DOI: https://doi.org/10.52756/ijerr.2023.v32.00X *Int. J. Exp. Res. Rev.*, Vol. 32: 00-00 (2023)

Original Article | *Peer Reviewed* | *Open Access*

International Journal of Experimental Research and Review (IJERR)

ISSN: 2455-4855 (Online)
www.iaph.in
ISSN 2455-4855

Safety evaluation of a polyherbal formulation: Acute and sub-acute toxicity using Wistar Albino rats

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Article History:

Received: 0th Jul., 2023
Accepted: 0th Aug., 2023
Published: 30th Aug., 2023

Keywords:
VNC, Traditional formulation, Musculoskeletal diseases, Sub-acute toxicity, Surveillance group

Abstract: Vatrog Nashak Churna (VNC) is a traditional polyherbal formulation for musculoskeletal diseases. Although the safety and mechanism of toxicity of the individual herbs have been explored, the formulation remains undocumented in the literature. Research into its sub-acute toxicity will strengthen its pharmacological outline and encourage its investigation as potential future treatment. Rats were split into three groups (n¼12) by OECD TG 407 (OECD, 2008). The limit test determined the necessary amount of VNC. The control groups were given an identical volume of vehicle, while the dosing and monitoring groups were given VNC (1000 mg/kg/day, p.o. for 14 days). The duration of the post-treatment surveillance period was extended by 14 days in order to evaluate reversibility. Deaths, toxic reactions, and weight shifts were all recorded. On days 15 and 29, the rats were killed while under anaesthesia so that blood samples could be collected to analyze for haematological and biochemical markers. Histopathological studies and evaluation of a wide range of biochemical and hematological parameters indicated that VNC has no appreciable harmful effect on body weight, erythropoiesis, or leucopoiesis. This study may assist scientists in determining appropriate levels for longer-term sub-chronic investigations so that VNC may be considered safe for short-term use. Sub-chronic and chronic toxicity tests must also assess long-term safety.

Introduction

Compared to basic plant materials and extracts, herbal formulations have become popular because of their lower dose, greater convenience, and simpler administration (Ganesan et al., 2021). These preparations are widely used as therapeutic medicines for various conditions that negatively affect patients' quality of life.

Traditional medicines are extensively used in underdeveloped countries because of low cost, widespread availability, and widespread acceptance of the idea that they are safe (Okaiyeto et al., 2021; Ozioma, 2019). Many synthetic medications are thought to relieve symptoms by acting on a specific molecular target. Chronic illnesses like musculoskeletal disorders and soon have been shown to benefit from multi-target responses of herbal medications, which are also effective at restoring health (Jahromi et al., 2021; Kunnumakkara et al., 2022). The safety of the active phytochemicals from these plants must be established before they can be employed in the pharmaceutical industry, even though many natural plant extracts traditionally have stood the test of time regarding toxicity and bad effects. Although the efficacy of herbal formulations has been demonstrated in pharmacological research or clinical evaluation, it is necessary to ensure their safety to obtain their maximal advantages (Mensah et al., 2019).

Toxicological investigations (acute, subacute, subchronic, and chronic) are important because they will make sure safe use of phytochemicals and potentially prevent onset of any unwanted consequences (Rehman

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and Saleem, 2022; Yang et al., 2022). Toxicity studies are seen as essential for medications intended for use in chronic illnesses. Crude extracts of Pygmeaopremna herbacea (Roxb.) Mold, Soymida febrifuga (Roxb.) A. Juss., and Cissus repanda (Wight & Arn.) Vahl is found in the polyherbal preparation Vatrog Nashak Churna (VNC). Sundaran et al. (2020), Varicella et al. (2012), and Rastogi et al. (2005) all cite the usage of these plants in traditional medicine as a treatment for bone-related issues. To date, there have been no conclusive safety studies of VNC. Acute toxicity research and a sub-chronic oral toxicity study were conducted on female Wistar albino rats to determine the safety profile of the VNC at the therapeutic dose level.

According to OECD TG 425 (OECD, 2008), the 4R rule for animal studies is followed when determining acute toxicity (Prieto et al., 2014). In addition, sub-acute toxicity studies are often conducted when preliminary data from acute toxicity studies becomes available (OECD, 2008). It aids in the establishment of doses for longer-term sub-chronic investigations. It sheds light on the potential dangers of short-term, recurrent exposure to chemicals or drugs. Sub-acute toxicity studies in rodents are also considered essential for moving forward with clinical trials and eventually marketing pharmacological compounds (Mane et al., 2023).

There may be reports of sub-acute toxicity from the chosen formulation, but none have yet been found in the literature. Therefore, it was decided to test for the compound's short-term and long-term effects. Present study was designed to determine safety of VNC using the parameters of test guidelines for signs and symptoms of toxicity (including histopathology) in Wistar rats. This investigation will enrich its ethnopharmacological profile and give scientists a leg up when designing sub-chronic and chronic studies of its toxicity. Provide an environment for investigating methods of mitigating its potential toxicity.

Materials and methods

Chemicals

The Chhattisgarh Council of Science and Technology's central laboratory in Raipur, India, supplied the NaCl solution (0.9% w/v). A traditional healer in the Bilaspur district of Chhattisgarh, India, grew the herbs used to make VNC, which were then purchased. It was well-dried and garbled.

Experimental animals

The research facility provided young adult female and male Wistar Albino rats with good health. Working institute provided basic animal feed. They were subjected to a typical climatic regime of 22°C and light-dark cycle of 12 hours (8.00 am-8.00 pm). Rats of both sexes were kept in clean polypropylene cages. Animals were given access to clean water and a pelleted meal as part of a normal feeding program. Each rat was given a week to adjust to their new environments before the experiment begun.

Ethical approval

The Institutional Animal Ethical Committee of the Chhattisgarh Council of Science and Technology, Raipur, India, has approved the experimental design of the current study (10/IAEC/CCOST/2023). Animals were cared for in accordance with the recommendations of the Committee for Control and Supervision of Experiments on Animals (CPCSEA), India.

Dose selection assay (Limit Test)

According to OECD TG 425 (OECD, 2008), one rat was orally given a single SA dosage (2000 mg/kg b.w.). Then, after monitoring mortality and morbidity, four additional rats were given the same amount. For 14 days, every rat was closely monitored for signs of death or toxicity. The limit test was used to determine the LD50 value and the dose for the subacute toxicity investigation.

Sub-acute Toxicity

In accordance with OECD TG 407, 36 rats (18 male and 18 female) were divided into three groups of 12 rats each (OECD, 2008). Rats were identified at the individual level by marking them. Rats of both sexes were housed in numbered cages and kept apart from one another. Table 1 shows the different rat groups.

Table 1. Toxicology study animal list.

Sr. No.	Group	Code for Male Rats	Code for Female Rats	Dosing	No. of Days
01	Control Group	1A	1B	NaCl solution (0.9 % w/v)	14
02	Dosing Group (VNCd)	2A	2B	VNC (1000 mg/kg/day, p.o.)	14
03	Surveillance Group (VNCs)	3A	3B	VNC (1000 mg/kg/day, p.o.)	28

Mortality rates, clinical symptoms, weight, food and water intake have all been documented regularly for each animal. The first three hours of any given day are important. We were monitored for indicators of mortality, as detailed in Table 2. On day 15, all the animals in both the control and the dosage groups were killed to conduct

the necessary analyses. The surveillance group was maintained for an extra 14 days post-treatment to assess reversibility or recovery. Every rat was checked daily to ensure they weren't dying. Clinical symptoms, body mass index, and caloric and fluid intake were tracked for 21 and 28 days in real life. Rats were used in the final assessments and were put to death on day 29. Figure 1 and Figure 2 offer a simplified representation of the experimental setup.

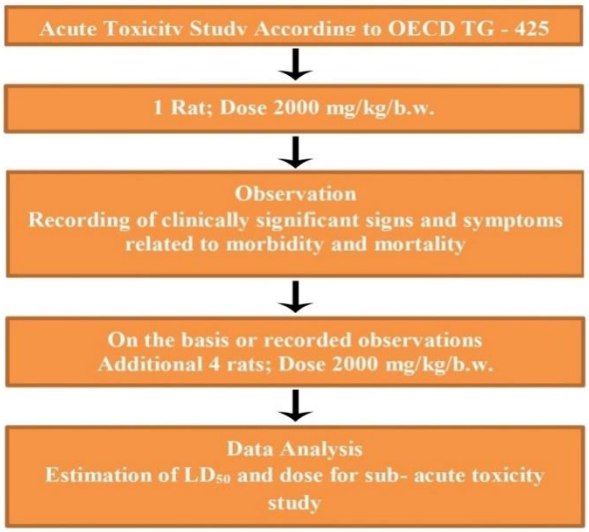

Figure 1. Experimental design for acute toxicity study

In-life Clinical Observations

The mortality rate of the animals was checked twice daily. Clinical symptoms were documented on days 0, 1, 7, and 14 during handling and in the open field for animals in the control and dosage groups, as shown in Table 2. Since we did not notice any hazardous or life-threatening changes in the animals, we planned to monitor them every week following a period of between 0 and 1 day of supervision (Smith et al., 2018). The animals in the monitoring group were re-examined on days 21 and 28 for good measure.

Table 2. List of significant clinical signs and symptoms

Sl. No.	Sign and symptoms
1	Fur and skin
2	Eyes and mucosal membranes
3	Respiratory functions
4	Cardiovascular functions
5	ANS and CNS
6	Somatomotor action (movement of the body)
7	Behavior pattern
8	tremors observations
9	Convulsions
10	Lethargy
11	Diarrhea
12	Sleep and coma
13	Salivation

Body weight

Before animals were sorted, they were weighed twice. The control and dosage groups reported their body weights on Day 1 (before dose), Days 7 and 14 (fasting body weight before sacrifice), and Day 15 (after sacrifice). The animals in the surveillance group had their weights taken weekly on days 21, 28, and 29 (just before they were sacrificed). After the study period, the average weight gain for each group was determined (Olayode et al., 2019).

Hematological analysis

At the end of study, central laboratory at the Chhattisgarh Council of Science and Technology in Raipur, India, anesthetized all surviving animals with halothane and collected blood samples via retro-orbital plexus puncture and cardiac puncture in pre-calibrated tubes coated with EDTA for hematological estimation (da Silva et al., 2014). Table 3 lists the relevant parameters.

Table 3. List of tested hematological parameters

Sl. No.	Particulars
1	Hemoglobin concentration (HBG)
2	Packed cell volume (PCV)
3	Red blood cells count (RBC)
4	Platelet count (PLT)
5	Mean corpuscular hemoglobin (MCH)
6	Mean corpuscular hemoglobin concentration (MCHC)
7	Mean corpuscular volume (MCV)
8	Total white blood cell (TWBC)

Serum biochemistry

Biochemical measurements were made from the non-heparinized blood samples. Serum was extracted from coagulated blood samples by centrifuging them at 2000g. New tubes were used to keep the serum at -20°C until analysis could be performed (Khan et al., 2016). Analytical-grade spectrophotometry kits were used to make estimates of the serum biochemical variables. Table 4 has the following set of parameters.

Table 4. List of tested serum biochemical parameters.

Sl. No.	Particulars
1	Blood glucose (BG)
2	Creatinine (CR)
3	Total billirubin (TB)
4	Albumin (ALB)
5	Total protein (TP)
6	Alkaline phosphatase (ALP)
7	Alanine transaminase (ALT)
8	Aspartate transaminase (AST)
9	Triglyceride (TG)
10	Lactate dehydrogenase (LDH)
11	Total cholesterol (TC)
12	High-density lipoprotein (HDL)

DOI: https://doi.org/10.52756/ijerr.2023.v32.00X

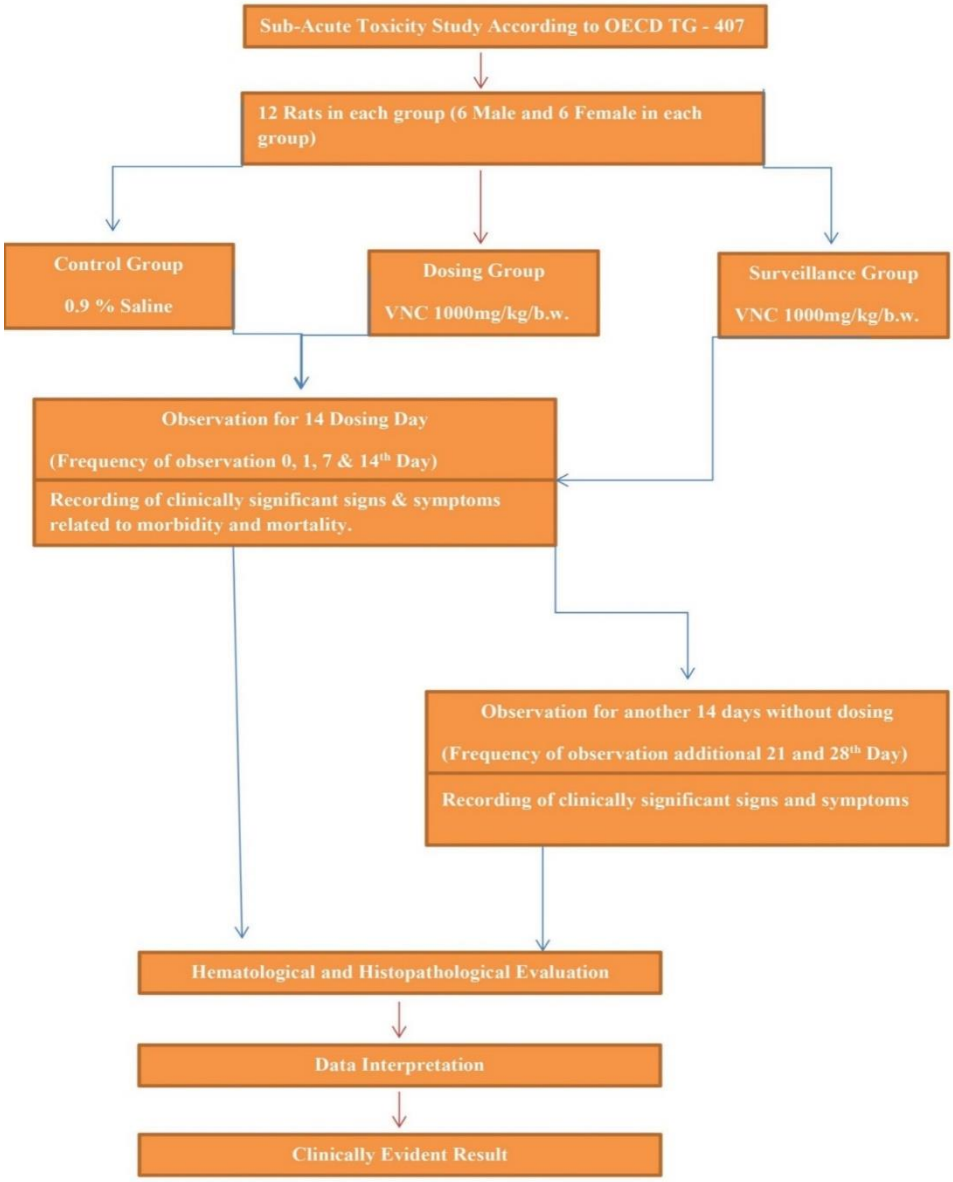

Figure 2. Experimental design for sub-acute toxicity study

Gross necropsy

On days 15 and 29, designated as their ultimate sacrifice days, all remaining animals were anaesthetized and dissected. During the necropsy, the exterior body surface, all orifices, the skeletal system, and bodily cavities (including thoracic, abdominal, cranial, and pelvic) were all examined to look for lesions. (Porwal et al., 2017).

Histopathological examinations

The kidneys, liver, and heart were all taken as samples for histological analysis. The tissues were immediately fixed in 10% formalin after being removed from the body, dried using a series of ethanol treatments, and then embedded in paraffin. Hematoxylin and eosin staining was applied so that slices cut with a rotary microtome to a thickness of 4-5 m could be examined under a microscope under a microscope. Under a microscope, organs of the treated and subacute groups were compared to those of the control group (Nigatu et al., 2017). The slides were made at a functioning research facility. An expert from the Chhattisgarh Council of Science and Technology in Raipur, India, examined all histopathological alterations.

Statistical analysis

Utilizing GraphPad Instat 3.0 (GraphPad Software, San Diego, CA), the data were examined. A one-way ANOVA was used to analyze the data, and it was followed by a post hoc Dunnett's multiple range test. The data were summarized using a mean SD. $P < 0.05$ was chosen as the criterion for statistical significance.

Results

Limit test

After receiving a single oral dose of 2000 mg/kg/p.o. of VNC, all five rats survived and exhibited no adverse effects. More than 2000 mg/kg may be the LD50 value. After reviewing available literature and OECD TG 407,

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the 1000 mg/kg dose was selected for the sub-acute toxicity investigation since it was deemed safe. (OECD, 2008).

In-life clinical observations

The death rate of all animals was checked twice daily. From the beginning of the trial until its conclusion, clinical indicators were documented weekly through handling and in open field. There was no death in any of the research groups at any time during or after the experiment. No obvious clinical indications were noticed in the animals of the dosage group or the surveillance group when compare to the control group during handling or open-field observation of the experimental rats. Table 6 provides a quick summary of the data.

Table 6. Observational findings of selected in-life clinical parameters

Sl. No.	Sign and symptoms	Inference
1	Skin and fur	No significant change
2	Eyes and mucous membranes	No significant change
3	Respiratory functions	Normal
4	Cardiovascular functions	Normal
5	Autonomic and Central nervous systems	Normal
6	Somatomotor activity (movement of the body)	Normal
7	Behavior pattern	Normal
8	Observations of tremors	Normal
9	Convulsions	Absent
10	Diarrhea	Absent
11	Lethargy	Absent
12	Sleep and coma	Normal sleep
13	Salivation	Normal

Body weight

Figure 03 and 04 shows a statistical summary of the weight changes throughout time. It was regularly taped once a week. Animals in the dosage and surveillance groups had healthy weight gains similar to those in the control group.

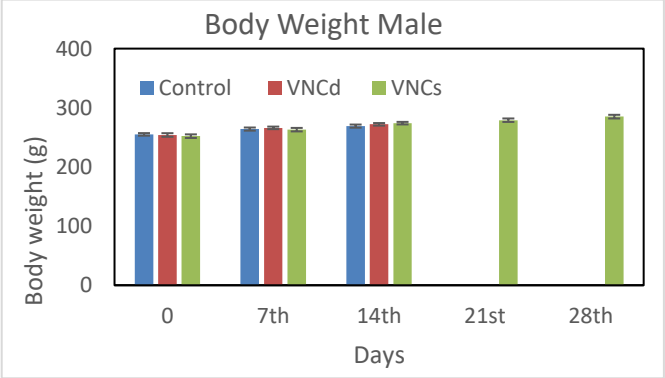

Figure 3. VNC effect on body weight of male rats

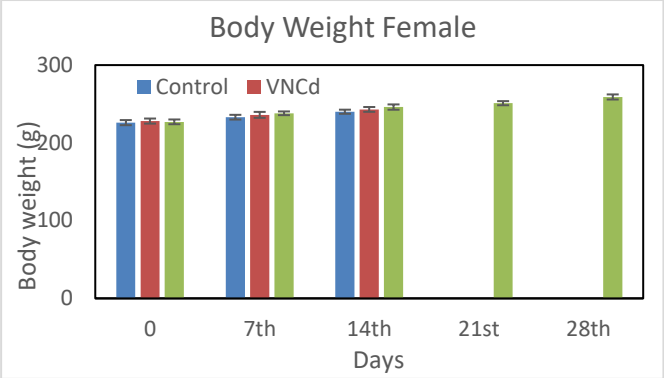

Figure 4. Effect of VNC on body weight of female rats

Hematological examination

Table 7 displays the findings of a hematological analysis. There was no statistically significant rise in HGB, RBC, PCV, PLT, or MCH in the male rats given the therapy; though, there was an amplify in MCV ($p < 0.05$) and MCHC level ($p < 0.01$). Only mean corpuscular hemoglobin (MCH) level increased significantly ($p < 0.05$) in the female treatment group. All hematological parameters in male and female satellite groups were observed to change ($p < 0.01$) but to remain within normal range.

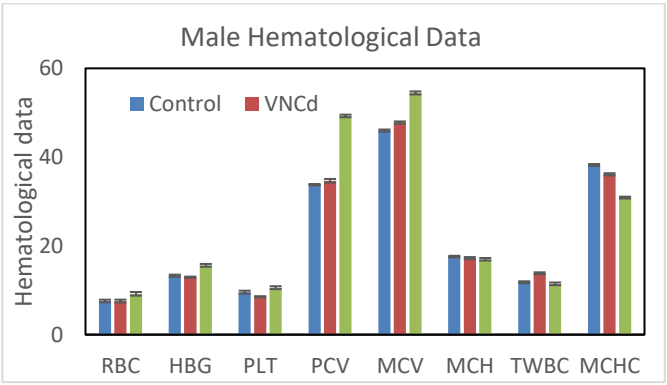

Figure 5. Result of VNC on hematological parameters of male rats

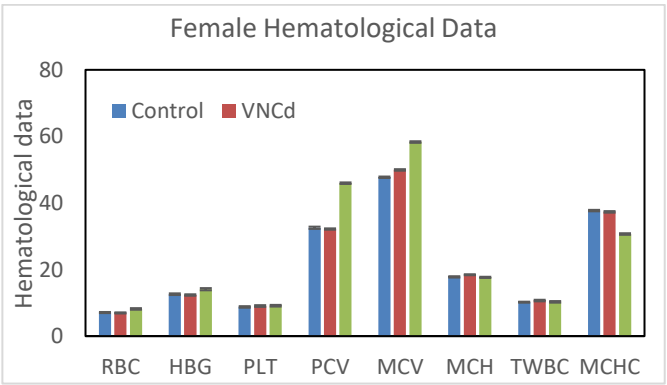

Figure 6. Result of VNC on hematological parameters of female rats.

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Table 7. Haematological findings

Parameters	Male Group			Female Group		
	Control	VNC^d	VNC^s	Control	VNC^d	VNC^s
RBC	07.58±0.30	07.56±0.32	09.20±0.41*	07.08±0.17	06.96±0.17	08.16±0.24
HBG	13.28±0.24	12.94±0.13	15.61±0.32*	12.59±0.25	12.34±0.22	14.09±0.33*
PLT	09.57±0.33	08.53±0.13	10.58±0.33	08.76±0.24	09.04±0.26	09.13±0.25
PCV	33.79±0.15	34.67±0.41	49.28±0.29*	32.55±0.34	32.17±0.20	45.94±0.24*
MCV	45.98±0.24	47.79±0.25	54.46±0.35*	47.72±0.22	49.92±0.23	58.3±0.24*
MCH	17.58±0.19	17.24±0.24	16.98±0.29	17.79±0.20	18.43±0.15*	17.65±0.18
TWBC	11.81±0.21	13.83±0.21*	11.45±0.30	10.19±0.15	10.68±0.24	10.29±0.24
MCHC	38.24±0.20	36.13±0.24	30.88±0.21*	37.71±0.22	37.32±0.23	30.67±0.24*

Table 8. Blood serum findings

Parameters	Male Group			Female Group		
	Control	VNC^d	VNC^s	Control	VNC^d	VNC^s
BG	88.95±0.33	78.92±0.24*	91.61±0.20*	97.93±0.23	91.42±0.29	93.14±0.21*
CR	0.50±0.02	0.46±0.04*	0.49±0.02	0.48±0.06	0.51±0.03	0.52±0.04*
TP	6.67±0.21	6.84±0.25	7.21±0.23*	7.43±0.29	7.52±0.25	7.44±0.29
ALB	4.89±0.17	4.77±0.25	5.02±0.21*	4.93±0.21	5.04±0.22*	5.06±0.22*
TB	0.19±0.03	0.22±0.03*	0.20±0.02	0.23±0.02	0.21±0.02	0.22±0.02
AST	84.24±0.21	86.95±0.38	85.98±0.22	73.15±0.25	76.71±0.29*	71.44±0.34*
ALT	31.63±0.21	30.54±0.37	30.23±0.30	29.05±0.66	31.94±0.21	30.78±0.22
ALP	261.86±0.21	245.51±0.37*	265.93±0.21	151.81±0.22	125.54±0.37*	150.89±0.24
LDH	360.85±0.222	360.81±0.23	356.34±0.24*	473.08±0.21	364.84±0.22*	403.05±0.19
HDL	20.38±0.30	21.64±0.23	22.03±0.21*	21.27±0.33	22.13±0.31	24.21±0.33*
TG	110.29±0.34	104.87±0.21*	107.73±0.21	107.94±0.22	113.82±0.21*	110.25±0.22
TC	81.67±0.21	87.43±0.33*	83.66±0.20	87.34±0.32	87.89±0.26	87.38±0.30

Mean ± SD, appreciably different at *p<0.05 in comparison to control group.

Blood serum biochemistry

As shown in Table 8, blood was biochemically analysed, with measurements taken for BG, CR, TP, albumin, TB, AST, ALT, ALP, LDH, and a lipid profile (HDL, TC, and TG). All biochemical indicators were discovered to be unaffected in the treatment and satellite groups when compared to the corresponding normal control groups, with the exception of a significantly lower ALP level (p 0.01) in the female treatment group that was regained in the satellite group.

Data are represented as mean ± SD, significantly different at *p<0.05 in comparison to control group.

Histopathological findings

In this investigation, histopathological data were obtained from all three organs. Neither the treatment nor the satellite groups showed any pathological alterations relative to the healthy controls (Figure 09). Here are the breakdowns in finer detail:

Heart: healthy cardiac muscle fibres were seen in heart tissue sections, which were grouped in a dense, compact pattern, had their full lengths and had typical cellular striations and nuclei.

Kidney: The glomeruli and renal tubules in the cortex and medulla of the kidneys were healthy upon examination of tissue sections.

Liver: Hepatocytes, the portal triad, and the central vein were all present and functioning in the liver tissue sections. Hepatocytes were organized in cords, and their nuclei and cell membranes appeared undamaged.

All histological sections of important bodily organs showed no signs of inflammation or metabolic alterations.

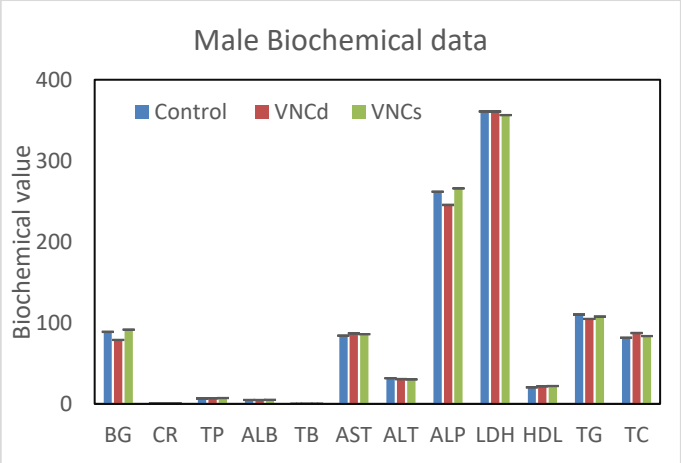


Figure 7. Effect of VNC on biochemical parameters of male rats.

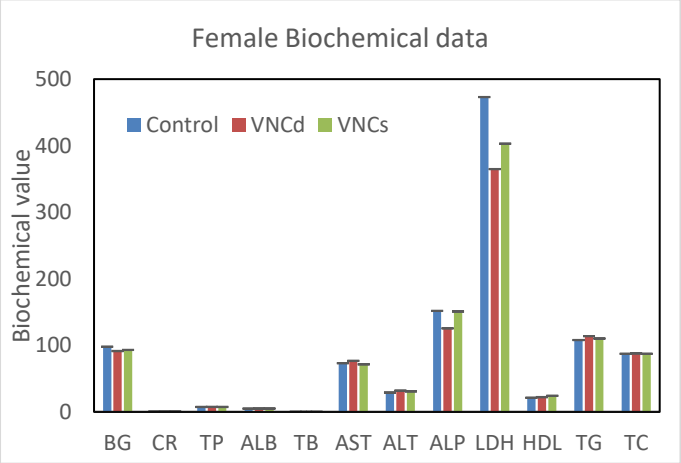


Figure 8. Effect of VNC on biochemical parameters of female rats.

Discussion

VNC is used to treat bone issues. One must take the drug for an extended period to experience its full therapeutic value; as result, its harmful or unwanted consequences cannot be disregarded. There is a lack of systematic toxicity data in the existing scientific literature. Consequently, it was determined to perform a sub-acute toxicity study, the results of which would not only improve its ethano-pharmacological profile but also shed light on potential fitness risks connected with its repeated administration over a short period. The trial will also aid in determining doses for the sub-chronic investigations that are being planned for the longer term. As seen in Figure 1, the research was organized per OECD TG407 (OECD, 2008) and previously published research.

Limit testing with single dose of VNC (2000 mg/kg p.o.) (OECD TG 425) was used to determine the dose used in sub-acute toxicity research. The effects of the toxins and the number of dead rats were closely monitored. After 14 days, there were no signs of toxicity or deaths reported. Medications' toxic effects often lead to toxic symptoms and even death (Mirza and Panchal, 2019). This indicated that a dose of VNC of 1000 mg/kg might be safe for sub-acute toxicity testing, but LD50 value for SA may be higher than 2000 mg/kg. Malaise or covert toxicity may be present long before overt symptoms appear if toxic indicators, food, and water consumption are considered. If an animal isn't feeling well, it won't consume as much food and/or drink as much water as usual. Since BMI is a proxy for overall health, Figure 03 and 04 displays how VNC affects weight.

Body weight increased sufficiently as compared to control group. Clinical observations performed during the trial in animals assigned to the dosage and surveillance groups showed that oral administration of VNC during the sub-acute toxicity study period had no adverse effect on rat development and physiological functions. The current sub-acute toxicity investigation estimated biochemical and hematological assessments because they are among the essential indicators for gauging the safety of the formulation (Ghauri et al., 2022). There was no recovery in the surveillance group, and the male rats in the therapy group exhibited no signs of improvement in HGB, RBC count, PCV, PLT count, or MCH [Table 7)]. They were excluding MCV and MCHC. All hematological indicators were higher in the monitoring group; however, this was not deemed hazardous because the increases were within the normal range. This is explained by the fact that VNC has an anemic effect and a negative effect on erythropoiesis, as demonstrated by a drop in hemoglobin, red blood cell count, and other relevant measures (Pluncevic et al., 2019).

The men's total white blood cell (WBC) counts did not differ between the treatment and control groups; however, there were rises in neutrophil and falls in lymphocyte counts, with no recovery in the surveillance group; these findings may support the anti-inflammatory action of VNC. The absence of any significant changes in WBC count in the female treatment and satellite groups, with the exception of eosinopenia in the surveillance group, further supported the general safety of VNC. Therefore, the findings supported the hypothesis that VNC is not harmful to the physiology of the red bone marrow.

Table 8 shows the impact of VNC on many biochemical markers. Hepatic health can be measured by measuring AST, ALT, and ALB levels. The dosage group, the surveillance group, and normal control group all showed no statistically major differences. No notable changes in liver structure were noted in histological investigations, either. VNC could not be considered

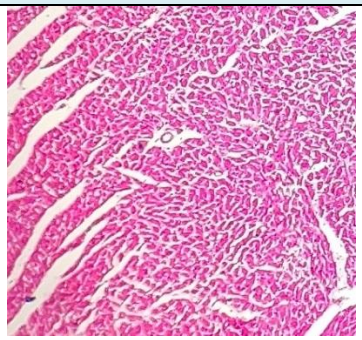
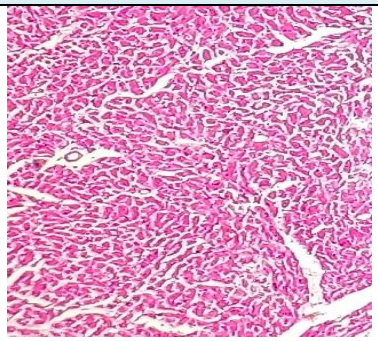
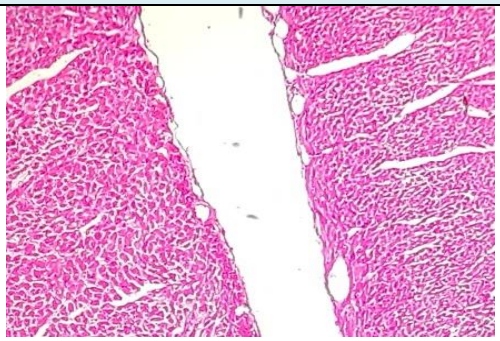
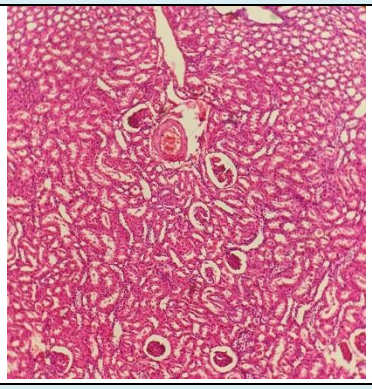
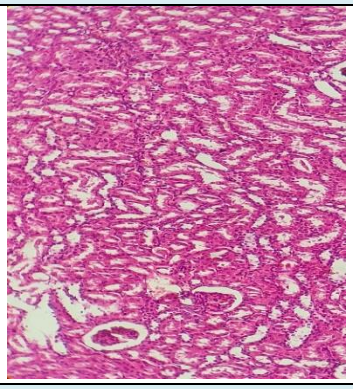
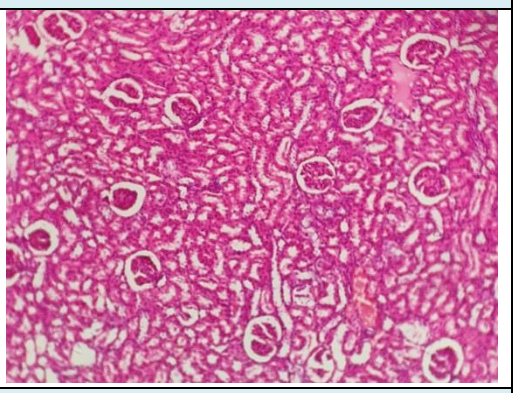
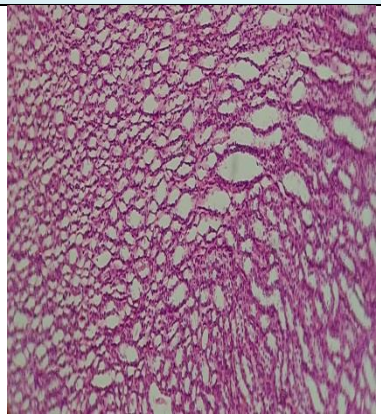
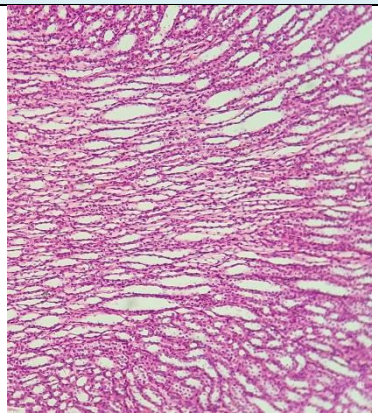
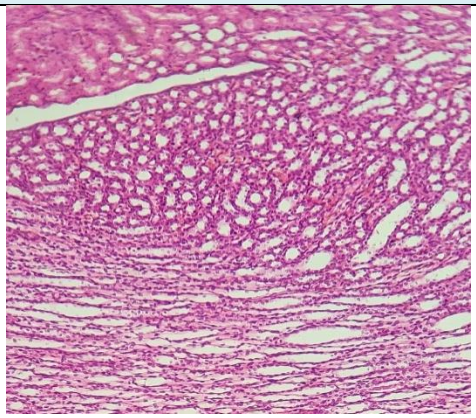
Organ	Control	VNCd	VNCs
Heart			
			
Kidney			
			
Liver			
			

Figure 9. Histological observations of selected vital organs

DOI: https://doi.org/10.52756/ijerr.2023.v32.00X

hazardous to the liver because there was no statistically significant difference in BG and CR values between the dose and control groups. To generate organic radicals and inorganic phosphate, ALP enzymes catalyse the hydrolysis of phosphate esters (Saif et al., 2005). Low alanine aminotransferase (ALP) levels were reported in the dosing group, with no recovery in the surveillance group of female rats; low ALP levels are an indicator of Wilson disease, also known as hepato-lenticular degeneration, associated with pernicious anaemia, hypophosphatasia, and hypercupremia; however, this was not seen in the male dosing group, and was not supported by histopathological studies of the liver. As a result, it's possible that this effect is not as hazardous as first thought.

Renal function can be measured by creatinine clearance, uric acid, and lactate dehydrogenase. However, Saka et al. (2014) found no evidence of a statistically significant difference between the dosing and monitoring groups of rats. These results suggested that oral administration of VNC over the subacute study period might be safe for kidneys, as it might not alter kidney morphological aspects. Haematological test reports indicating that the VNC is not affecting the digestive system, the metabolism process, the cardiac, or the renal functioning are supported by non-significant changes in renal and lipid parameters (TG, HDL, and TC) and by characteristic histological findings of the heart and liver (Aamir et al., 2019). Therefore, it's possible that VNC won't mess with your electrolyte levels. Histopathological examinations provided additional proof that VNC did not cause any harm to vital organs within the body. Thus, it may be stated that VNC does not significantly damage essential internal body organs.

Conclusion

Traditional practitioners have used VNC, a polyherbal preparation, to treat musculoskeletal ailments. According to recent studies, safety is also a key concern regarding phytochemicals. The sub-acute toxicity of VNC has not been systematically studied. As a result, it was decided to perform a sub-acute study as a precursor to longer-term trials. Physical, hematological, and biochemical measures showed no significant changes in VNC-treated rats during or after the sub-acute toxicity trial. This meant there was no interference with the physiology of internal body organs or the processes of erythropoiesis and leukopoiesis. Histopathological examinations turned up no evidence of structural abnormalities. Present study may aid researchers in defining doses for longer-term sub-chronic investigations, so VNC may be regarded as safe for short-term use. Sub-chronic and chronic toxicity tests must also assess long-term safety.

Conflict of interest

The authors state that they do not have any known conflicting interests in the work.

References

Aamir, K., Khan, H. U., Hossain, C. F., Afrin, M. R., Shaik, I., Salleh, N., ... & Arya, A. (2019). Oral toxicity of arjunolic acid on hematological, biochemical and histopathological investigations in female Sprague Dawley rats. *Peer. J.*, *7*, e8045.https://doi.org/10.7717/peerj.8045

da Silva, A. R., Moreira, L. D. R., Brum, E. D. S., de Freitas, M. L., Boligon, A. A., Athayde, M. L., ... & Brandão, R. (2014). Biochemical and hematological effects of acute and sub-acute administration to ethyl acetate fraction from the stem bark Scutia buxifolia Reissek in mice. *Journal of Ethnopharmacology*, *153*(3), 908-916. https://doi.org/10.1016/j.jep.2014.03.063

Ganesan, M. R., Settu, D. K., Murkunde, Y., & Duraipandian, C. (2021). Pharmacological and pharmacokinetic effect of a polyherbal combination with Withania somnifera (L.) Dunal for the management of anxiety. *Journal of Ethnopharmacology*, *265*, 113337. https://doi.org/10.1016/j.jep.2020.113337

Ghauri, A. O., Mohiuddin, E., Rehman, T., & Siddiqui, H. S. M. (2022). Acute and subacute toxicity studies of a poly herbal formulation used for diabetes. *Pakistan Journal of Medical Sciences*, *38*(6), 1668. https://doi.org/10.12669%2Fpjms.38.6.5928

Jahromi, B., Pirvulescu, I., Candido, K. D., & Knezevic, N. N. (2021). Herbal medicine for pain management: efficacy and drug interactions. *Pharmaceutics*, *13*(2), 251. https://doi.org/10.3390/pharmaceutics13020251

Khan, S., Choudhary, S., Kumar, A., Tripathi, A. M., Alok, A., Adhikari, J. S., ... & Chaudhury, N. K. (2016). Evaluation of sesamol-induced histopathological, biochemical, haematological and genomic alteration after acute oral toxicity in female C57BL/6 mice. *Toxicology Reports*, *3*, 880-894. https://doi.org/10.1016/j.toxrep.2016.03.005

Kunnumakkara, A. B., Hegde, M., Parama, D., Girisa, S., Kumar, A., Daimary, U. D., ... & Aggarwal, B. B. (2023). Role of Turmeric and Curcumin in

Prevention and Treatment of Chronic Diseases: Lessons Learned from Clinical Trials. *ACS Pharmacology & Translational Science*, *6*(4), 447-518. https://doi.org/10.1021/acsptsci.2c00012

Mane, V., Killedar, S., More, H., Gaikwad, A., & Tare, H. (2023). A Novel Rp-Hplc Gradient Elution Technique For Bioanalytical Method Development And Validation For Estimating Gallic Acid In Wistar Rat Plasma. *International Journal of Applied Pharmaceutics*, *15*(2), 153–160. https://doi.org/10.22159/ijap.2023v15i2.47278

Mensah, M. L., Komlaga, G., Forkuo, A. D., Firempong, C., Anning, A. K., & Dickson, R. A. (2019). Toxicity and safety implications of herbal medicines used in Africa. *Herbal Medicine*, *63*, 1992-0849.

Mirza, A. C., & Panchal, S. S. (2019). Safety evaluation of syringic acid: subacute oral toxicity studies in Wistar rats. *Heliyon*, *5*(8). https://doi.org/10.1016/j.heliyon.2019.e02129

Nigatu, T. A., Afework, M., Urga, K., Ergete, W., & Makonnen, E. (2017). Toxicological investigation of acute and chronic treatment with Gnidia stenophylla Gilg root extract on some blood parameters and histopathology of spleen, liver and kidney in mice. *BMC Research Notes*, *10*(1), 1-13. https://doi.org/10.1186/s13104-017-2964-3

OECD (2008). Acute Oral Toxicity: Up-and-Down Procedure (OECD TG 425). pp. 477–490.

OECD (2008). Repeated Dose 28-Day Oral Toxicity Study in Rodents (OECD TG 407). pp. 477–90.

Okaiyeto, K., & Oguntibeju, O. O. (2021). African herbal medicines: Adverse effects and cytotoxic potentials with different therapeutic applications. *International Journal of Environmental Research and Public Health*, *18*(11), 5988. https://doi.org/10.3390/ijerph18115988

Olayode, O. A., Daniyan, M. O., & Olayiwola, G. (2020). Biochemical, hematological and histopathological evaluation of the toxicity potential of the leaf extract of Stachytarpheta cayennensis in rats. *Journal of Traditional and Complementary Medicine*, *10*(6), 544-554. https://doi.org/10.1016/j.jtcme.2019.05.001

Ozioma, E. O. J., & Chinwe, O. A. N. (2019). Herbal medicines in African traditional medicine. *Herbal Medicine*, *10*, 191-214.

Pluncevic G. J., Gontarev, S., Dejanova, B., Todorovska, L., Stojmanova, D. S., & Manchevska, S. (2019). Red blood cell variables in children and adolescents regarding the age and sex. *Iranian Journal of Public Health*, *48*(4), 704.

Porwal, M., Khan, N. A., & Maheshwari, K. K. (2017). Evaluation of acute and subacute oral toxicity induced by ethanolic extract of *Marsdenia tenacissima* leaves in experimental rats. *Scientia Pharmaceutica*, *85*(3), 29. https://doi.org/10.3390/scipharm85030029

Prieto P, Burton J, Graepel R, Price A, Whelan M, Andrew W (2014). EURL ECVAM strategy to replace, reduce and refine the use of animals in the assessment of acute mammalian systemic toxicity, EUR 26704. Luxembourg (Luxembourg): Publications Office of the European Union; 2014. JRC90611. https://dx.doi.org/10.2788/86684

Rastogi, S., Pandey, M. M., Kumar, K., Rawat, A. K. S., & Pushpangadan, P. (2005). Pharmacognostical Evaluation of Roots of *Pygmaeopremna herbacea* (Roxb.) Mold. *Natural Product Sciences*, *11*(2), 58-62.

Rehman, M. H. U., Saleem, U., Ahmad, B., & Rashid, M. (2022). Phytochemical and toxicological evaluation of Zephyranthes citrina. *Frontiers in Pharmacology*, *13*, 1007310. https://doi.org/10.3389/fphar.2022.1007310

Saif, M. W., Alexander, D., & Wicox, C. M. (2005). Serum alkaline phosphatase level as a prognostic tool in colorectal cancer: a study of 105 patients. *The journal of Applied Research*, *5*(1), 88.

Saka, W. A., Akhigbe, R. E., Popoola, O. T., & Oyekunle, O. S. (2012). Changes in serum electrolytes, urea, and creatinine in Aloe vera-treated rats. *Journal of Young Pharmacists*, *4*(2), 78-81. https://doi.org/10.4103/0975-1483.96620

Smith, D., Anderson, D., Degryse, A. D., Bol, C., Criado, A., Ferrara, A., ... & Voipio, H. M. (2018). Classification and reporting of severity experienced by animals used in scientific procedures: FELASA/ECLAM/ESLAV Working Group report. *Laboratory Animals*, *52*(1_suppl), 5-57. https://doi.org/10.1177/0023677217744587

Sundaran, J., Vasanthi, M., Kamalapathy, M., Bupesh, G., & Sahoo, U. (2020). A short review on pharmacological activity of Cissus

quadrangularis. *Bioinformation*, *16*(8), 579. https://doi.org/10.6026%2F97320630016579

Varicola Karunasree, Veeresham, C., Rao, K. R. S., & Asres, K. (2012). Antioxidant, 5-Lipoxygenase inhibitory and anticancer activities of *Soymida febrifuga* A. *Juss. Mol. Cli.n Pharmacol., 3*, 134–42.

Yang, M. Y., Song, Z. Y., Gan, H. L., Zheng, M. H., Liu, Q., Meng, X. T., ... & Fan, H. Y. (2022). Non-clinical safety evaluation of salvianolic acid A: acute, 4-week intravenous toxicities and genotoxicity evaluations. *BMC Pharmacology and Toxicology*, *23*(1), 83. https://doi.org/10.1186/s40360-022-00622-1

How to cite this Article:

Vaibhav Tripathi, Chandra Kishore Tyagi and Wasim Raja (2023). Safety evaluation of a polyherbal formulation: Acute and sub-acute toxicity using Wistar Albino rats. *International Journal of Experimental Research and Review*, 32, 00-00.

DOI : https://doi.org/10.52756/ ijerr.2023.v32.00x