ARTICLE

HEPATIC AND HEAMATOLOGICAL EFFECTS OF SMART HERBAL PURIFIER: A NIGERIAN POLY HERBAL REMEDY IN MALE SPRAGUE-DAWLEY RATS.

Author

Zelinjo Nkeiruka Igweze [1], Anthonet Ndidiamaka Ezejiofor[2], Orish Ebere Orisakwe [2], and Atuyobedia Obianime [3]

Affiliation

[1]Department of Pharmacology and Toxicology. Madonna University, Elele, Rivers State, Nigeria.
[2]Toxicology Unit, Faculty of Pharmacy, University of Port-Harcourt, Rivers State, Nigeria
[3]Department of Pharmacology. College of Health Sciences University of Port-Harcourt, Rivers State, Nigeria.

Correspondence

Anthonet Ndidiamaka Ezejiofor,
Email: ndidiezejiofor@yahoo.com
Phone no: +2347065384114.

Abstract

There is a resurgence of interest in the use of herbal supplements in Nigeria arising from the unsubstantiated belief of the safety of the supplements. The hepatotoxicity and heamatological profile of Smart Herbal Purifier (SHP) a formulation of herbal components in male Sprague-Dawley (SD) rats was investigated in the present study. One hundred and twenty SD rats were divided into four groups of thirty rats each. Group 1 (control) and was given only feed and water *ad libitum*. Groups 2, 3 and 4 received SHP at 48, 240 and 480 mg/kg orally for 90 days repeated dose toxicity study and 30 days post treatment in which SHP was withdrawn from the animals. Body and liver weights, fluid and feed intake, histopathology of liver, biochemical and heamatological parameters were measured. The RBC and WBC increased in the high treatment groups. There was a significant increase in aspartate aminotransferase (AST) in the high treatment group. Alanine aminotransferase (ALT) increased in a dose dependent manner but was not significant. The liver showed mild periportal lympocytic infiltration. SHP may not be as safe as it is claimed and we recommend more in-depth risk assessment.

Key words: herbal supplements, hepatotoxicity, risk assessments, public health.

Introduction

During the past decades, the general population globally has developed an unparalleled interest in self-medication with natural therapies especially herbal medicine. (Mackowiak, *et al.*, 2001; Blumenthal, *et al.*, 1998,) These herbal supplements are taken routinely without medical consultation or disclosure. There are bold and popular perceptions that, because the products are natural, they are safe and that they have been used for centuries without harmful effects. The long history of use for herbal supplements is not a guarantee of safety, particularly with long-term use, at high doses, or with co-medications and/or co-morbidities (Elvin-Lewis, 2001).

Although scientific research had made major strides in isolation and identification of active components of herbal origin, however, the systematic assessment of the toxicity, standardization of components of the herbal drugs for public consumption is a major concern of public health dimension because "safe" no longer confers safety. Although several studies have been carried out on the safety and toxicity of herbal formulas, these studies are insignificant compared with herbal formulas available for public use (Ha, *et al*., 2011; Shin, *et al*., 2011; Hyekyung, *et al*.,2010; Li-Hua, *et al.*, 2011). Smart herbal purifier (SHP) is a registered polyherbal with the following declared herbs on the label: *Morinda lucida 30%, Cassia alata 30%, Nauclea blend 40%* commonly sold in Nigerian. Like most unregulated products, there is indiscriminate use of SHP without information on the safety. It is prescribed for various conditions such as low sperm count, weakness of the body, erectile dysfunction and in the language of the manufacturers as a 'general body tonic'. Among the most serious safety concerns for herbal supplements is the potential for liver injury. Many herbal products have been associated in case reports with rare but severe cases of liver failure (Estes, *et al*., 2003;Teschke, *et al*., 2003).The liver holds a unique position in the human body because of its gastrointestinal connections and varied functions. Liver receives large amount of nutrients and noxious compounds entering the body through the digestive tract and portal vein (Dioka, *et al*., 2002).

As a result of its continuous involvement, it is susceptible to toxic injuries caused by certain agents and hence any damage to hepatic cells will disturb body metabolism (Patel, *et al*., 1988). Liver toxicity from drugs, and herbal remedies is currently an increasingly relevant health issue. In developing countries, adaptation of traditional medicine as a complementary to orthodox medicine is fast advancing (Salahdeen & Yemitan, 2006), amidst paucity of proof of safety as

aforementioned (Mosihuzzaman & Iqbal Choudhary, 2008).

To add to the fund of knowledge in this area, the present study was undertaken to evaluate the effect of SHP on the liver of male Sprague – Dawley rats.

Materials and Methods

Source of SHP

SHP is a poly herbal formula and was obtained from the manufacturers. It is an encapsulated coarse powdered herbal mixture without the specification of the plant parts. Each 500mg capsule contained the composition *Morinda lucida* (30%), *Cassia alata* (30%), and *Nauclea blend* (40%). The manufacturers recommended dose is six capsules/day equivalents to 3.0g/day. Smart herbal purifier capsule was purchased in June 2012 from the manufacture's outlet in Port-Harcourt, Nigeria.

Chemical contents of SHP using GC/MS.

The SHP capsules used were analyzed for respective marker compounds by gas chromatography /mass spectrophotometer (GC/MS) method (Canini, *et al*., 2007).

Animal husbandry

Sprague Dawley rats weighing 140 – 180g were obtained from the Faculty of Veterinary Medicine, University of Nigeria Nsukka. Animals were housed singly under standard laboratory conditions (ambient room temperature, 12 hr high/dark cycle) and all had free access to standard rat chow (Top Feeds Premier Feeds Flour Mills Nig. Plc., Lagos State) and tap water. All animal experiments were conducted in accordance with internationally accepted practice for laboratory animal and approved by the Animal Ethics Committee of our University.

Sub chronic oral toxicity study.

Weight matched rats divided into four groups of 30 animals each were used. The test groups 2, 3 and 4 received 48, 240 and 480mg/kg of SHP incorporated into the 5g of feed while the control group 1 did not receive the SHP. To ensure that the test groups ingested the exact dose of SHP, the test groups received the SHP-5g feed mixture firstly prior to *ad libitum* feeding. The animals were observed for signs of toxicity and mortality throughout the experimental 90 days and the 30 day recovery period. The feed and fluid intakes were taken daily while body weight was taken weekly. Whole blood was obtained from ocular orbit vein from each of the rats. At the end of 30, 60, 90 days, 5 rats from each group (groups 2 – 4) were sacrificed under ether anaesthesia at the end of each time-line. In the 30 – day post treatment experiment i.e. after 90 days, SHP was withdrawn from the test groups 2 - 4, but had access to standard rat chow and water. At the end of the withdrawal study i.e. after 120 days, rats were also sacrificed under ether anaesthesia.

Haematological parameter procedures

The white blood cell (WBC), red blood cell (RBC), haemoglobin (HGB), mean cell volume (MCV), mean corpuscular haemoglobin (MCH), mean corpuscular haemoglobin concentration (MCHC), platelet (PLT), haematocrit HCT, and granulocytes GRAN were determined using a fully automated haematology analyzer. (Mindray Auto Hematology analyser BC 2300 (Mindray Diagnostic China).

Determination of biochemical parameters

For biochemical analysis, blood was collected in non – heparinized bottle, centrifuged at 3000g at 40ºC for 10 minutes. Serum was separated and alanine amino transferase (ALT), aspartate amino transferase (AST) and alkaline phosphatase (ALP) were measured using commercial Randox test kits

Histopathological Examination:

The liver were excised, weighed, and fixed in 10% neutral formalin for at least 24 h. They were processed in an automatic tissue processor, and embedded in paraffin wax. Thick sections of 5 µm were cut on a rotary microtome by serial sectioning until the entire thickness of the liver was sectioned. Staining was done by haematoxylin and eosin (H&E) staining method, and photomicrography carried out using a Leitz light microscope.

Statistical Analysis

The data were expressed as mean ± S.E.M. Data were evaluated using Mann Whitney (Graph pad Prism version 5 software). Groups were considered to be significantly different if $p \leq 0.05$.

Results

GC/MS profiling.

Analysis of the SHP extract by GC/MS 6.2.0.0:B30 software version revealed seven major peaks. The peaks in the GC/MS chromatogram are shown in Fig.1 Major compounds of the extract are: **(1)** 4-Benzolyn-2-amino acetaldehyde **(2)** 3,7-dimethylocta-1,6-dien-3-yl acetate**. (3)** 3-fluoro-p-anidine **(4)** Stigmast-4-en-3-one **(5)** 2-phenyl-1,4-benzopyrone **(6)** Beta-sitosterol **(7)** 9,10-anthracenedione

.

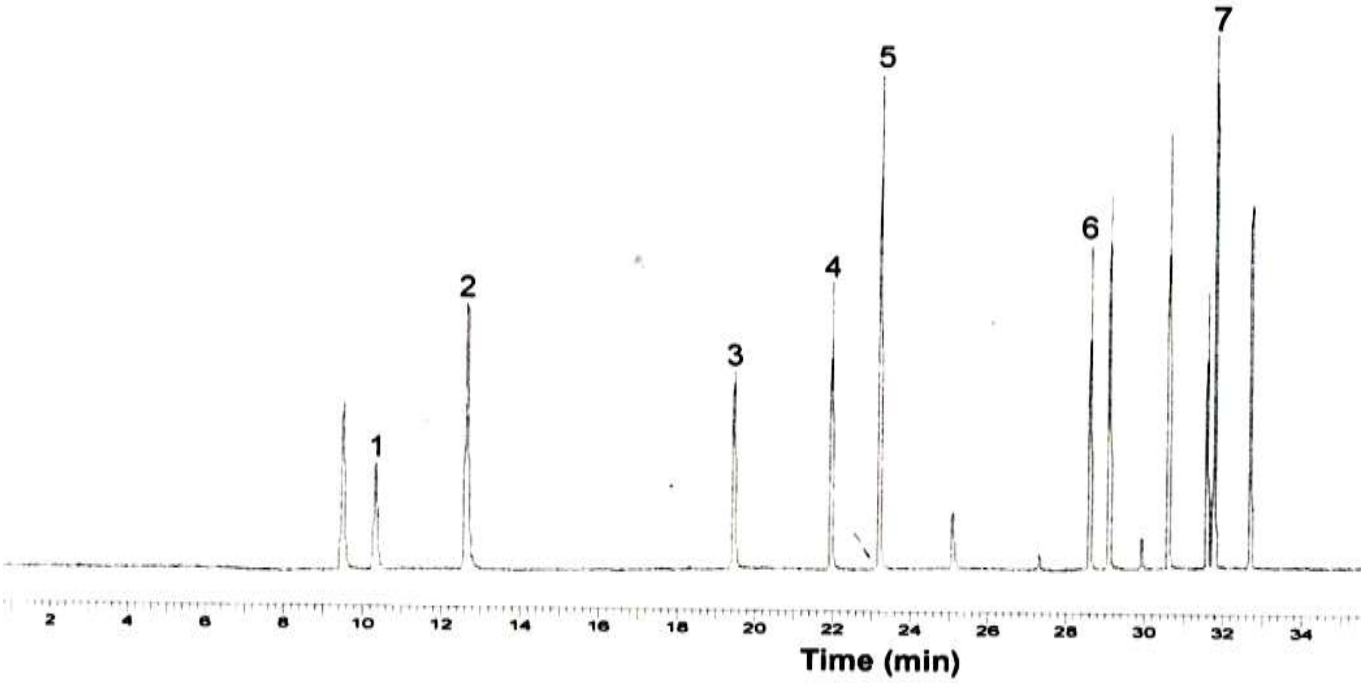

Fig 1. Gas chromatography of components of Smart herbal capsule

Acute toxicity Test: There were no deaths and no signs of toxicity in all the groups.

Effect on the Body weight, fluid and feed intake

The effect of SHP on the body weight, fluid and feed intake, mean body weight, percentage weight gain and feeding efficiency for animals at 48, 240, 480 mg/kg/day are shown in Table 1. The Sprague Dawley rats in the control had lower mean body weights compared to other treatment groups but the percentage weight gain of the control and the feeding efficiency was higher in the control than in all the treatment groups. There was no significant variation in the fluid intake between the control and 48 and 240 mg/kg groups but there was a significant difference between the control and the 480 mg/kg treatment group ($p<0.05$) from the 30th day.

The absolute and relative weights of the liver are shown in Table 1. The absolute weight of the liver in the 480 mg/kg SHP treated group) was significantly increased when compared with the control controls ($p < 0.05$). There was no significant change in the relative weight of the liver in all the SHP treated groups when compared with the control.

Table I: Effect of SHP on body weight, liver weight (absolute and relative), feed and fluid intake, feed efficiency, mean weight gain, mean percentage weight gain

PARAMETERS	TREATMENT			
	CONTROL	48mg/kg	240mg/kg	480mg/kg
INITIAL WEIGHT (g)	129±2.88	135±1.15	153±0.98	177±1.48
WEIGHT AT DAY 90(g)	274±3.35	260±2.63	282±2.31	285±3.05
WEIGHT GAIN (g)	145	125	129	108
MEAN % WT. GAIN	52	48	45	37
ABSOLUTE LIVER WT (g)	7.56±0.53	7.96±0.41	7.95±0.31	9.51±0.04
RELATIVE.LIVER WT	3.04±0.01	3.20±0.20	3.08±0.23	3.38±0.07
FLUID INTAKE (ml)	25.61±0.84	26.27±1.02	25.01±0.71	31.96±2.72
FEED INTAKE (g)	895.2±110.8	1219±89.01	1420±89.39	1334±104**
FEED EFFICIENCY (%)	19.91±4.3	9.41±0.60	8.20±0.55	10.88±1.20

Values are mean± S.E.M. n= 30 rats.
**statistically significant compared to control ($p<0.01$)

Effect of SHP on Heamatological Parameters

The administration of SHP did not significantly affect the RBC, HCT, MCV, MCH, MCHC, PCT and LYMPH (Table 2). However the RBC and WBC increased from 5.94±0.08 (control) to 6.31 ± 0.44 ($\times 10^6$ mm^{-3}) in 240mg/kg SHP and 11.22±6.57 to 13.77 ± 6.58 ($\times 10^9$/L) in 480 mg/kg SHP respectively.

Table II: Effect of SHP on the hematological parameters of male rats

		Treatment (SHP mg/kg)		
Parameters	Control	**48**	**240**	**480**
RBC	5.94±0.08	6.21±0.14	6.31±0.44	6.26±0.29
Hb	120.1±5.31	125.9±2.88	125.5±4.45	127.3±7.68
HCT	33.75±2.41	33.35±0.89	35.03±1.30	35.67±4.10
MCV	56.74±3.34	56.45±2.67	55.97±2.91	56.52±3.91
MCH	20.15±0.60	20.26±0.52	20.01±0.91	20.24±0.34
MCHC	357.2±11.0	360.7±9.0	359.0±3.31	361.9±20.28
PLT	423.3±40.24	491.9±35	461±45.09	492.7±48.72
PCT	0.42±0.03	0.49±0.00	0.45±0.04	0.48±0.04
WBC	11.22±6.57	9.51±4.18	10.49±4.46	13.77±6.58
LYMPH	5.77±3.15	5.05±2.66	5.60±2.52	6.75±2.80

RBC=Red blood cell (×106 mm−3), Hb=Hemoglobin concentration (g/dl), HCT=Hematocrit (%), MCV=Mean corpuscular volume (FL), MCH=Mean corpuscular hemoglobin (pg), MCHC=Mean corpuscular hemoglobin concentration (g/dl), PLT= Platelets (×109/L), PCT= Plateletcrit(Retics)(%), WBC=White blood cell (×109/L), LYMPH= Lymphocyte (%). Values are mean± S.E.M. n= 30 rats.

Effect of SHP on Biochemical parameters

The effect of sub chronic administration of Smart herbal capsule on biochemical parameters is presented in Table 3. A 90- day ingestion of SHP caused increase in serum ALT but the changes were not significant, however the AST increase was significant in the 480 mg/kg SHP group when compared with the control($p < 0.05$). There were no significant changes in ALP. At the end of the 30 day withdrawal period, there was a slight change in the values in the blood chemistry between the treated and control groups.

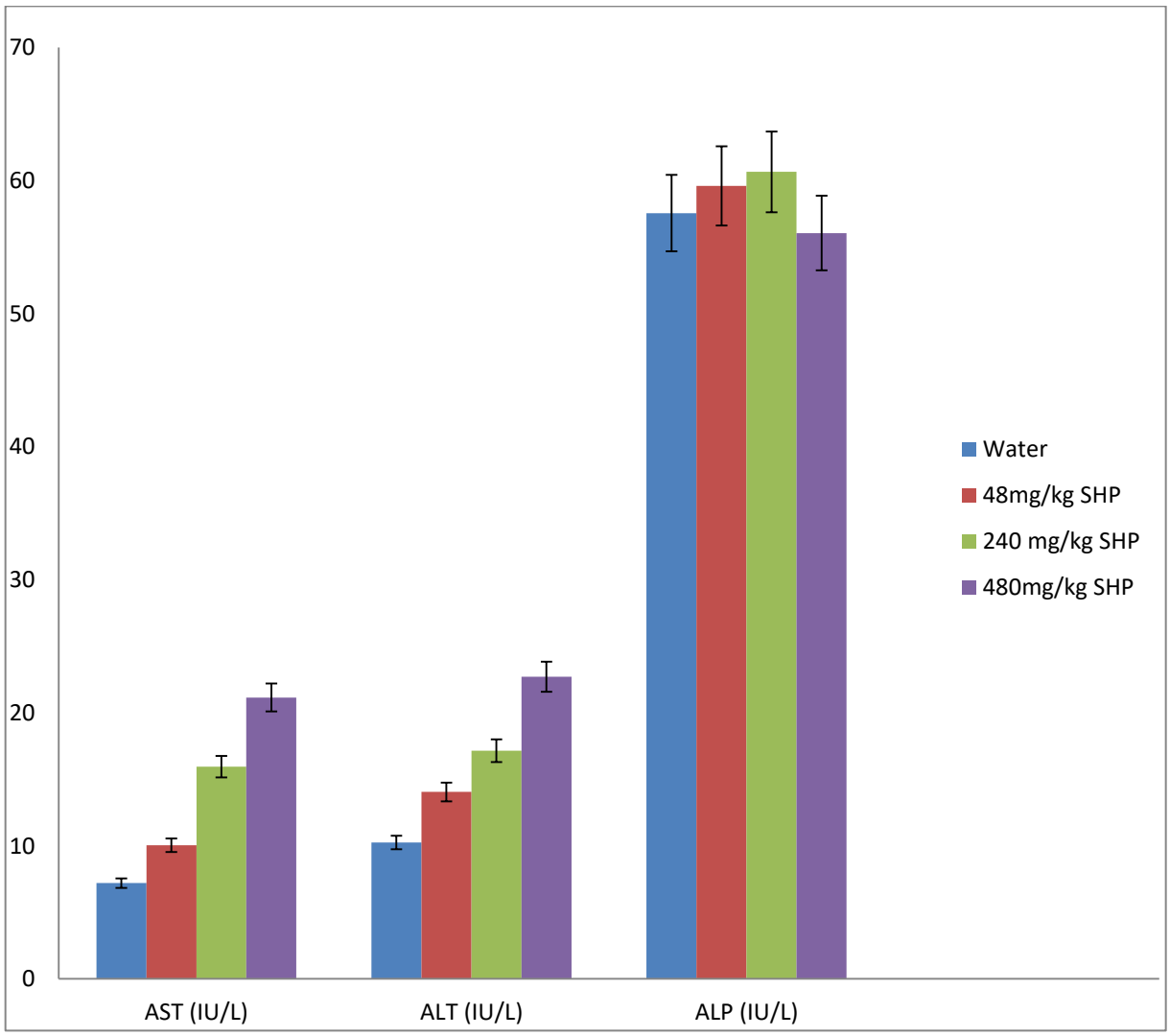

Figure 2: Effect of SHP on liver enzymes

Histopatghological result:

The liver histology results showed no destruction to the hepatocyte and the architecture except mild lymphocytic infiltration around the periportal region of the liver of the 480 mg/kg SHP group (**Figure 3)**.

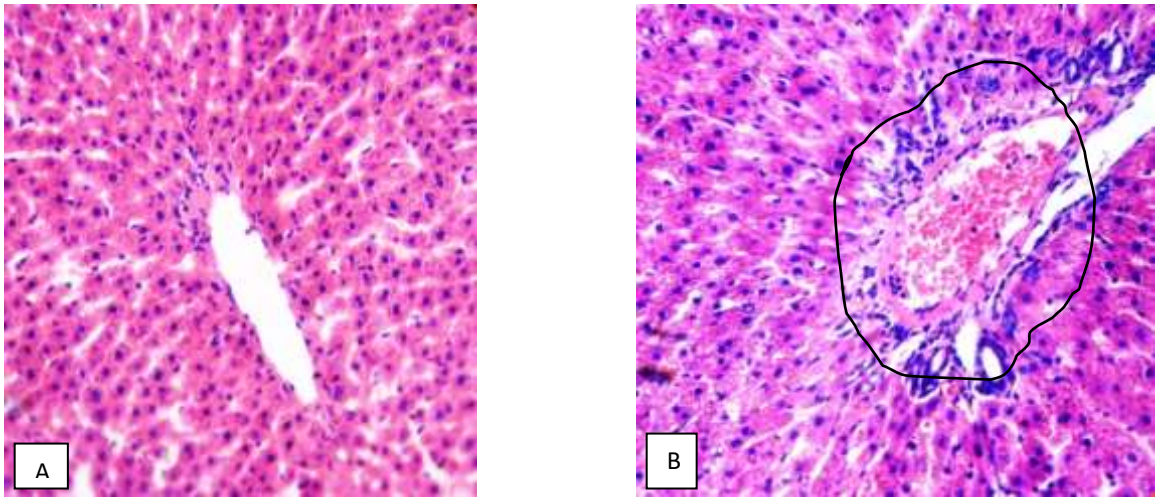

Figure 3: Photomicrograph of animal liver section from animals treated with SHP and control for 90 days. **A**: showing unremarkable hepatocytes around a central venule **B**: High dose group (480mg/kg) representative animal showing mild periportal lymphocytic infiltration (encircled area) **X400** H&E.

Discussion

The evaluation of the safety of drugs and plant products is presently carried out in animals. In risk assessments there is a better correlation between rats and humans, whereas it is less predictive when mice is used (Olson, *et al.*, 2000).Numerous studies have therefore investigated the chronic or subchronic effects in rats, including the extrapolated usable doses in humans (Hyekyung, *et al*., 2010; Li-Hua, *et al.*, 2011; Latilou, *et al.*, 2008).

Also the 90-day subchronic test has been used in safety assessment studies as a reliable toxicity time protocol (Oloyede, *et al*., 2012; Allan, *et al*., 2012).In traditional Nigerian medicine as it is found in others, be it Ayuverda or Chinese traditional medicine, multi-component and principally plant derivatives are used for disease prevention, symptom amelioration and treatment without prescription and duration of therapy. The latter has led to chronic ingestion of these herbals. Due to the indiscriminate use of herbal drugs globally, especially in developing nations like Nigeria with seldom proof of efficacy, the safety profile of these herbal products become sine qua non for public health reasons (Firenzuoli & Gori, 2007; Lynch & Berry, 2007; Jordan, *et al*., 2010).

In this study, we examined the hematological and hepatic effects of SHP, a finished product composed of three herbs, in a 90-day repeated dose study. Pattern and extent of increase in body weight is one of the core indicators of generalized toxicity (Heywood, 1983).

Agbor, *et al* (2012), concluded in a study that *Morinda lucida*, one of the components of SHP decreased the body weight of rat in a dose dependent manner. Organ weight is another measurement

that is an indicator of toxicity. Kluwe, (1981) and Simmoni, *et al* (1995) stated that change in organ weight either absolute or relative or both with a change in one of the biochemical parameters is an indicator of organ toxicity and a pointer to the toxicity potential of the substance in question. SHP administration increased the absolute weight of the liver in all the groups but was significant in the highest dose group ($p<0.001$) when compared with the control. There was no significant difference in the relative weight of the control compared with the treatment groups.

Indeed the trasaminases (AST and ALT) are well-known liver enzymes used as indicators of liver function (Hilaly, *et al*., 2004) and a biomarker that predicts possible toxicological assault on the liver. Generally, perturbation of parenchymal cells of the liver by xenobiotics or drugs results in elevation of both transaminases (AST and ALT) in the blood (Wolf, *et al*., 1972). AST has both mitochondrial and cytoplasmic origin and any elevation could be taken as a first sign of cell damage that leads to the appearance of these enzymes in the serum (Mdhluli, 2003). Therefore the increases observed in AST and ALT activities in this study suggest that the chronic administration of SHP did interfere with the integrity of the parenchymal cell. However the increase was only significant in the high dose group. One of the major enzymes involved in hepatobiliary evaluation is alanine aminophosphatase (ALP). ALP levels above normal are mainly associated with blocked bile duct although this enzyme is also concentrated in the kidney and bone. The liver, via bile excretes ALP, whenever liver function is compromised the excretion of bile by the hepatocytes diminishes and this results in the increased levels in the serum ALP (Rajesh & Latha ,2004).There was no increase in the ALP values, suggesting that SHP did not obstruct bile excretion nor caused congestion and therefore SHP has no tendency to cause cholestasis.

In risk evaluation, analysis of blood parameters is very relevant because any change in the hematological system have a stronger correlation value for human toxicity when the data is extrapolated from animal studies using rats (Olson, *et al*., 2000). The hematological profile of SHP showed no significant difference between the control group and the treatment group. SHP may have no effect on hematopoiesis. Histopathological examination of the liver showed lymphocytic infiltration within the periportal area in the highest dose (480 mg/kg). The periportal area of the liver is the first area of the hepatic acinus to be exposed to a toxin being delivered via the blood (Huxtable, 1988) and more likely to manifest hepatocellular malfunction. Although, the histopathological result may be said to be mild at this stage, a chronic study is suggested in order to unravel the full histological distortion by SHP. In conclusion this study has provided an insight and data on the sub chronic oral administration of SHP for any future clinical or in vivo studies.

References

1. Agbor, G.A., Tarkang, P., Fogha, J.V., Biyiti, L.F., Tamze,V., Messi, H.M., et al.(2012) .Acute and subacute toxicity studies of aqueous extract of Morinda Lucida stem bark. J Pharmacol Toxicol, 7(3):158-65.

2. Allan, J.J., Bhide, R.M., Agarwal, A. (2012). Safety assessment of Zigbir : A Polyherbal formulation in Sprague-Dawley Rats Journal of Toxicology Volume 2012, Article ID 589520, 10 pages doi:10.1155/2012/589520

3. Blumenthal, M., Brusse, W.R., Goldberg, A., Gruenwald, J., Hall, T., Riggins, C.W., Rister, R.S. (1998). The Complete German Commissions E- Monographs. Therapeutic Guide to Herbal Medicines. The American Botanical Council, TX, USA, 45: 67-68.

4. Canini, A., Alesiani, D., D'Arcangelo, G., Tagliatesta, P. (2007). Gas chromatography-mass spectrometry analysis of phenolic compounds from Carica Papaya leaf. J Food Comp Analysis 20(7): 584-90.

5. Dioka, C., Orisakwe, O. E., Afonne, O.J., Agbasi, P.U., Akumka, D.D., Okonkwo, C.J., Ilondu, A.N. (2002). Investigation into the Haematologic and Hepatotoxic effects of Ribacin in rats.J Health Sci 48(5): 393-8.

6. Elvin-Lewis, M. (2001). Should we be concerned about herbal remedies. J Ethnopharmacol 75: 141-64.

7. Estes, J. D., Stolpman, D., Corless, C.L., Ham, J.M., Schwartz, J.M., Orloff, S.L. (2003). High prevalence of potentially hepatotoxic herbal supplement use in patients with fulminant hepatic failure. Arch.Surg, 138:852-858.

8. Firenzuoli, F., Gori, L. (2007).Herbal medicine today: clinical and research issues in

subacute toxicity studies. Toxicol. Appl. Pharmacol, 57: 414-24.

9. Ha, H., Lee, J.K., Lee, H.Y., Seo, C.S., Lee, M. Y., Shin, H.K. (2011). Geno toxicity assessment of a herbal formula Ojeok-san. J Ethnopharmacol, 135: 586-89.

10. Heywood, R. (1983). Long term toxicity In M.Balls.R.J Riddle and A.N.Worden (Eds).Animals and alternatives in toxicity testing, London Academic press.

11. Hilaly, J.E., El.Israili, Z., Lyoussi, B. (2004).Acute and chronic toxicological studies of Ajugaiva in experimental animals. J Ethnopharmacol 91: 43-50.

12. Huxtable, C.R. (1988).The liver and exocrine pancreas. In W.F.Robinson and C.R.R Huxtable (Eds).Clinicopathologic Principles for Veterinary Medicine. Cambridge University press, 194-215.

13. Hyekyung, H., Jun Kyoung, L., HoYoung, L., Chang-Seo, S., Jung-Hoon, K., Mee-young, L., Woo Suk, K., Hyeun Kyoo, S. (2010). Evaluation of safety of the herbal formula Ojeok-san: Acute and sub-chronic toxicity studies in rats. J Ethnopharmacol 131 : 410–416.

14. Jordan, S.A., Cunningham DG, Marles RJ. (2010). Assesment of herbal medicinal products: challenges, and opportunities to increase the knowledge base for safety assessment. Toxicol Appl Pharmacol 243: 198-216.

15. Kluwe, W.M..(1981) Renal function test as indicators of kidney sub acute toxicity studies. Toxicol.Appl. Pharmacol 57: 414-24.

16. Latilou, S., Zafar, H.I., Badia, L. (2008). Acute and chronic toxicity of lypholized aqueous extract of tanacetum Vulgare leaves in rodents. J Ethnopharmacol 117: 221-7.

17. Li-Hua, M., Huang, Z., Ring, L., Yuan, H., Yue, G. (2011). Acute and subchronic oral toxicity assessment of the herbal formular Kai-Xin-San. J Ethnopharmacol 138: 351-57.

18. Lynch, N., and Berry, D. (2007).Differences in perceived risks and benefits of herbal, over the counter conventional medicines, and the implications of this for safe and effective use of herbal products. Compl Ther Med 15: 84-91.

19. Mackowiak, E.D., Parikh, A., Freely, J. (2001). Herbal product education in the United States pharmacy schools: Core or elective program? Am.J Pharm.Ed 65: 1-6.

20. Mdhluli, M. (2003). Toxicological and antifertility investigations of oleanolic acid in male vervet monkeys (Chlorocebus aethiops). PhD Thesis. Discipline of Physiological Sciences, University of the Western Cape, Cape Town, South Africa .

21. Mosihuzzaman, M., Iqbal Choudhary, M. (2008). Protocols on safety, efficacy, standardization, and documentation of herbal medicine. Pure Appl Chem 80(10): 2195-2230.

22. Oloyede, A.M., Okpuzor, J., Omidiji, O.O. (2012).91-day oral toxicity evaluation of a herbal preparation used in the management of breast tumour in southwestern Nigeria using mice. J Pharmacol and Tox 7(4): 199-205.

23. Olson, H., Betton, G., Robinson, D., Thomas, K., Monro, A., Kolaja, G., et al.(2000). Concordance of the toxicity of

Pharmaceuticals in humans and animals. Reg Tox and Pharmacol 32:56-67.

24. Patel, R.B., Raval, J.D., Gandhi, T.P., Chakravarty, B.K. (1988). Hepatoprotective effect of Indian Medicinal Plants Part 1. Indian Drugs, 25(6): 224-5.

25. Rajesh, M.G., Latha, M.S. (2004). Preliminary evaluation of the antihepatotoxic activity of Kamilari, a polyherbal formulation. J Ethnopharmacol 91(1): 99-104.

26. Salahdeen, H.M., Yemitan, O.K. (2006). Neuropharmacological effects of aqueous leaf extract of Bryophyllum Pinnatum in mice. Afr J Biomed Res 9, 101-7.

27. Shin, I.S., Seo, Y,.B., Ha, H.K., Lee, M.Y., Huang, D. S., Kim, J.H., Shin, H.Y. (2011). Subchronic toxicity of Sipjeon-tang (SDT) in Sprague-Dawley rats. Reg Tox Pharmacol 59: 375-84.

28. Simmons, J.E., Yang, R.S., Berman, E. (1995).Evaluation of complex mixtures containing organics and metals: advantages and disadvantages of the use of renal world complex mixtures. Environ. Health Perspect 103: 67-71.

29. Teschke, R., Gaus, W., Loew, D. (2003). Kava extracts:Safety and risks including rare hepatotoxicity Phytomed 10: 440-446.

30. Wolf, P.L., Williams, D., Tsudaka, T., Acosta, L. (1972). Methods and techniques in clinical chemistry. John Wiley and Sons, USA .

