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Research Article

SUB-ACUTE TOXICITY STUDY OF HERBAL BLOOD TONIC (HAMEBUILD) IN ALBINO RATS.

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ABSTRACT

Hamebuild is widely used in Nigeria for restoration of vitality and to facilitate recovery after ill-health. Acute and sub-acute toxicity profiles of hemebuild were evaluated in Wistar albino rats. The control group received 10 ml normal saline/kg, while group 2 received oral daily doses of 1000 mg hamebuild /kg for 28 days. The effects of the hamebuild on, body weight, hematological and biochemical parameters, relative organ weight and rat`s internal organ histopathology were evaluated. The oral LD_{50} of the extract was estimated to be greater than 5,000 mg/ kg body weight. The study showed that hamebuild did not have significant influence on the hematological, biochemical and histo-pathological parameters of rats. The positive safety profile of hamebuild may explain the rationale behind the continual use of hamebuild in Nigeria.

Keywords: hamebuild, toxicity, herbal medicine, liver function, histology.

INTRODUCTION

Herbal preparations are often referred to as health supplements in most countries and are sold in pharmacies and open market. Most of these preparations contain more than one herb, with each herb having its pharmacological/toxicological effect(s). Ethnopharmacology has focused more attention on the study of pharmacological effects and toxicities of one herb under mining the synergistic, side effect nullifying and harmful effect that may result from their combination. It is therefore necessary to study the effects of herbal preparations as they are presented to the public for consumption. In this study we evaluated the acute and sub-acute toxicity of the herbal preparation; hamebuild, sold in Nigerian drug market.

Hamebuild Herbal Blood Tonic Mixture (200ml) is manufactured by Hameko Naturalist Hospital Ltd (Plot 2 and 3 morolab street kagini district Abuja). Its contains eight herbs, (Urtical dioica 10%, Rumex acetose 15%, Pulygonum Aviculare 10%, Nasturtium Officinalis 10%, Eugenia Tomentosa 10%, Taraxacum Officinale 10%, Panax Gingseng 10%, and Trigonella Foecum 10%), Gamma Globulin 5%, and water. It is claimed to enhance quick recovery from illness and to improve vitality in adult and children above 6 years. Adult are advised to take 2 table spoonful three times daily, while children are to be given 1 table spoonful three times daily (orally). The manufacturer advices storage below 25^0c and reported that it is well tolerated when taken after food.

Plants in the preparation have been reported to possess some pharmacological/toxicological actions. Polygonum aviculare, LINN, (Polygonaceae) is an annual growing plant. It contains, water, protein, fat, carbohydrate, fiber and Ash. It is used in folkloric medicine as an antiphlogistic, astrigent, cardiotonic, antiemetic, diuretic, purgative, haemostatic agent, and in the treatment of dysentery, hemorrhoid and dieresis, (Sofowora, 1984, Dalziel, 1956).

Panax gingseng, LINN (Araliaceae) is an adaptogenic herb that is rich in ginsenosides. Ginsenosides has been reported to increase vitality, (Emilia, *et al.,* 2000). Polygonum aviculare, is a slow growing perennial plants. It is used in traditional medicine as an antioxidant, adaptogen, aphrodisiac, a nourishing stimulant, and in the management of type two (II) diabetes mellitus, (Ren-You, *et al.,* 2010), sexual dysfunction (in men) and as an antibacterial agent, (Hediat, *et al.,* 2009). Trigonella foenum LINN (Leguminoceae) is an herbaceous plant that is reported to contain, antioxidant, (Solomon, *et al.,* 2002), carminative, demulcent, expectorant, laxative and stomachic agents. It is commonly used to enhance breast size, general body health, increase breast milk production and sexual desire, reduce blood sugar and as an antioxidant, (Sreeja, et al., 2010). Other uses are for the control of fever, sore throats, wounds healling, swollen glands, and skin irritation. Phytochemical studies revealed that it contains Diosgenin, yamogenin, titogenin, neotigogens, and 4 hydroxy isoleucine. Diosgenin has been reported to have anticancer activity, (Jayadev, *et al.*, 2004).

Nasturtium officinale (Brassicaceae), commonly known as watercress, is a fast growing, aquatic or semi aquatic, perennial plant native to Europe and central Asia and one of the oldest known leaf vegetable consumed by humans. It is rich in iodine, iron, calcium, folic acid, vitamin A and C, and is used for its stimulant, (Gonçalves, *et al*., 2009), diuretic, expectorant, digestive aid, antioxidant, (Yazdanparast, *et al*., 2008). It is of benefit in the management of hypothyroidism, probably because of the high iodine content, (Suzana, *et al*., 2012). Rumex acetosa LINN, (Polygonaceae) is commonly known as Garden Sorrel or Sorrel. Its leaf is edible and is often used in soups, stew and sauces or added to salad. It has been reported to possess anti-inflammatory, (Suleymana, *et al.,* 1999), antioxidant, anti ulcer, (Ji Yeong, *et al.,* 2012), and antimicrobial, (Magdalena, *et al.,* 2011) properties. Taraxacum officinale (Asteraceae), is commonly known as Monks-head, blowball, or Lion's-tooth. It is used to make dandelion wine (Lovell, and Rowam, 1991), and for its antioxidant, immune call proliferative and tumor call growth inhibitory activities, (Sung-Hyeon, *et al*., 2005). The roots have been used to make coffee like drink and the plant was used by Native Americans as a food (salad) and medicine, (Dalziel, 1956). It contains flavonoids, cinnamic acids, luteolin 7-glucoside, and coumarins, (Christine, *et al.,* 1996).

Gamma globulins are a class of proteins found in blood, and are identified by their position after serum protein electrophoresis. The most important gamma globulins are the immunoglobulins (IgS), commonly known as antibodies. Gamma globulin injections are usually given in an attempt to temporarily boost a patient`s immunity against diseases especially patient that cannot produce gamma globulin naturally due to immune disorders, such as X – linked agammaglobulinemia and hyper IgM syndrome, (Rebecca, *et, al.,* 1991). Gamma globulin injection is mostly given to patients that have been exposed to hepatitis A or measles, or to make a kidney donor and recipient compatible regardless of blood type or tissue match, (Stanley, *et al*., 2006). It is used in treatment of immunological disease, (such as idiopathic thrombocytopenia purpura (ITP)), in diseases in which the platelets are being attacked by antibodies leading to serious low platelet count, (Jorg Fehr, *et al.,* 1982). The rationale for it oral use is not understood, since proteins are digested in the gastrointestinal tract.

Urtica Dioica (Uticaceae) is common known as stinging Nettle. It is used for symptomatic relief of lower urinary tract symptoms, as

diuretics, coloring, astringent and haemostatic agent, cleansing tonic and blood purifier. Eugenia tomentosa (Myrtaceae) is eaten fresh or use to make juice, ices, liqueurs or sweets, (Dalziel, 1956; Duke and Ayensu, 1985).

This preparation seems to contain herbs rich in body replenishing chemicals, but the rationale for the combination is not clear. Safety of this combination was evaluated to ascertain sub acute toxicity of the combination in wister rats.

MATERIALS AND METHOD

COLLECTION OF HERBAL PREPARATION

Freshly prepared herbal product (Hamebuild) was obtained from Hameko naturalist Hospital LTD in 2010 by Patrick O. Olurunfemi of the Department of Pharmaceutical Microbiology, University of Jos, Jos, Plateau State, Nigeria. Hamebuild was stored in the laboratory cabinet till use. Composition of Hamebuild was obtained from the manufacturer and compared with listed contents on the product label. The product contains eight (8) plants, gamma globulin and water.

Animals

Male wistar albino rats (130 - 200 g) obtained from the animal house of the Department of Pharmacology University of Jos was used for this study. The rats were fed with standard laboratory diet, given water *ad libitum* and maintained under laboratory conditions of temperature 28 ± 1°C, relative humidity 11 ± 1% and 12 h light and 12 h dark cycle.

Acute toxicity (LD50) study

Acute toxicity study was evaluated using the method describe by Lorke (1983). In the first phase, nine rats, randomly divided into three groups of three rats per group were given 10, 100 and 1000 mg /kg orally (via a canula), respectively. The rats were observed for signs of toxicity and death for 24 h and then for 14 days. Based on the results of the phase one study, the procedure was repeated using another set of three rats randomly divided into three groups of one rat each, given 1600, 2900 and 5000 mg/kg body weight, respectively. For 14 days, the rats were observed for signs of toxicity which include but not limited to paw-licking, salivation, stretching on the floor and wall of cage, and death.

Sub-acute toxicity study

Sub-acute toxicity study was evaluated using the method described by Otimenyin, *et al*., (2010) in wistar rats. Group of 5 male rats received hamebuild (1000 mg/Kg) orally daily for 28 days and the second group (control) of 5 male rats were given 10 ml/kg of distilled water daily for 28 days. Daily body weights of the rats were monitored during the period of administration. After 28 days, animals were allowed to fast overnight, anaesthetized with petroleum ether and blood samples were collected from the rats via cardiac puncture into heparinized tubes for hematological analysis and non heparinized centrifuge tubes for biochemical analysis. The animal were then sacrificed and liver, kidneys, lungs, spleen, testis, stomach and heart were collected, weighed and preserved in 40 % formalin for histopathological studies.

HAEMATOLOGICAL ANALYSIS

Haematological analysis was carried out using standard physiological methods, (Rinder and Dabieh, (1979), briefly

Clothing time

A drop of blood was collected on a clean and clears surface of a glass slide, the tip of an office pin was passed across the drop of blood once every 5 seconds, until the pin lift up little threads of formed fibrin. The time was noted and the procedure was repeated for both the test and control animals.

Packed cell volume (PCV)

Blood was collected in a heparinized capillary tube by allowing the blood to enter the tube by capillary method. One end of the capillary was sealed with crista seal. The blood containing capillaries were arranged into micro-centrifuge and centrifuged at12,000 rev/min for 5 minutes. The resultant packed cell volume (PCV) was measured with microhaematocrit reader and values obtained were recorded and percentage calculated.

$$\text{PCV} = \frac{\text{Height of Packed cell column}}{\text{Height of whole blood column}} \times 100$$

Hemoglobin estimation (Hb)

Blood Sample (0.02 ml) was washed in 4 ml of dropskin's solution. The mixture was allowed to stand at room temperature for 5 minutes to ensure complete reaction. The absorbance was taken using spectrophotometer at wavelength of 540 nm within 6 hours of dilution. The standard was read against blank. Hemoglobin level was then calculated from the readings.

Red blood cell count (RBC)

Red blood cells were counted after making a 1:200 dilution of blood in formal citrate solution. The resultant mixture was allowed to stand for 5 minutes for development. Improved Neubauer counting chamber was filled (with the aid of Pasteur pipette) with blood after it was charged with cover glass already in position avoiding air bubbles and over or under floating. It was incubated for 2 minutes at room temperature for the cells to settle. The chamber was mounted on microscope and stained cells were counted using x10 or x40 lens in the central large square of the chamber.

White Blood Cells WBC

Leucocyte were counted after making a 1:20 dilution in Turk solution the mixture was allowed to stand for 5 minutes for development, the nucleus stains deep blue – black. Improves neubauer counting chamber was filled using Pasteur pipette, avoiding air bubble and over floating. The slide was then incubated for 2 minutes for the cells to settle. Leucocyte cells were counted under the microscope using x 10 or x 40 lens in the four corner large squares.

Platelet count

Platelets were counted after making 1:20 dilution of the blood with 1% ammonium oxalate solution. The mixture was allowed to stand for 30 minutes (for lysing of red cells) and then filled into the counting chamber using Pasteur pipette, the charged chamber was incubated for 20 minutes for platelets to settle. Counting was done microscopically using x 10 lens in one of the large four corner square of the chamber.

Differential white blood cell count

Blood film was made on a slide, allowed to air dry, stained with leishman solution and air dried. The slide was examined under the microscope, and base on the differences in the nucleus of monocytes, leucocytes, basophiles, neutrophiles and eusinophiles, they were counted and noted.

Biochemical Studies

Blood samples were collected by cardiac puncture with the aid of syringe, transferred into centrifuge bottle, and centrifuged at 4000 rpm for 15 minutes until the serum was petitioned from blood cells. The serum was separated and stored in the refrigerator till use. Biochemical assay were carried out using kit methods, (Otimenyin, *et al*., 2010).

Histopathological studies

The Liver, Kidney, Heart, Lungs, Spleen, Stomach and Uterus were isolated from sacrificed animals, grossly examined for any pathological changes and then fixed in 10 % saline for 5 days at room temperature. The fixed organs were washed in running tap water for 2 minutes, dehydrated, waxed with ascending grades of alcohol (50%, 70%, 80%, 90%, 95% and absolute alcohol) for 2 hours and cleared in xylene for 2 hours. They were infiltrated with molten paraffin wax at melting point of 50 degree Celsius for 48 hours, embedded in molten paraffin wax to obtain blocks of tissues

and allowed to cool. The blocks were trimmed to remove excess wax and expose the tissue for sectioning.

The tissues were sectioned using a microtone which was set at 5 micrometer and floated on water bath maintained at 45 degree Celsius. The tissues were then picked on slide and allowed to dry on a hot plate maintained at 60 degree Celsius, and stained. Prepared slices were observed and photographed under the microscope. Test rats` organs were compared with the control rats` organs by pathologist and observations were noted and recorded, (Lee, and Luna, 1960).

Statistical analysis

Results were expressed as mean ± SEM. Statistical analysis of data was carried out using one –way analysis of variance and students T-test. Significant differences were determined using a Student's t-test and the differences were considered significant if $p < 0.05$.

RESULTS

Acute toxicity studies

The LD_{50} was found to be greater than 5000 mg/kg

Sub-acute toxicity studies

Table 1: Effect of herbal blood tonic mixture (hemabuild) on body weight of rat

Days	Control	hamebuild, 1000 mg/kg
Day 1	187.31±19.46	145.00±14.49
Day 7	202.09±19.53	162.05±17.43
Day 14	194.12 ± 15.03	182.19± 16.32
Day 21	210.05 ± 21.21	185.08 ± 2.88
Day 28	210.93 ± 23.71	176.25 ± 2.39

Values are expressed as mean ± S.E.M. P < 0.05 when rats weight on day one was compared to the weight on days 21 and 28. N= number of animals = 5

Slight increase (P < 0.05) in the daily weight of animals treated with hamebuild was observed. This observation was similar to weight changes observed in control group.

Table 2: Effect of herbal blood tonic mixture (hamebuild) on weight of internal organ of rats

Parameters	Control	Hamebuild 1000 mg/kg
Heart	0.75±0.064	0.65 ± 0.02
Liver	6.30 ±0.21	5.52 ±0.21
Lungs	1.45 ± 0.22	1.35 ± 0.13
Spleen	0.72 ± 0.62	0.82 ± 0.13
Kidney	0.75 ± 0.95	0.67 ± 0.47
Stomach	2.42 ± 0.24	2.20 ± 1.77

Value are expressed as mean ± S.E.M

N = Number of animals per group = 5

*** Significant difference from control p < 0.05**

The blood tonic mixture (hamebuild) did not alter the integrity ($P < 0.05$) of internal organs of rats treated with Hamebuild.

Table3:Effect of herbal blood tonic mixture (hamebuild) on haematological parameters

Parameters	Control	Test (1000 mg/kg)
RBC	4.27 ± 0.22	4.22 ± 0.22
WBC	5.22 ± 0.85	5.42 ± 0.20
PLT	85.75 ± 2.01	79.50 ± 3.79
CT (Mins)	40.00 ± 10.00	51.00 ± 2.67*
PCV	51.75 ± 0.47	50.00 ± 2.67
Hb	17.25 ± 0.16	16.60 ± 0.90
NEUt	44.50 ± 0.64	38.50 ± 6.86
LYMP	54.50 ± 1.19	60.50 ± 6.84
EOST	0.50 ± 0.28	0.75 ± 0.25
Mono	0.50 ± 0.28	0.25 ± 0.25

Data are expressed as mean ± S.E.M
N = Number of animals per group = 5
***Significant difference from control P <0.05**
RBC = Red blood cells ($x10^6mm^{-3}$)
WBC = White blood cells ($X10^0mm^{-3}$)
PLT = platelet ($X10^3mm^{-3}$)
CT = Clotting time (mins)
PCV = Packed cell volume (%)
Hb = Hemoglobin concentration (gldl)
Neut = Neutrophiles (%)
Lymp = Lymphocyte (%)
Eosi = Eosinophile (%)
Mono = Monocyte (%)
Baso = basophile (%)

Hamebuild significantly ($P > 0.5$) increased the clotting time of the treated animal when compared to control rats.

Table 4: Effect of hamebuild on biochemical parameters of rats

Parameters	Control	Hamebuild 000mg/kg
Urea (mg/dl)	38.70 ± 4.53	29.20 ± 6.09
Albumin (gldl)	4.64 ± 0.55	4.69 ± 0.18
Cholesterol (mg/dl)	153.82 ± 7.25	119.20 ± 4.85*
Triglyceride (mg/dl)	70.69 ± 6.52	39.65 ± 7.64*
Creatinine (mg/dl)	0.36 ± 0.27	0.81 ± 0.39
Total protein (g/dl)	12.41 ± 0.35	11.14 ± 1.21

Values are expressed as mean ± S.E.MN = Number of animals per group*Significant difference (P>0.05) from control

Hamebuild significantly ($P>0.05$) reduced blood cholesterol and triglyceride level in rats.

Table 5: Effect of hamebuild on rat`s liver enzymes

Parameters	Control	Test (mg/kg)
ALT (u/l)	18.33 ± 11.33	33.00 ± 13.61
ALT (u/l)	25.66 ± 71.13	27.00 ± 6.00
ALP (u/l)	116.54 ± 71.13	105 ± 42.74

Values are expressed as mean ± S.E.M
N = No. of animal per group
*** Significant different from control p<0.05**
Hamebuild insignificantly (P>0.05) altered liver enzymes.
ALT = Alanine transferase
AST = Aspartate transaminase
ALP = Alkaline Phosphatase

Table 6: Effect of hamebuild on body electrolytes

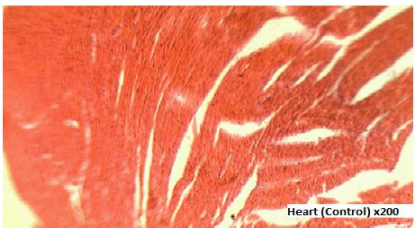
Parameters	Control	Test (mg/kg)
Calcium (mg/dl)	6.08 ± 0.52	7.70 ± 0.45
Sodium (Mequiv/L)	70.45 ± 6.81	70.86 ± 2.27
Potassium Mequiv/l)	9.35 ± 1.87	11.42 ± 1.18

Values are expressed as mean ± S.E.M
N = No. of animal per group = 5
*** Significant different from control p<0.05**
There is no significant difference in the electrolytes concentration compare to control.

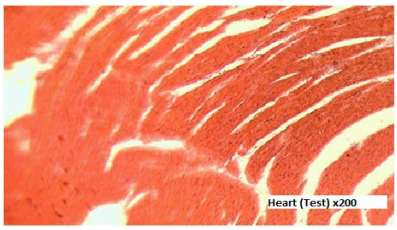
EFFECT OF HAMEBUILD ON HISTOLOGY OF INTERNAL ORGANS

Gross examination of isolated tissues revealed that there were no significant or detectable/pathological abnormalities in all the tissue examined. Signs of pathology were not observed on microscopic examination of the internal organs (i.e kidney, liver, lungs, spleen, stomach and heart) of animals treated with hamebuild.

HISTOPATHOLOGICAL RESULTS

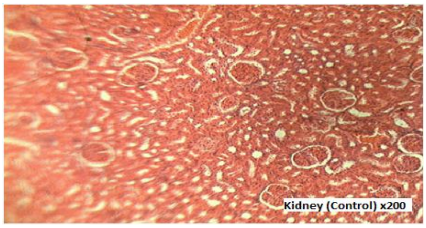


Control group showing normal cells

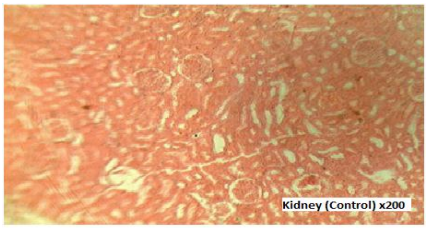


Heart cells of rat treated with hamebuild

Figure 1: Effect of hamebuild on heart cells

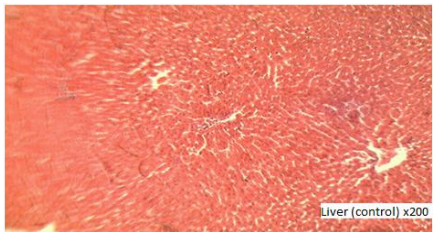


Control group showing normal cells

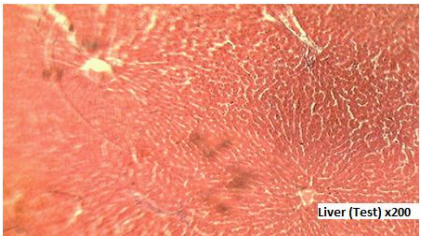


Kidney cells of rat treated with Hamebuild

Figure 2: Effect of hamebuild on kidney cells

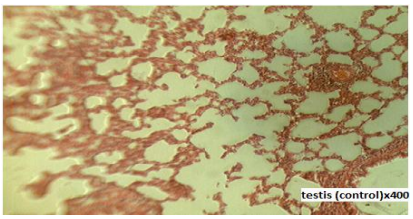


Control group showing normal cells

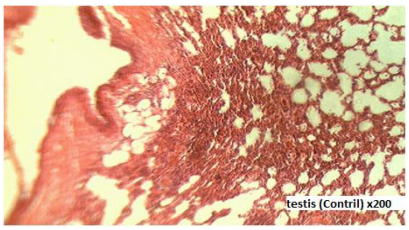


Liver cells of rat treated with Hamebuild

Figure 3: Effect of hamebuild on liver cells

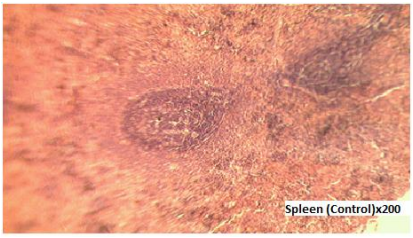


Control group showing normal cells

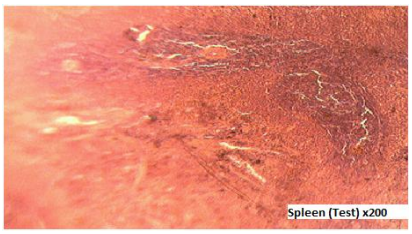


Lungs of rat treated with Hamebuild

Figure 4: Effect of hamebuild on lungs



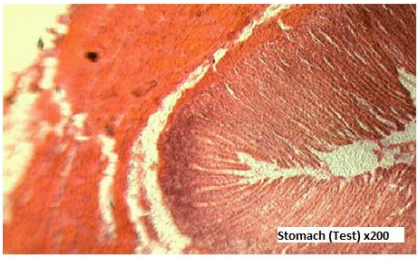
Control group showing normal cells



Spleen of rat treated with Hamebuild

Figure5: Effect of hamebuild on spleen

Control group showing normal cells



Stomach cells of rat treated with Hamebuild

Figure 6: Effect of hamebuild on stomach

DISCUSSION

In the twentieth century, many of the drugs used in orthodox medicine are produced via total synthesis of natural product, or synthesis from natural products precursors and/or their analogs. However, in the past few years, phytotherapy has regained a new interest. Over the past years, the preoccupation with the excesses of industrial civilization and the threats they represent to human physical and moral health, lead to an increase in the number of individuals which maintained an attitude of reconciliation with nature. This has contributed to the noticed increase in the use of medicinal plants.

The pharmacological activity of most herbal medicines has been linked to the presence of alkaloids, triterpenoids, flavonoids, saponins, steroids, tannins, lactones, quinones, glycosides and other compounds in the herbs. This discovery has led to the synthesis of valuable drugs used in orthodox medicine, the discovery of new receptors and better understanding of disease conditions. Less than 30 % of medicinal plants have been studied for their pharmacological actions, suggesting the need for systematic examination of the pharmacological potential, (de Medeiros, et al 2000) and toxicity of medicinal plants.

Single Plant is rarely used for the management of disease conditions. Two or more plants are often combined together for the management of disease conditions. Plants are also combined with animal parts or inanimate materials. Pharmacologists have focused attention on the evaluation of single herbs in herbal preparations, under mining the possible synergistic and/or toxicity that may have resulted from the uses of plant combinations. The need for the evaluation of herbal preparation (as presented to the public) for pharmacological activity and toxicity cannot be over emphasized. The results from this study have clearly shown that there could be synergism, side effect nullifying, toxicity and abolition of toxicity when plants are presented in mixture with other plants.

Results obtained in rats showed that the herbal blood tonic mixture (hamebuild) is well tolerated, no mortality was recorded at the dose of 1000 mg/Kg, the prescribed dose in man, after 28 days daily administration of hamebuild.

During the 28 days sub-acute test, hamebuild did not affect ($P<0.05$) normal increase in body weight as well as water and food consumption. The general increase in weight observed in the control and test animals is probably due to the normal growth associated with balanced feeding and good animal handling. Behavioral changes observed with the plants did not reveal emotional alteration and depression, apart from relative calmness of treated rats.

The weight of internal organs, (namely, kidney, liver, spleen, heart, stomach, and testis) of test animals was not different ($p<0.05$) from the weight of the control. There was no organ swelling, atrophy or hypertrophy observed in treated rats. This implies that the hamebuild did not have damaging effect on the rats organ or induce inflammation in the organs examined.

Hamebuild significantly prolonged clotting time in the treated animals, revealing that the preparation may delay the formation of fibrin. This effect may not be unrelated to the antithrombin activity of one of the constituents of the preparation, (de Medeiros, *et al*., 2000). Values of other hematological indices studied did not show significant difference from untreated animal`s values. The inability of the preparation to increase red blood cells as showed in the packed cell volume, (PCV), and hemoglobin concentration showed that the preparation may not be beneficial to anemic patients. It is possible that anemic patients may respond differently to hamebuild, since the test animals used in this study were healthy rats, and hamebuild contains plants rich in carbohydrate, minerals and iron. Thus, supporting claims by traditional healers.

Hepatic function has been monitored by the evaluation of the serum levels of transaminaeses (ALT and AST) and alkaline phosphatase. The three maker enzymes did not increase significantly in test animals compared to the control. Hamebuild did not cause any significant change in biochemical parameters analyzed, except for the decrease in the cholesterol and triglyceride levels in the rat`s blood. This observation is healthy, as it implies that hamebuild will reduce the formation of atherosclerosis and reduce the risk of stroke and cardiovascular disorders. This result suggests that hamebuild have anti-dyslipidaemic activities. Dyslipidaemia is an elevated concentration of total cholesterol and low density lipoprotein, which increases the risk of cardiovascular disease, while high density lipoprotein confers protective effect. Thus, herbal preparation like hamebuild which has antidyslipidemic effect may exhibit protective effect against cardiovascular diseases.

Renal function was evaluated by determining the plasma concentration of urea and creatinine. Decrease in creatinine concentration might be due to rapid clearance of the creatinine by the kidney, which is an indicator of preservation of kidney`s integrity. The histopathological studies, which did not show any abnormality of the kidney, also supports the preservation of kidney`s integrity.

There was no significant alteration in the serum levels of electrolytes analyzed (calcium, sodium and potassium). This is an indication that the integrity of the kidney was not compromised by the hamebuild, as supported by the histopathological and creatinine results.

Urtica dioica, though, reported to possess antiviral, (Uncini, *et al*., 2005), cholesterol and triglyceride lowering, (Daher, *et al*., 2006), and hepatoprotective, (Turkdogan, *et al*., 2003) effects, has been reported to be slightly toxic, (Tita, *et al*., 1993) with intravenous LD_{50} of 1310 mg/kg, (Baraibar, *et al.,* 1983). Its toxicity has been attributed to the presence of hydro soluble constituents, (suspected to have a pyran-coumarin structure), a substance eliminated by boiling, (Baraibar, *et al.*, 1983). Toxic effects observed in horses were apparent neurological disorders, which manifested as ataxia, distress and muscle weakness, and urticaria, (Bathe, 1994). In another study, U. dioica was reported to protect the liver against the hepatotoxic effect of carbon tetrachloride, (Turkdogan, *et al.,* 2003). Urtica dioica contains flavonols (quercetin and rutin), which confer it genotoxic activity in somatic cells of Drosophila, (Graf, *et al.,* 1994). The results above showed that the presence of the other herbs in the preparation nullified the toxic effects of U. dioica, without nullifying the beneficial effects, but rather potenciated its cardioprotective, (an activity also exhibited by N. officinale, (Yazdanparast, *et al.*, 2008), and antithrombin activities.

T. foenum, used alone is relatively nontoxic (with LD_{50} of 10 g/kg, oral and 4 g/kg, intraperitoneal), (Fedelic et al., 2009) but not without mild central nervous stimulation, rapid respiration and tremors at high doses, (Abdel-Barry, *et al.*, 1997, Javan, *et al.*, 1997). Results from this study showed that the reported mild central nervous stimulation, rapid respiration and tremors were nullified by the presence of other herbs in the preparation. The presence of galactomannan, carbohydrates and sugar, amino acid, fatty acid, vitamins, and saponins, (Fedelic, *et al.*, 2009) in T. foenum may explain why the preparation is claimed to be useful for the restoration of health after illness, though this was not proved in this study, but cannot be disproved, since the response of healthy and sick subjects to drugs may vary. T. foenum contains fibers, flavonoids, polysaccharides, saponins, polysaccharides fixed oils and some identified alkaloids viz., trigonelline and choline, (Fedelic, *et al.,* 2009), which may be responsible for its nociceptive and anti-inflammatory effects, (Javan, *et al*., 1997), activities that are beneficial to recovering patients.

Histopathological examination of the organs revealed that the integrity of the organs examined were preserved, there was no abnormality of the organs, no enlargement, atrophy or hypertrophy of all the organs examined and analyzed. This implies that the drug is well tolerated at prescribed dose and that it does not have any noticeable toxic effect nor cause necrosis of internal organs. Distortion or alteration in the functions of the organ would have complicated the disease conditions being managed. This observation supports the nullifying effect of plant in the preparation on the toxic effect of toxic plants in the preparation.

The gastro-intestinal walls` integrity was not compromised; this was revealed by the absence of ulcers, and presence of active villa. The testicular cells were normal, and showed no hypertrophy. This supports its use for the restoration of vitality. Heart tissues were not affected by hamebuild as shown in the results obtained from histological studies. This further proved that hemabuild has good safety profiles.

CONCLUSION

The herbal blood tonic mixture (hamebuild) was found to be nontoxic and well tolerated at prescribed doses in rats. The increase

in clotting time, reduction in triglyceride and cholesterol revealed that the preparation may be beneficial to geriatric patients as it will prevent atherosclerosis, cardiovascular diseases and stroke. The delayed clotting observed also showed that the herbal preparation will not be ideal for hemophilic patients.

RECOMMENDATION

Chronic toxicity, mutagencity and carcinogenicity studies are necessary to further support the safety of the herbal preparation-hamebuild.

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