***

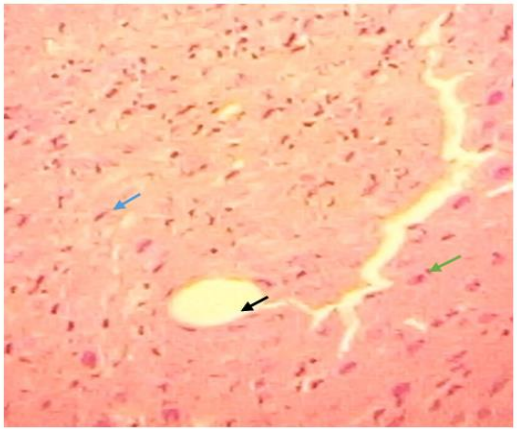

FIGURE 1: ***Liver section of animals administered with 30% extract showed central vein (black arrow), kupffer cell (blue arrow), and radiating chords of hepatocytes (green arrow). (H&E. X 400).***

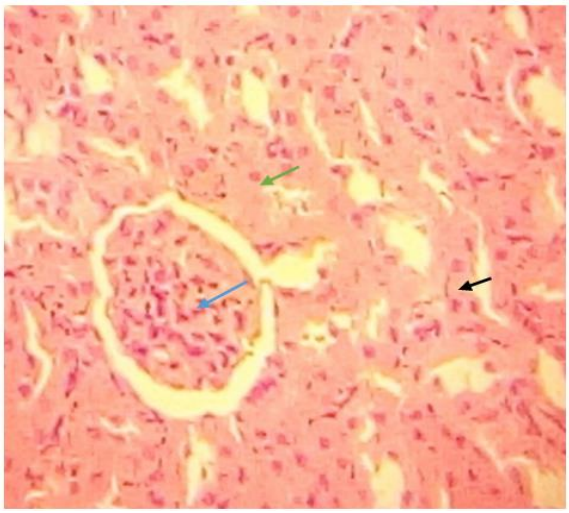

FIGURE 7: ***Kidney section of animals administered with 30% extract showed apparently healthy renal tubule (black arrow), podocyte (green arrow), and glomeruli tuft of capillaries (blue arrow). (H&E. X 400).***

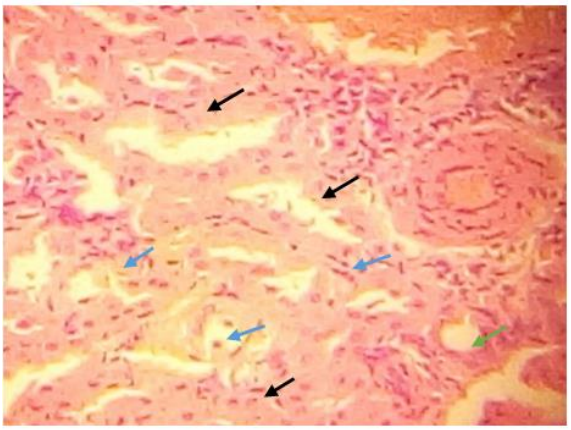

FIGURE 8: ***Kidney section of animals administered with 40% extract induced interstitial hemorrhage (black arrow) and inflammatory cells (blue arrow). (H&E. X 400).***

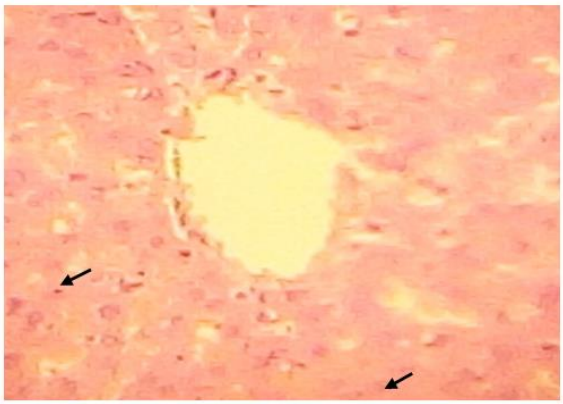

FIGURE 2: ***Liver section of animals administered with 40% extract induced degenerating hepatocytes (black arrow). (H&E. X 400).***

DISCUSSION

Polyherbal formulations are herbal medicines that contain a combination of two or more herbs in a single formulation for increased therapeutic effectiveness and decreased toxicity of individual herbs.[11] These formulations have been used in traditional medicine for centuries and are becoming increasingly popular in modern medicine due to their potential therapeutic benefits and perceived low toxicity.

In the acute toxicity study, experimental animals were exposed to relatively higher doses of Bukumol® PHF (up to 5000 mg/kg), there were no recorded signs of toxicity or adverse effects, and there was no reported mortality among the study animals. Acute toxicity tests are used to determine the lethal dose/concentration of a substance that causes death in 50% of the test population (LD/LC50) during short-term exposure.[4] According to the Organization for Economic Cooperation and Development (OECD) Guidance Document for Acute Oral Toxicity Testing (OECD, 2001)[9], compounds with LD50 values greater than 5000 mg/kg are generally considered to be relatively safe since values above this are non-classified. Thus, Bukumol® PHF can be considered nontoxic at acute administration since the extracts were well tolerated and there was no observed mortality.

Similarly, during subacute exposure, there were no observed clinical signs of local or systemic toxic effects, and there was no death reported from both control and study groups. All animals sustained their levels of activity and responded appropriately to stimuli. Traditionally, the alteration (increase or decrease) in the body weight of an animal is an indicator of the adverse effect of drugs and chemicals. This parameter has generally been employed to assess physiological impact of drugs and PHF.[12] In this study, there was no significant difference in the observed weight between the control and experimental animals. Thus, the extract has no deleterious effect on appetite as well as on growth of the animals.

In the hematological study, only the mean corpuscular hemoglobin concentration (MCHC) showed a significant difference as it was observed to decrease ($p < 0.05$) in the experimental group compared to the control group, and this decrease was seen in the study group that received the highest dose of PHF (2000 mg/kg). MCHC is a red blood cell (RBC) indicator, which is a combination of both mean corpuscular hemoglobin (MCH) and mean corpuscular hemoglobin (MCV). MCHC is used for classifying and assessing different types of anemia and also used for measuring the average hemoglobin concentration in each RBC.[12] Low MCHC indicates Iron deficiency states with or without anemia. The decrease in MCHC

observed in the experimental animals can be attributed to blood loss as interstitial hemorrhage was noticed in the kidneys of some study animals during histopathological assessment. This suggests that continual long-term use of Bukumol® PHF can lead to internal hemorrhages that can precipitate a decrease in MCHC value from the resultant iron deficiency leading to anemia.

The liver and kidneys both play important synthetic and excretory functions that are vital for maintenance of the body's homeostasis. This vital role also exposes them to both exogenous and internal toxins as they are at the frontline of waste management. While the liver monitors the digestive tract for ingested toxins, the kidneys scan the blood for circulating toxins. So, the integrity of both organs serve as an indirect indicator of body's exposure and response to toxins.[13] In this study, assay of biochemical parameters for both organs were performed to obtain insights into possible toxic effects of the PHF under study. Markers of kidney function assayed (Urea and Creatinine) revealed normal functioning of the kidney at the end of the observatory period (28 days after administration of Bukumol® PHF), and with non-significant a dose dependent increase in Urea levels in the experimental groups compared to the controls. Although the relationship between the administered dose and assayed creatinine levels in the study group was not as certain as that of urea, the highest level of creatinine was seen in the 2000mg/kg dose group (which was the highest dose administered to the experimental group).

All the parameters assayed for the liver function also showed a dose-dependent increase across the experimental groups. The assay was done at 28 days after PHF administration. For instance, Glutamic Oxaloacetic Transaminase (GOT) levels were significantly increased ($P < 0.05$) in the study group that received a PHF dose of 2000 mg/kg compared to the control group. GOT, also known as Aspartate Aminotransferase, plays a key role as an enzyme in amination-deamination reactions of the urea cycle. Although it can be found in various tissues of the body, its highest concentration is in the liver and heart. Since GOT is not restricted to the liver, the observed increase in its activity in this study can reflect either hepatic and/or extra-hepatic toxicity. However, it is important to note that this relative increase in GOT level observed fell within the normal range of values for the species[14], and there was no associated decline in measured cumulative metabolic function of the liver.

The excretory function of the liver, our assay also explored its synthetic role. The liver produces most of the proteins found in the extracellular fluid with albumin being one of its

major products. So, blood protein levels tend to drop during prolonged periods of liver pathology.[5] In our study, although total protein (TP) level was elevated across the experimental group compared to the control, the increase wasn't dose dependent as the highest value was recorded in the 1500 mg/kg dose group. One probable cause of this increase in TP level might be from extracellular production by cells of the immune system in response to PHF-induced inflammation of internal organs. This is in keeping with findings of inflammatory infiltrates in the liver and kidney samples of the experimental groups.[5]

Histopathological examinations of the liver and kidneys of both control and the study groups showed increased numbers of Kupffer cells in the liver and podocytes in the kidneys of the study group who received the PHF at a dose of 1500mg. Kupffer cells are the resident liver macrophages and play an essential role in maintaining liver function. Under physiological conditions, they are the first innate immune cells and protect the liver from bacterial infections.[14] This might suggest that the PHF is hepatoprotective at dose of 1500 mg/kg. In a similar study, podocytes play an essential role in preventing plasma proteins (albumin, globulin, fibrinogen *et al*) from escaping into the glomerular ultrafiltrate.[14] Notions of beneficial effects of the PHF on the liver and kidneys are however dispelled by the concomitant findings of presence of inflammatory infiltrates on the kidney and pockets of degenerating hepatocytes in samples from the experimental group administered 2000 mg/kg of PHF.

CONCLUSION

The acute toxicity study indicated the oral LD_{50} value of the PHF was greater than 5000 mg/kg making it relatively safe. While for the subacute toxicity, at the end of the 28 days' observatory period, it was showed that the PHF was well tolerated by the rodents at lower doses but demonstrated toxicity at higher doses (2000 mg/kg) to the liver and kidney as determined by hematological, serum biochemical, and histological analyses.

These results provide valuable preliminary data on the toxic profile of the PHF. Therefore, further assessments (such as studies of genotoxicity, sub-chronic toxicity, reproductive toxicity, and compounds toxicity) are required to proceed to clinical studies of this plant formulation.

ACKNOWLEGMENT

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CONFLICT OF INTEREST

No conflict of interest.

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