JOURNAL OF NATURAL REMEDIES
DOI: 10.18311/jnr/2023/29566
SHORT COMMUNICATION

Toxicity Status of Nano Powder of Stem Bark of *Bauhinia Variegata* Linn. on Experimental Animals

Prachi Vishwakarma[1*], Shailesh V. Deshpande[1] and Snigdha Das Mandal[2]

[1]Department of Kayachikitsa, Parul Institute of Ayurveda, Parul University, Vadodara - 391760, Gujarat, India; dr.prachee.vi@gmail.com
[2]Department of Pharmacology, Parul Institute of Pharmacy and Research, Parul University, Vadodara - 391760, Gujarat, India

Abstract

The assumption that medicinal plants are less toxic or cause fewer adverse effects due to their natural composition is debatable. Hence, this study was conducted to evaluate the safety and toxicity of Nano *Kanchanara* stem bark powder (NKSP) on liver function. The oral acute toxicity of NKSP was conducted in accordance with OECD Guidelines 423 and was carried out with six (6) female *Wistar albino* rats, divided into two groups of three rats each. Test rats were orally administered a single dose of 2000 mg/kg of NKSP and were observed for changes in behavior and mortality for the next 14 days, while the Control Group was given feed and water ad libitum. The data obtained from the oral acute toxicity study indicated that there were non-significant (P<1.000) decreases in body weight of rats in the treatment groups of NKSP at 2000 mg/kg. There were no elevations in specific liver function enzymes, whereas there were increases in serum SGOT, SGPT, and albumin levels. Plasma total proteins and globulin, total bilirubin and serum creatinine were within the normal range. Albeit, hydropic changes and steatosis in liver lesions at NKSP 2000 mg/kg. The study suggests that oral administration of NKSP is safer at dose levels less than 2000 mg/kg/day.

Keywords: *Bauhinia variegata* Linn., Liver Toxicity, Medicinal Plant, Safety

1. Introduction

Ayurveda emphasizes maintenance and promotion of health, along with a vivid description of diseases and their management. Herbal drugs are used singly and in combinations[1]. Out of the various *Panchavidha kalpana* mentioned *Churna* is one form of *Kalpana*[2].

A particle having dimensions between 1nm (nanometer) and 100 nm is considered as a nanoparticle. Nanotechnology is the branch of applied science where the particle size of the drug is reduced to the nanoscale (10^{-9}), i.e. **1–200 nanometer**[3,4]. Nano is derived from the Greek word '**Nanos**' which means dwarf or extremely small. It can be used as a prefix for any unit to describe a billionth of that unit[5,6].

In the present study, nanoparticles of nano *Kanchanara (Bauhinia variegata* Linn.) (Figure 1) *twak churna* was made by High Energy Ball Milling (HEBM) Technology. As this was the new dosage form, the safety of nano *Kanchanara twak churna* was established in the experimental study.

The advantage of nanoparticles in the present study is that the method used for their preparation is the simplest, as it is the mechanical method (HEBM), where the herbal drug is grounded to nano form[7,8]. It is an environmentally friendly method as no chemical processing is used[9,10]. In future prospects, it will reduce the exploitation of plants on a large scale and help in mass production[11,12]. This will also help in maintaining quality standards of drug and drug preparation[13]. Also, as the dosage is reduced, it will be better for palatability[14].

The assumption that medicinal plants are less toxic or cause fewer adverse effects due to their natural composition is debatable. Hence, this study was conducted

Article Received on: 14.02.2022 Revised on: 10.09.2022 Accepted on: 18.10.2022

(a)

(b)

Figure 1(a, b). *Kanchanara* (*Bauhinia variegata* Linn.) grown in Botanical Garden of Parul Ayurved College, Vadodara.

to evaluate the safety and toxicity of Nano *Kanchanara* stem bark powder (NKSP) on liver function[15-16]. The study concludes that Nano *Kanchanara* is safer at dose levels less than 2000 mg/kg whereas it may raise blood parameters Vis SGOT, SGPT, and Albumin levels.

2. Materials and Methods

2.1 Collection of *B. variegata* Linn. Stem Bark and Preparation of Nano Powder

The *Kanchanara* stem bark was purchased from M/S Shree Enterprises, Siyapura, Vadodara, Gujarat. The plant specimen was certified with Reference No. PU/PIA/DG-Certi-43 at the Raw Drug Authentication Committee, Department of Dravyaguna, Parul Institute of Ayurved, Parul University, Vadodara, Gujarat. The sample was cleaned and air- and shade-dried at room temperature for two weeks. To make a fine powder, the stem bark was pounded, pulverised, and sieved. The fine powder was then processed into Nano *Kanchanara* powder using HEBM (High Energy Ball Milling) methodology at the Institute of Applied Research, Bhosari, Pune-411026, Maharashtra, India.

2.2 Experimental Animals

Female rats of 16 to 18 weeks of age, weighing between 200 and 300 g, were used for the acute toxicological studies. The animals were housed in cages, fed a normal pelleted diet, given water *ad libitum,* and maintained under laboratory conditions of a 12 h light/12 h dark cycle with a two-week acclimatization prior to the start of the study. The research was conducted in line with international guidelines for laboratory animal use (CPCSEA). The study received approval from the Institutional Animal Ethical Committee (Project No. 984/2019-07), at Parul Institute of Pharmacy and Research, Parul University, Vadodara, Gujarat.

2.3 Acute Toxicity Studies (*In vivo*)

Acute toxicity evaluation (19) of NK was evaluated as per OECD TG 423 with a 2000 mg/kg dose. A total of six (6) female *Wistar albino* rats were used after two weeks of acclimatization. Rats were randomly divided into two group as Test and Control where $n = 3$. The rats were fasted for 12 hr prior to treatment with free excess water. The Control Group was treated with distilled water, and the other group received a single oral dose of 2000 mg/kg body weight of Nano *Kanchanara* Stem Bark Powder (NKSP). Following 30 minutes of continuous observation for any changes in weight, general behavior, food and water consumption, and mortality, treated animals were followed intermittently for 4 to 24 hours after NKSP ingestion. Following the therapy, the rats were monitored for up to 14 days (Tables 1 and 2).

2.4 Collection of Data and Specimen

The experimental animals were fasted overnight and sacrificed. The blood was withdrawn from the retro-orbital route and collected in a heparinized specimen bottle. The sample collected was analyzed for Hemogram, Liver Function Test and Serum Creatinine at Central Clinical Laboratory, Parul Sevashran Hospital, PIMSR, and Vadodara Clinical Laboratory, Vadodara. The liver, heart, kidney, brain, stomach, and intestine were excised, cleaned and cleared from connective tissue; they were stored in 10% Formalin Solution, and sent for Histopathologic evaluation at the Vadodara Clinical Laboratory, Vadodara.

2.5 Biochemical Analyses

Complete Blood Count (CBC), Liver Function Tests, which included aspartate aminotransferase (AST), alanine aminotransferase (ALT), alkaline phosphatase (ALP), gamma-glutamyl transferase (GGT) and total bilirubin and Serum Creatinine.

2.6 Histopathology Evaluation of Sectioned Liver Tissue, Brain Tissue and Kidney Tissue

The excised organs were prepared as blocks, and sections, stained and then slides were prepared and later examined in the microscope and photographed at 100x and 400x.

2.7 Statistical Analysis

The data are expressed as mean and standard deviation (mean ± SD) as applicable. Statistical analysis was done using IBM® SPSS® software version 26, and differences were considered significant at $P<0.05$ using a two-way analysis of variance (ANOVA) test.

3. Results

3.1 Acute Toxicity Study of Nano Stem Bark Powder of *B. variegata* Linn. in *Wistar* Rats

In the acute toxicity study, oral administration of a single dose of up to 2000 mg/kg did not exhibit any mortality or signs of toxicity during the observation period of 14 days. No significant changes in body weight loss were detected (Table 1). The data show that oral administration of NKSP given to *Wistar* rats was relatively safe; no deaths were recorded (Table 1).

3.2 General Behaviour and Mortality

Administration of NK for 14 consecutive days did not induce behavioral changes at any point in time in treated rats compared to the control group. No mortality was observed during the experimental period.

3.3 Effect on Body Weight

There was a progressive decrease in the body weights of rats during the acute toxicity study. The significance of weight reduction is not significant in each group, which reflects that the drug NK is not toxic (Figure 2).

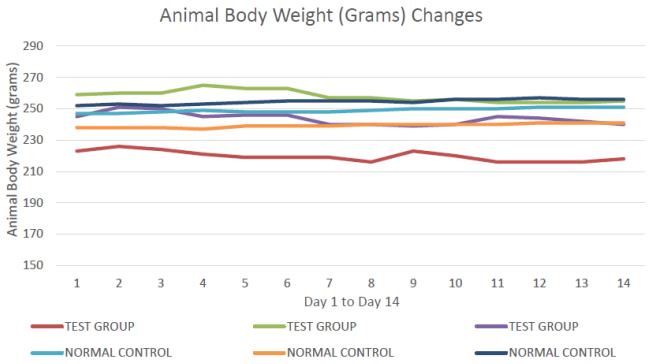


Figure 2. Animal body weight in grams as observed during 14 days period.

Table 1. Acute toxicity study of Nano *Kanchanara* Stem Bark Powder of *B. variegata* Linn. administered to female rats; behavioural changes and mortality table during 14 days period

Groups	Dose (NKSP)	Behavioral Changes	Eating Habit	Sleep	Mortality
I Test	2000mg/kg	NØ	NØ	NØ	NØ
II Control	DW	NØ	NØ	NØ	NØ

NØ=No significant observation, NKSP (Nano *Kanchanara* Stem Bark Powder), DW=Distilled Water, n=3

Table 2. Acute toxicity study of Nano *Kanchanara* Stem Bark Powder of *B. variegata* Linn. administered to female rats; effect on body weight after 14 days period. Where, Group 1-Test, Group 2-Normal Control

Parameters	Nano *Kanchanara*		
	Mean	SD	P value
Group 1 Test	240.5	3.6	1.000
Group 2 Normal	247.667	1.4	1.000

Weights are expressed as Mean, SD (Standard Deviation), n=3, P value significant at <0.05

3.4 Effect on Liver Function Parameters

The effects of acute administration of NKSP on hematological parameters is illustrated in Tables 3, 4, 5, 6, 7, 8 and 9. Single-dose administration of NK did not cause any significant change in hematological parameters tested at 2000 mg/kg dose when compared to normal control.

3.4.1 SGPT

Group 2 shows a significant P value, i.e 0.003 and Group 1 shows non-significant P value i.e. 0.23.

3.4.2 SGOT

Group 2 shows significant P value, i.e 0.003 and Group 1 shows non-significant P value, i.e. 0.1 respectively.

3.4.3 Total Bilirubin

Group 1 shows non-significant P value, i.e. 0.17 and Group 2 shows significant 0.04 respectively.

3.4.4 Total Protein

Group 1 and 2 shows significant P value, i.e. 0.02 and 0.003 respectively.

3.4.5 Albumin

Group 1 and 2 shows significant P value, i.e. 0.05 and 0.00 respectively.

3.4.6 Globulin

Group 1 and 2 shows significant P value, i.e. 0.06 and 0.00 respectively.

3.4.7 AG Ratio

Group 1 and 2 shows significant P value, i.e. 0.0 and 0.00 respectively.

Table 3. One sample T-Test Analysis of SGPT (Within the Group). Where, Group 1-Test and Group 2- Normal Control

Parameters	Nano *Kanchanara*	Within Group Analysis
	Mean ± SD	P
Group 1	163 ± 165.54	0.23
Group 2	76 ± 7.00	0.003

Table 4. One sample T-Test Analysis of SGOT (Within Group). Where, Group 1-Test and Group 2-Normal Control

Parameters	Nano *Kanchanara*	Within Group Analysis
	Mean ± SD	P
Group 1	451.00 ± 267.07	0.1
Group 2	166.33 ± 14.98	0.003

Table 5. One sample T-Test Analysis of Total Bilirubin (Within the Group). Where, Group 1-Test, and Group 2-Normal Control

Parameters	Nano *Kanchanara*	Within Group Analysis
	Mean ± SD	P
Group 1	0.76 ± 0.62	0.17
Group 2	0.30 ± 0.30	0.04

Table 6. One sample T-Test Analysis of Total Protein (Within the Group). Where, Group 1-Test, and Group 2-Normal Control

Parameters	Nano *Kanchanara*	Within Group Analysis
	Mean ± SD	P
Group 1	6.13 ± 1.62	0.02
Group 2	6.33 ± 0.65	0.003

Table 7. One sample T-Test Analysis of Albumin (Within the Group). Where, Group 1-Test, and Group 2-Normal Control

Parameters	Nano *Kanchanara*	Within Group Analysis
	Mean ± SD	P
Group 1	4.02 ± 1.59	0.05
Group 2	4.03 ± 0.15	0.00

Table 8. One sample T-Test Analysis of Globulin (Within Group). Where, Group 1-Test, and Group 2-Normal Control

Parameters	Nano *Kanchanara*	Within Group Analysis
	Mean ± SD	P
Group 1	2.79 ± 1.21	0.06
Group 2	2.1 ± 0.10	0.00
Group 3	2.3 ± 0.15	0.00

Table 9. One sample T-Test Analysis of AG Ratio (Within the Group). Where, Group 1-Test and Group 2-Normal Control

Parameters	Nano *Kanchanara*	Within Group Analysis
	Mean ± SD	P
Group 1	1.45 ± 0.08	0.00
Group 2	1.72 ± 0.07	0.00

The data expressed in Figure 3(a) indicate that the P value is non-significant (P>0.05) in Test and Confirmatory groups for SGPT, whereas the P value is significant (P<0.05) in Normal Control Group. The P value for SGOT is non-significant (P>0.05) for Test group whereas it is significant (P<0.05) for Confirmatory and Normal Control Group.

The data expressed in Figure 3(b), indicate that the P value is non-significant (P>0.05) in Test and Confirmatory group for Total Bilirubin, whereas P value is significant (P<0.05) in Normal Control Group. The P value of Total protein is significant (P<0.05) in Test, Confirmatory and Normal Control Group. The P value is significant (P<0.05) in Test and Confirmatory group for Albumin, whereas the P value is non-significant (P<0.05) in Normal Control Group. The P value for Globulin is non-significant (P>0.05) for Test group whereas it is significant (P<0.05) for the Confirmatory and Normal Control Group. The P value of the AG Ratio is significant (P<0.05) in Test, Confirmatory and Normal Control Group.

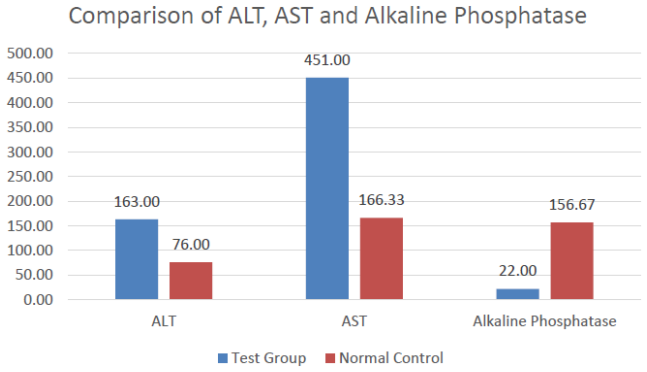


Figure 3(a). Effect of 2000 mg/kg dose of NKSP on SGPT(IU/L) and SGOT(IU/L) after 14 days. Data are Mean ± SD (Standard Deviation).

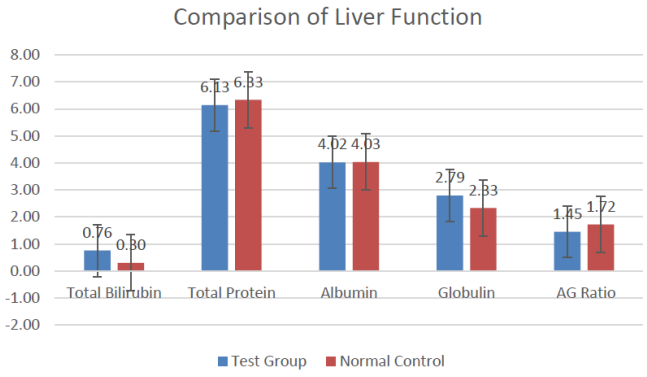


Figure 4(b). Effect of 2000 mg/kg dose of NKSP on Total Bilirubin (mg/dl), Total Protein (gm/dl), Albumin (g/dl), Globulin (g/dl), A/G Ratio after 14 days. Data are Mean ± SD (Standard Deviation).

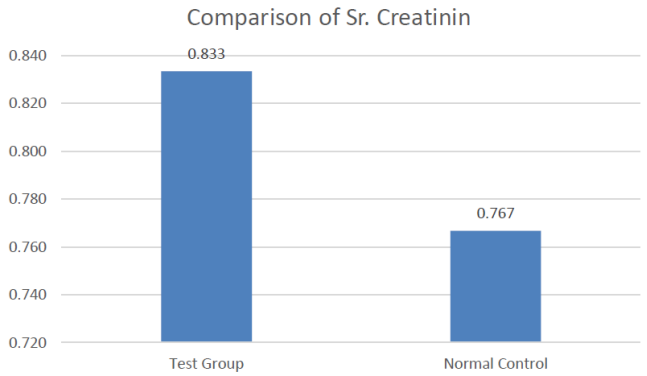


Figure 3(c). Effect of 2000 mg/kg dose of NKSP on Sr. Creatinine (mg/dl) after 14 days. Data are Mean ± SD (Standard Deviation).

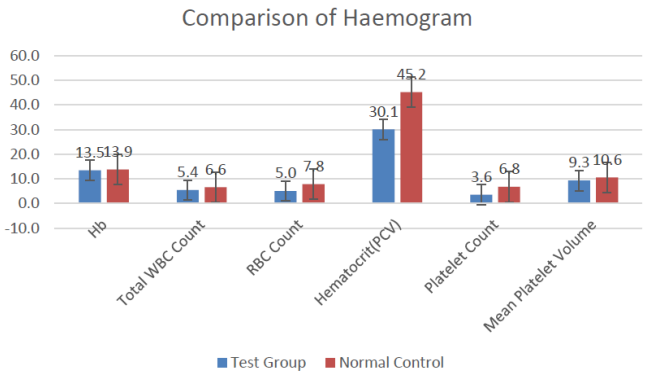
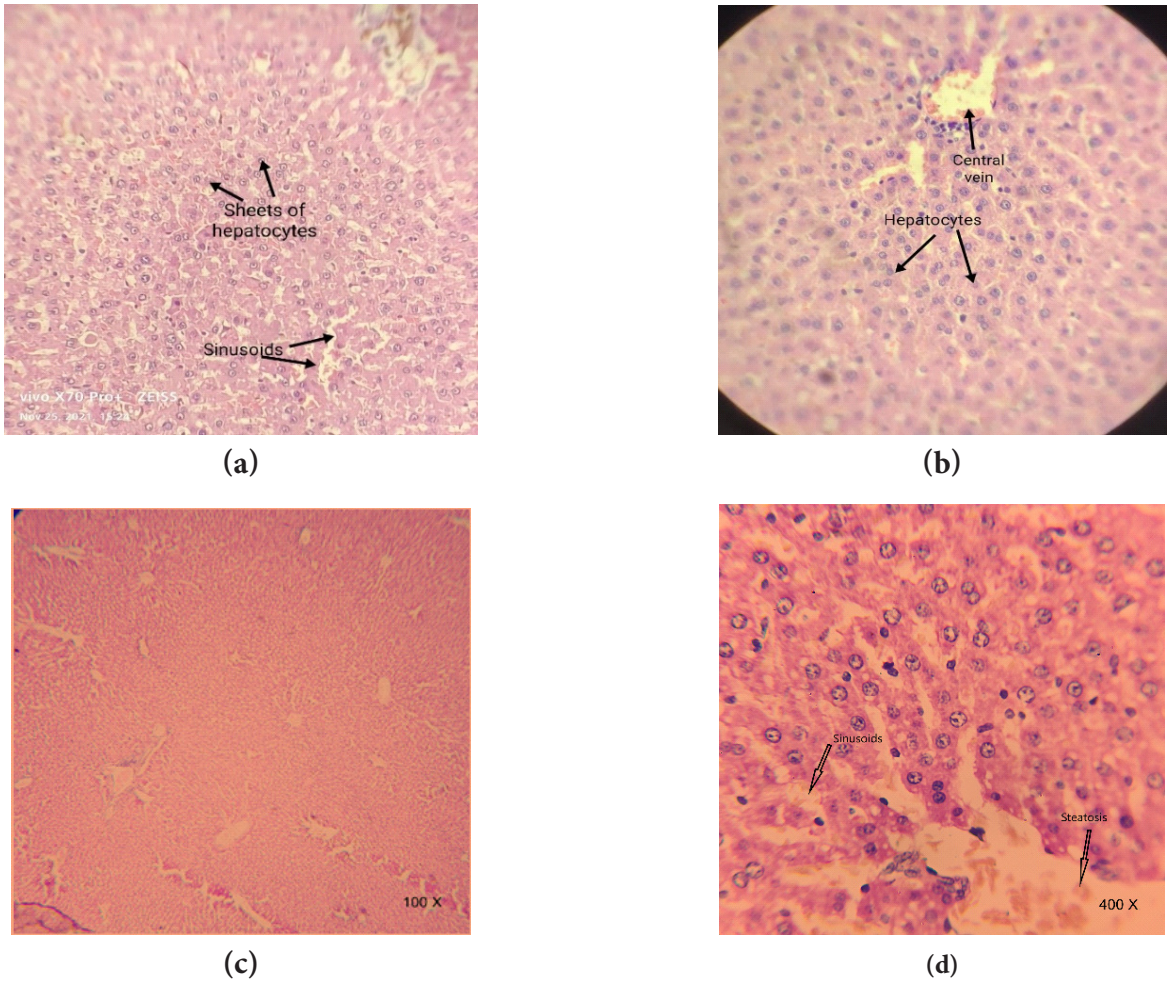


Figure 3(d). Effect of 2000 mg/kg dose of NKSP on Hb (gm/dl), Total WBC Count (109/L), RBC Count (million/mm3), Haematocrit (%), Platelet Count (10*3/uL), and Mean Platelet Values (fL) after 14 days. Data are Mean ± SD (Standard Deviation).

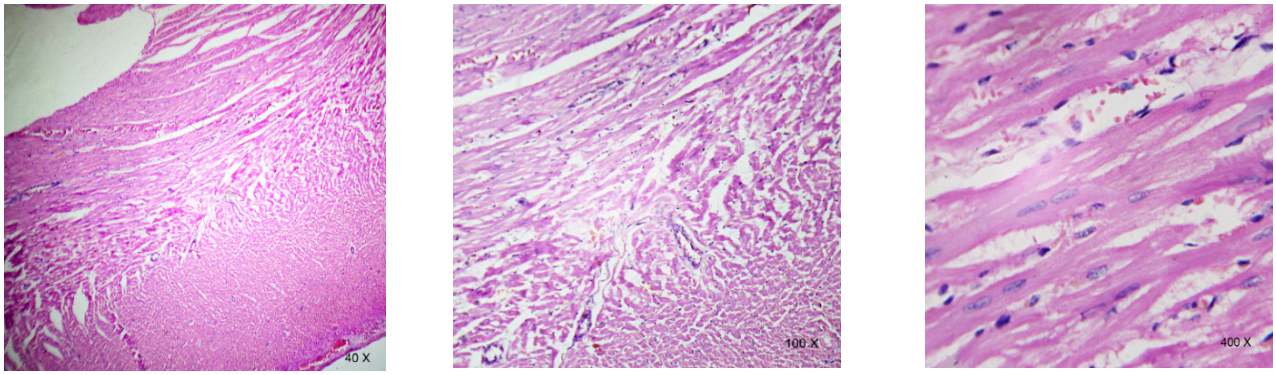
3.5 Histopathology Report of NKSP Induced Changes in Liver



(a) (b) (c) (d)

Figure 4 **(a, b)**. Pictograph of Normal control rats with clear Central Vein (CV) and hepatocytes. **(c, d)**. Pictograph of test group rats with steatosis and hydropic changes.

3.6 Histopathology Report of NKP Induced Changes in Heart



(a) (b) (c)

Figure 5. **(a)** - Pictograph of sectioned heart of control rats indicates normal myocardial fibrils. **(b)** - Pictograph of sectioned heart of test rats indicates normal myocardial fibrils. **(c)** - Pictograph of sectioned heart of confirmatory rats indicates normal myocardial fibrils.

3.7 Histopathology Report of NKP Induced Changes in Brain

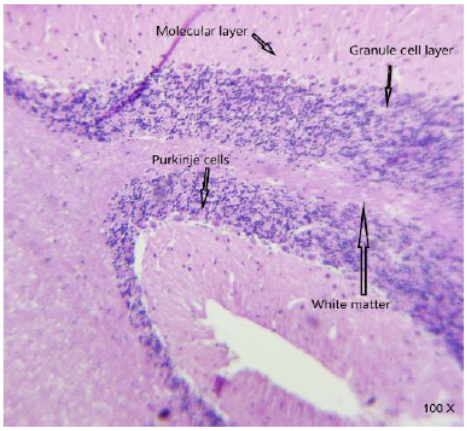
Normal microscopic structure observed in test and control group. Figure 6 Brain tissue.

3.8 Histopathology Report of NKP Induced Changes in Kidney

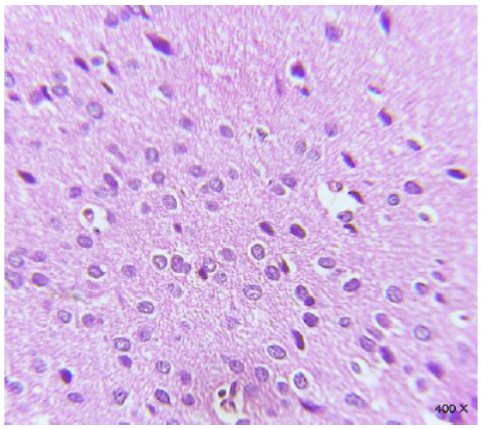
Normal microscopic structure observed in test and control group.

4. Discussions

Acute oral toxicity was carried out to establish toxicity of NKSP at 2000mg/kg single dose. In the present study oral administration of NKSP at 2000 mg/kg dose did not exhibit any signs of behavioural signs of toxicity, eating and sleeping patterns and mortality in the period of 14 days. This concludes that NKSP is safe until dose 2000 mg/kg with no observable side effects.

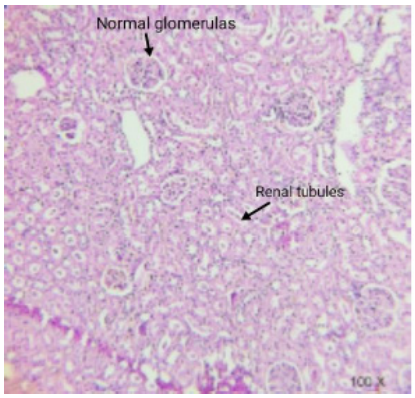


(a)

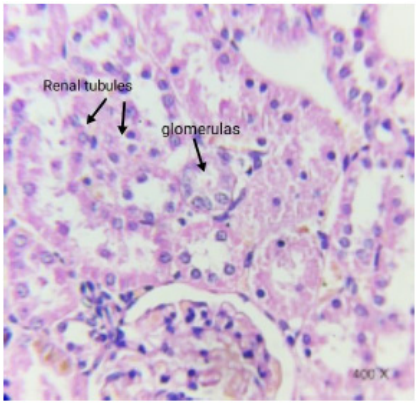


(b)

Figure 6(a, b). Normal pictograph of brain tissue.



(a)



(b)

Figure 7(a,b). Normal pictograph of Kidney tissue.

A gradual reduction in weight was observed in the period of 14 days, but there was no sudden loss in weight seen which implies that there was no harmful change in the body or organs. While in between the groups reveal non-significant (P>0.05) changes i.e. there are no observable changes and which is not associated with toxic nature of NKSP.

Assessing Liver Function Test report suggests that there are elevations in SGPT, SGOT levels specifically. Raised levels of SGPT in all groups suggests acute injury to liver tissue. Elevations in these enzymes are specific to Liver damage[17]. Elevation of SGOT in test group and not in normal control group suggests that their might be raised SGOT levels after administration of NKSP. This suggests that there is some sort of acute damage to the liver tissue causing raised levels of SGPT and SGOT in blood plasma[18]. Administration of NKSP also reports elevations in Albumin in Test group. Albumin is specific to liver and it indicates alterations in liver function. The data indicates that there might be elevations in specific liver function enzymes after administration of NKSP at higher doses.

Histopathological images Test group of rats reveal that there are acute reversible damage to liver tissue. There are hydropic changes and steatosis seen at certain places in liver. Histopathology images of heart, brain and kidney tissue indicates normal myocardial structures at 2000 mg/kg dose of NKSP.

5. Conclusions

The NKSP administered to *wistar albino* rats at 2000 mg/kg dose is relatively safe, although it may cause alterations in specific liver function enzymes SGPT and SGOT and albumin protein implying reversible liver damage at acute level. There were hydropic changes seen at certain places in liver parenchyma, cardiac myofibril, heart, brain and kidney exhibited normal structure. This concludes that NKSP is safe at dose levels lower than 2000 mg/kg.

6. Acknowledgements

This research was supported by Dr. Hemant Toshikhane, Dean, Parul Institute of Ayurveda, Parul University, Vadodara, Gujarat. who provided insight and expertise that greatly assisted the pre-clinical research.

We thank Dr. Vaishali Deshpande, Professor, Department of Kayachikitsa, Parul Institute of Ayurveda and Research, Parul University, Vadodara, Gujarat for assistance in nano technology and Mr. V. V. Mujumdar, Founder and Managing Director, Institute of Applied Research, Pune, Maharashtra for his immense experience in the field of precise herbal nano-material manufacturing.

7. References

1. Chopra A. Ayurvedic medicine and arthritis. Rheumatic Disease Clinics of North America. 2000; 26(1):133-44. https://doi.org/10.1016/S0889-857X(05)70127-7
2. Mr Y, Mitti JG, Sharma VS. Review on Churna Kalpana.
3. Morganti P. Use and potential of nanotechnology in cosmetic dermatology. Clinical, cosmetic and investigational dermatology: CCID. 2010; 3:5. https://doi.org/10.2147/CCID.S4506
4. Jain KK, Jain KK. The handbook of nanomedicine. NJ: Humana Press. 2008. https://doi.org/10.1007/978-1-60327-319-0
5. Boholm M. The use and meaning of nano in American English: Towards a systematic description. Ampersand. 2016; 3:163-73. https://doi.org/10.1016/j.amper.2016.10.001
6. Boholm M, Boholm A. The many faces of nano in newspaper reporting. Journal of Nanoparticle Research. 2012; 14(2):1-8. https://doi.org/10.1007/s11051-012-0722-y
7. Murthy CK, Lakshman SV, Buddhudu S. Indian Science Congress. Lacknow. 1985; 72:4.
8. Balaz M. Environmental Mechanochemistry. Springer International Publishing. 2021. https://doi.org/10.1007/978-3-030-75224-8
9. Kumar P, Mahajan P, Kaur R, Gautam S. Nanotechnology and its challenges in the food sector: A review. Materials Today Chemistry. 2020; 17:100332. https://doi.org/10.1016/j.mtchem.2020.100332
10. Dhand C, Dwivedi N, Loh XJ, Ying AN, Verma NK, Beuerman RW, Lakshminarayanan R, Ramakrishna S. Methods and strategies for the synthesis of diverse nanoparticles and their applications: A comprehensive overview. Rsc Advances. 2015; 5(127):105003-37. https://doi.org/10.1039/C5RA19388E
11. Rajendran D, Thulasi A, Jash S, Selvaraju S, Rao SB. Synthesis and application of nano minerals in livestock industry. Animal nutrition and reproductive physiology (recent concepts). Satish Serial Publishing House, Delhi. 2013; 517-30.
12. Pal G, Rai P, Pandey A. Green synthesis of nanoparticles: A greener approach for a cleaner future. InGreen synthesis, characterization and applications of nanoparticles. Elsevier. 2019. 1-26. https://doi.org/10.1016/B978-0-08-102579-6.00001-0
13. Nijhara R, Balakrishnan K. Bringing nanomedicines to market: regulatory challenges, opportunities, and uncertainties. Nanomedicine: Nanotechnology, Biology and Medicine. 2006; 2(2):127-36. https://doi.org/10.1016/j.nano.2006.04.005
14. Pham K, Li D, Guo S, Penzak S, Dong X. Development and *in vivo* evaluation of child-friendly lopinavir/ritonavir pediatric granules utilizing novel *in situ* self-assembly nanoparticles. Journal of Controlled Release. 2016; 226:88-97. https://doi.org/10.1016/j.jconrel.2016.02.001
15. Dr Xiaorui Zhang. WHO guidelines on safety monitoring of herbal medicines in pharmacovigilance systems. Geneva. 2004. Annexure 3(4)Part 2: 2-5.
16. https://www.sciencedaily.com/terms/nanoparticle.htm
17. Osagie-Eweka SE, Orhue NE, Omogbai EK, Amaechina FO. Toxicity Status of Methanol Leaf Extract of *Simarouba glauca* on Rat Liver. https://www.researchgate.net/publication/34696724
18. Abid R, Mahmood R. Acute and sub-acute oral toxicity of ethanol extract of *Cassia fistula* fruit in male rats. Avicenna Journal of Phytomedicine. 2019; 9(2):117.
19. Erhirhie EO, Ihekwereme CP, Ilodigwe EE. Advances in acute toxicity testing: strengths, weaknesses and regulatory acceptance. Interdisciplinary toxicology. 2018; 11(1):5-12. https://doi.org/10.2478/intox-2018-0001