**RESEARCH ARTICLE**

# Chronic Toxicity Investigations with Nilavembu Kudineer in Sprague-Dawley Rats

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**ABSTRACT**

The safety assessment of Nilavembu Kudineer (NVK), a polyherbal formulation with a wide spectrum of medicinal applications, was meticulously conducted in Sprague-Dawley rats. Over the course of 90 days, NVK was orally delivered at dosages of 100, 200, and 400 mg/kg to test for chronic toxicity. The results of this research provide important information on the relative safety of NVK and its possible effects on human health. Neither death nor any treatment-related adverse clinical manifestations were recorded during the chronic investigation. Feed intake and body weight increase were similar across animals given NVK, with the exception of a little decrease in females in the 200 and 400 mg/kg groups. No adverse findings were found in the ocular investigations performed as part of the safety study. Comprehensive analyses of urinalysis, hematological parameters, and biochemical markers subsequent to NVK administration identified minor alterations in select parameters at distinct dose levels, without any overarching systemic effects. Importantly, both gross and histopathological examinations revealed an absence of lesions directly attributable to NVK treatment. The findings contribute substantial insights to the realm of herbal medicine, providing a solid foundation for the responsible integration of NVK into diverse healthcare contexts.

**Keywords:** Nilavembu kudineer, NVK, Chronic toxicity, Polyherbal formulation.

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## INTRODUCTION

In the ancient tapestry of Siddha medicine, Nilavembu Kudineer (NVK), a classic gem in traditional Indian medicine, is highly esteemed. This herbal mixture, based on ancient wisdom, demonstrates the amazing healing synergy that comes with traditional methods. Fundamentally, Nilavembu fittingly called the "King of Bitters" is the source of NVK's strength. This marvel of nature possesses a wide range of immune-boosting properties, serving as a powerful anti-inflammatory, cholagogue, digestive aid, hepatoprotective agent, and antipyretic. Nevertheless, its impact goes beyond these classifications, reverberating throughout the domains of

Chinese medicine, Siddha, Unani, Homoeopathy, Ayurveda, and Tribal health. Furthermore, it is crucial to educate people about the potential exploitation of GAN technology and its ethical and societal ramifications. Addressing the changing landscape of GAN technology and image recognition will need responsible creativity and awareness in maintaining the integrity of digital information as we traverse the problems provided by GANs.[1-5] NVK is a monument to the ageless knowledge that resonates across generations as we walk through the halls of tradition and modernity. Its importance is international, resonating in the melodic chorus of diverse medical systems. Nilavembu's legacy thrives at NVK, capturing the spirit of age-old wisdom entwined with the never-ending pursuit of well-being.[6,7]

The powerful properties of nine different herbal elements are combined in NVK, an exceptional polyherbal preparation. These well-selected and aesthetically pleasing components consist of a wide range of useful components and a selection of botanical elements. *Andrographis paniculata*, commonly referred to as Nilavembu, is joined by the roots of *Plectranthus vettiveroides*, known as Vilamichamver. The list also encompasses the dried form of *Zingiber officinale*, or Sukku, adding a unique dimension. Adding to the mix is the stem of *Cyperus rotundus*, recognized as Koraikizhangu, offering its distinctive attributes. The whole plant of *Trichosanthes dioica*, known as Pei pudal, contributes its own botanical essence. Furthermore, the roots of *Vetiveria zizanioides*, commonly referred to as Vetiver, contribute their significance. The list expands to include the seed of *Piper nigrum*, known as Milagu, lending its distinctive character. Adding to the botanical

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medley is the whole plant of *Mollugo cerviana*, or Parpadagam. Lastly, the wood of *Santalum album*, also known as Sandanam, concludes this diverse assortment of botanical treasures. Each element brings its own unique qualities, contributing to the rich tapestry of botanical resources. Despite the wealth of pharmacological knowledge surrounding these constituent herbs, comprehensive safety evaluations following accepted testing methodologies remain essential. This is particularly crucial for ensuring the prolonged and sustainable use of NVK.[8-10] To address this, our study focuses on an intricate toxicological assessment of NVK, encompassing both acute and chronic oral toxicity tests in a rat model. While previous studies of acute toxicity study have shed light on certain aspects of NVK's safety and its antipyretic activity, our investigation aims to provide a more profound understanding.[11] We strive to establish the no observable effect level (NOEL) – a key determinant of the safe dosage range – and elucidate the nuanced interplay of these herbal constituents when administered in combination.[12-18]

Our approach integrates traditional wisdom with contemporary scientific rigor to unravel the complex safety dynamics of NVK. By doing so, we contribute to the body of knowledge surrounding herbal formulations and their potential for sustained therapeutic use. This study not only reinforces the positive attributes of NVK but also presents a comprehensive framework for evaluating the safety of polyherbal remedies in a holistic manner.

## MATERIALS AND METHODS

Nilavembu Kudineer powder was acquired from a government-authorized local store situated in the bustling metropolis of Chennai.

Using Sprague-Dawley rats, we performed a chronic oral toxicity study of NVK in accordance with the OECD's Guidelines 408 for oral toxicity, with just a few modifications to account for the unique circumstances of this investigation. All procedures involving the use of laboratory animals adhered to the "Institute Animal Ethics Committee (IAEC) guidelines, which were approved by the Committee for the Purpose of "Control and Supervision of Experiments on Animals (CPCSEA)". The work was granted approval under the reference number "IAEC-03/SES/2020/004", demonstrating our commitment to ethical and responsible animal research practices.

### Experimental Animals and Housing

In the current investigation, Sprague-Dawley rats aged between 6 and 8 weeks were carefully chosen as the study subjects. These rats were housed in controlled environments, maintaining room temperatures within the range of 20 to 24°C, humidity levels between 30 and 70%, ensuring adequate ventilation with 10 to 15 air changes per hour, and adhering to a 12-hours light-dark cycle. The rats were accommodated in polypropylene cages featuring stainless steel grill tops and clean rice husk bedding, fostering a comfortable and hygienic living space. Their nutritional needs were met with standard rodent pellet feed procured from M/s. ATNT Laboratories is based in Mumbai, India. Additionally, the rats had unrestricted access to reverse osmosis (RO) water, further contributing to their well-being. These meticulously controlled conditions were implemented to ensure the animals' overall health and welfare while sustaining a consistent and conducive setting for the duration of the study.[19,20]

### Chronic Oral Toxicity Study

Test guideline no. 408, which addresses a repeated dosage 90-days oral toxicity study in rats, is required by the Indian government's Ministry of Health and Family Welfare's Schedule Y of the Drug and Cosmetics (Second Amendment) Rules-2005",[19,20] a chronic oral toxicity study of NVK was conducted over the course of 90 days to examine any potential toxic effects of repeated oral exposure. About 100 Sprague-Dawley rats, fifty males and 50 females, were used in this study. These rodents, between the ages of 6 and 8 weeks old, were randomly split into four groups of ten male and ten female rodents. All of the NVK was made in 0.5% CMC and given orally once a day for 90 days at 0, 100, 200 and 400 mg/kg. Additionally, five rats of each gender were placed into supplementary satellite groups, corresponding to the control and high dose levels. These satellite groups received the respective treatments for the same 90-day period, followed by an extended observation phase of 28 days. After therapy ended, researchers monitored patients to see whether side effects vanished, lingered, or showed up later.

The female rats used in the research were neither pregnant nor ovulating, which is an important caveat. The dosing volume 10 mL/kg of NVK extract was consistent throughout all dose levels, including the placebo group. To prevent over- or under-dosing, each rat's dosage volume was modified depending on its most recent weight measurement. The rats were given their doses at around the same time every day. This meticulous approach ensured reliable and comprehensive data collection for the study's objectives.[19-23]

### Body Weight and Feed Intake

On the first day of the experiment and every week afterwards throughout the research, data on body weight and feed intake were painstakingly documented for all groups. During the post-treatment phase, same indicators were also meticulously tracked in the recovery groups. This approach ensured a thorough and continuous assessment of any potential changes or trends in body weight and feed consumption patterns throughout the entire study, including the recovery phase.

### Ophthalmoscopy

A hand slit lamp was used for examining the eyes of the control group and all therapy animals before the study officially began, as well as between weeks 13 and 17 for recovery groups following the induction of mydriasis with a 0.5% tropicamide solution

### Functional Observations

In week 13, grip strength (measured with a Digital Grip Strength Metre, Columbus), motor function, and acute response to visual, hearing, and proprioceptive stimuli were all assessed

in all animals. A useful observational battery was created to assess how the animals behaved in the home cage and open field settings.

### Clinical Pathology

Rats in the control and treatment groups went without food for one night at the end of week 13, whereas rats in the recovery groups went without food for two days at the end of week 17. The blood sample was drawn from retroorbital sinus. In-depth hematological and blood biochemistry tests were performed on these samples after treating them with anticoagulants such as potassium EDTA (1.5 mg/mL) and sodium heparin (200 IU/mL). The study's methodology includes a thorough evaluation of hematological parameters. Many other measurements were taken from the blood samples, such as the number of RBC, the number of reticulocytes, the hematocrit, the mean corpuscular hemoglobin, the mean corpuscular volume, the platelet count, and the WBC count. Careful manual differential leukocyte counts, including in-depth analysis of microscopic samples, were also required to guarantee precision. Using this methodical strategy, we were able to get accurate and trustworthy insights into the hematological profile of interest. For the Pt analysis, we employed a citrate bulb containing 100 mL of a sodium citrate arrangement at 3.8% per mL of blood. At 3000 rpm for 15 minutes, we centrifuged all of our blood samples.

Complete cholesterol, fatty substances, calcium, inorganic phosphorous, sodium, aspartate aminotransferase, alanine aminotransferase, soluble phosphatase, gamma-glutamyl transferase, lactate dehydrogenase, creatine, total protein, blood glucose, and blood urea nitrogen were all estimated from the serum.

This meticulous approach to data collection encompassed a broad spectrum of physiological indicators, ensuring a thorough understanding of the potential effects of the treatment and allowing for comprehensive analysis and interpretation.

### Urinalysis

In order to test for bilirubin urobilinogen, nitrite, glucose, ketones, proteins, and glucose oxidase activity in the urine. Control, treatment, and recovery groups all had urine samples taken on days 91 and 119. Microscopically, we noted the existence and frequency of analytes such pus cells, epithelial cells, casts, RBCs, and crystals.[21-22]

### Necropsy and Histopathology

At the conclusion of the experiment, all of the remaining rats were exsanguinated under the influence of carbon dioxide anesthesia and exposed to a thorough necropsy. This study recorded the absolute weights of organs with their relative weights (or organ/body weight ratios). This included the kidneys, liver, adrenals, lungs, brain activity, uterus, heart, and testes/ovaries. All the tissues and organs were conserved in 10% formalin and examined histopathologically.

### Statistical Analysis

The information underwent preliminary analysis using variance analysis (ANOVA) before further analysis with the student's t-test, Cochran t-test, and Dunnett's test. A *p-value* between 0.01 and 0.05 was chosen as the cutoff for statistical significance.

## RESULTS AND DISCUSSION

### Chronic Oral Toxicity Study

*Mortality and clinical investigations*

Throughout the 90-day dosing period and the subsequent 28-day post-dosing recovery period, animals receiving treatment with NVK at the chosen levels did not have any fatalities, unfavorable clinical symptoms, or toxic consequences.

*Body mass and feed consumption*

Over the course of the study, male rats in all treatment dose groups and recovery groups gained about as much weight as their counterparts in the control groups (Figure 1). Female rats treated to 200 and 400 mg/kg had 6.67 and 8.49% lower body weight increase compared to controls at the decision of the 90-day trial period. Over the course of the 28-day regaining period, females in the high dosage (400 mg/kg) group gained about as much weight as expected (Figure 2). At no point in the evaluation did the treatment groups vary significantly from one another in terms of feed consumption (Table 1).[24,25]

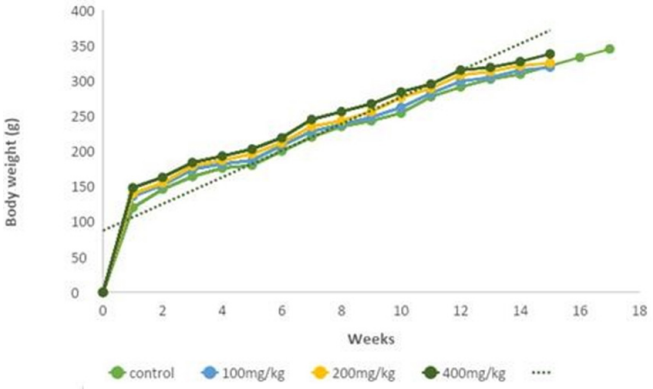


**Figure 1:** Alterations in male rats' mean body weights following oral administration of NVK for 90 days

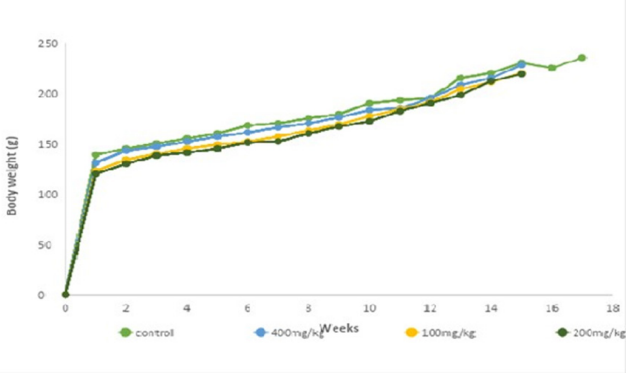


**Figure 2:** The effects of oral NVK administration on the mean body weight of female rats over 90 days

*Ophthalmoscope examination*

Ophthalmoscopic exams of rats from the control group and all other dosage groups treated with the substance did not detect any abnormalities.

*Hematological parameters*

Several statistically significant alterations in hematological parameters were discovered. The mean corpuscular volume (MCV) of men exposed to 100 mg/kg had significant reductions. Males in the 100, 200, and 400 mg/kg groups all had reduced mean corpuscular hemoglobin (MCH) and MCHC levels compared to the control group. The *p-value* for these decreases is less than 0.01, indicating their statistical significance. Females in the 100 mg/kg group had a significantly smaller mean corpuscular volume (MCV) than those in the (100, 200, and 400) mg/kg groups ($p < 0.01$). Hematocrit levels dropped significantly among women receiving a recovery dosage of 400 mg/kg (HCT; $p < 0.05$). In contrast, a number of increases were recorded. The 100 mg/kg group had substantially greater red blood cell (RBC) counts than the other two groups. The white blood cell count increase in the 200 mg/kg group was statistically significant ($p < 0.05$) in males. Increases in HCT were seen in the male 100 mg/kg group, female 200 mg/kg and female 400 mg/kg groups, respectively ($p < 0.01$). Females in the 200 mg/kg group also had a greater mean corpuscular volume (MCV) compared to individuals who were in the control group ($p < 0.05$). The study indicates notable changes in hematological parameters, including decreases in MCV, MCH, and MCHC in certain groups and increases in RBC, WBC, and HCT in others (Tables 1 and 2).[26-28]

*Serum biochemistry*

The blood biochemistry results showed no treatment-related alterations, with the exception of animals from the 400 mg/kg dose group having higher alkaline phosphatase levels ($p$ 0.01) and female rats from the 100 mg/kg group having lowered potassium levels (Tables 3 and 4).[29,30]

*Urinalysis*

None of the urine parameters showed statistically significant changes in response to the experimental conditions. This suggests that the tested doses did not have a notable impact on these specific urine-related measures.

*Organ sizes and gross pathology*

NVK therapy did not result in any noticeable pathological alterations in the animals. Although there were minor differences, organ weights were similar across treatment groups and controls overall. Notably, absolute and relative organ weights (including kidney, liver, adrenals, heart, testis, epididymis, ovaries, and uterus) differed significantly, with the exception of certain changes observed in females. In female animals, there was a noteworthy increase in relative organ weights ($p < 0.01$). In addition, the brain and spleen showed increased relative weights in rats given 100 and 200 mg/kg dosages. There was a statistically significant ($p < 0.05$) rise in relative adrenal weight in the 400 mg/kg recovery group. These findings suggest potential dose-dependent effects of NVK on organ weights, particularly in females and in specific organs at varying doses. Further investigation is required to elucidate the underlying reasons for these observed differences and their implications (Tables 5-8).[31-33]

*Histopathological evaluation stands as a pivotal cornerstone in the comprehensive assessment of toxicity*

Associated alterations in herbal drugs. Widely regarded as the gold standard, this approach offers invaluable insights into the intricate fabric of physiological responses. Figures 3 and 4

**Table 1:** Results of hematological tests performed on male rats following subchronic oral treatment of NVK for 90 days (mean SD)

| *Parameters* | *Control* | *100 mg/kg* | *200 mg/kg* | *400 mg/kg* | *Control recovery* | *400 mg/kg recovery* |
|---|---|---|---|---|---|---|
| Hemoglobin (g%) | 14.9 ± 0.7 | 14.8 ± 0.3 | 15.2 ± 0.7 | 15.3 ± 1.1 | 16.7 ± 0.6 | 16.2 ± 0.3 |
| Total RBC ($\times 10^6/\mu L$) | 6.8 ± 0.8 | 7.6* ± 0.3 | 7.1 ± 0.8 | 7.0 ± 1.0 | 8.1 ± 0.7 | 7.6 ± 0.4 |
| Hematocrit (%) | 36.5 ± 3.2 | 39.8** ± 0.8 | 39.7** ± 2.0 | 38.9* ± 2.7 | 46.0 ± 1.5 | 44.1# ± 1.0 |
| MCH (pg) | 22.1 ± 1.7 | 19.4** ± 0.6 | 21.5 ± 0.6 | 21.8 ± 1.0 | 20.9 ± 1.5 | 21.5 ± 0.9 |
| MCV ($\mu m^3$) | 53.6 ± 2.8 | 52.2 ± 1.5 | 56.3* ± 1.5 | 55.9 ± 2.9 | 57.3 ± 2.9 | 58.2 ± 2.2 |
| Platelets ($\times 10^3/\mu L$) | 482.8 ± 86.5 | 470.7 ± 69.6 | 432.6 ± 84.1 | 401.4 ± 67.0 | 467.2 ± 37.7 | 425.6 ± 48.2 |
| MCHC (%) | 41.2 ± 1.8 | 37.2** ± 0.4 | 38.2** ± 0.3 | 39.0** ± 0.3 | 36.4 ± 0.7 | 36.9 ± 0.5 |
| Total WBC ($\times 10^3/\mu L$) | 10.7 ± 3.7 | 8.9 ± 2.5 | 10.3 ± 3.8 | 8.5 ± 3.4 | 8.4 ± 2.1 | 8.9 ± 2.8 |
| Neutrophils (%) | 21.0 ± 3.8 | 21.2 ± 3.5 | 21.1 ± 3.4 | 21.1 ± 3.4 | 21.8 ± 4.3 | 21.2 ± 3.0 |
| Lymphocytes (%) | 75.4 ± 4.0 | 75.8 ± 3.5 | 75.6 ± 2.7 | 75.3 ± 2.9 | 75.2 ± 4.0 | 75.2 ± 3.2 |
| Eosinophils (%) | 1.3 ± 1.0 | 1.2 ± 0.8 | 1.1 ± 0.7 | 1.4 ± 0.7 | 0.8 ± 0.8 | 1.2 ± 0.8 |
| Monocytes (%) | 2.4 ± 1.2 | 1.9 ± 0.5 | 2.2 ± 1.0 | 2.2 ± 0.7 | 2.4 ± 1.1 | 2.5 ± 1.1 |
| Basophils (%) | 0.0 ± 0.0 | 0.0 ± 0.0 | 0.0 ± 0.0 | 0.0 ± 0.0 | 0.0 ± 0.0 | 0.0 ± 0.0 |
| Prothrombin Time (s) | 14.7 ± 2.4 | 14.5 ± 2.4 | 14.7 ± 2.6 | 15.1 ± 2.6 | 15.1 ± 2.9 | 15.8 ± 2.9 |

*P < 0.05 versus control group. **P < 0.01 versus control group. #P < 0.05 versus recovery control group.

**Table 2:** Results of hematological analysis (mean SD) in female rats exposed to subchronic oral NVK for 90 days

| *Parameters* | *Control* | *100 mg/kg* | *200 mg/kg* | *400 mg/kg* | *Control recovery* | *400 mg/kg recovery* |
|---|---|---|---|---|---|---|
| Hb (g%) | 14.9 ± 0.7 | 14.8 ± 0.2 | 15.2 ± 0.6 | 15.3 ± 1.1 | 16.7 ± 0.5 | 16.2 ± 0.3 |
| Rt (%) | 1.6 ± 0.4 | 1.5 ± 0.4 | 1.8 ± 0.6 | 1.7 ± 0.4 | 1.6 ± 0.3 | 1.6 ± 0.3 |
| Total RBC ($\times 10^6/\mu L$) | 6.8 ± 0.8 | 7.6* ± 0.3 | 7.1 ± 0.8 | 7.0 ± 0.9 | 8.0 ± 0.7 | 7.6 ± 0.4 |
| MCV ($\mu m^3$) | 53.6 ± 2.8 | 52.1 ± 1.5 | 56.2* ± 1.5 | 55.9 ± 2.9 | 57.3 ± 2.8 | 58.2 ± 2.2 |
| HCT (%) | 36.5 ± 3.2 | 39.8** ± 0.8 | 39.7** ± 1.9 | 38.9* ± 2.6 | 46.0 ± 1.5 | 44.1# ± 0.9 |
| MCHC (%) | 41.2 ± 1.8 | 37.1** ± 0.4 | 38.1** ± 0.3 | 39.0** ± 0.3 | 36.3 ± 0.7 | 36.9 ± 0.4 |
| MCH (pg) | 22.1 ± 1.6 | 19.3** ± 0.6 | 21.4 ± 0.6 | 21.8 ± 1.0 | 20.8 ± 1.5 | 21.5 ± 0.8 |
| Total WBC ($\times 10^3/\mu L$) | 10.6 ± 3.7 | 8.9 ± 2.4 | 10.2 ± 3.8 | 8.5 ± 3.4 | 8.3 ± 2.1 | 8.8 ± 2.8 |
| Platelets ($\times 10^3/\mu L$) | 482.8 ± 86.5 | 470.7 ± 69.5 | 432.6 ± 84.1 | 401.4 ± 67.0 | 467.2 ± 37.7 | 425.6 ± 48.1 |
| L (%) | 75.4 ± 4.0 | 75.8 ± 3.5 | 75.6 ± 2.7 | 75.3 ± 2.9 | 75.2 ± 4.0 | 75.2 ± 3.2 |
| N (%) | 21.0 ± 3.8 | 21.2 ± 3.4 | 21.1 ± 3.3 | 21.1 ± 3.4 | 21.8 ± 4.3 | 21.2 ± 3.0 |
| M (%) | 2.4 ± 1.2 | 1.9 ± 0.5 | 2.2 ± 1.0 | 2.2 ± 0.7 | 2.4 ± 1.1 | 2.4 ± 1.1 |
| E (%) | 1.3 ± 0.9 | 1.2 ± 0.8 | 1.1 ± 0.7 | 1.4 ± 0.7 | 0.8 ± 0.8 | 1.2 ± 0.8 |
| Pt (s) | 14.7 ± 2.4 | 14.5 ± 2.4 | 14.6 ± 2.6 | 15.1 ± 2.6 | 15.0 ± 2.9 | 15.8 ± 2.9 |
| B (%) | 0.0 ± 0.0 | 0.0 ± 0.0 | 0.0 ± 0.0 | 0.0 ± 0.0 | 0.0 ± 0.0 | 0.0 ± 0.0 |

*$P < 0.05$ versus control group. **$P < 0.01$ versus control group. #$P < 0.05$ versus recovery control group.

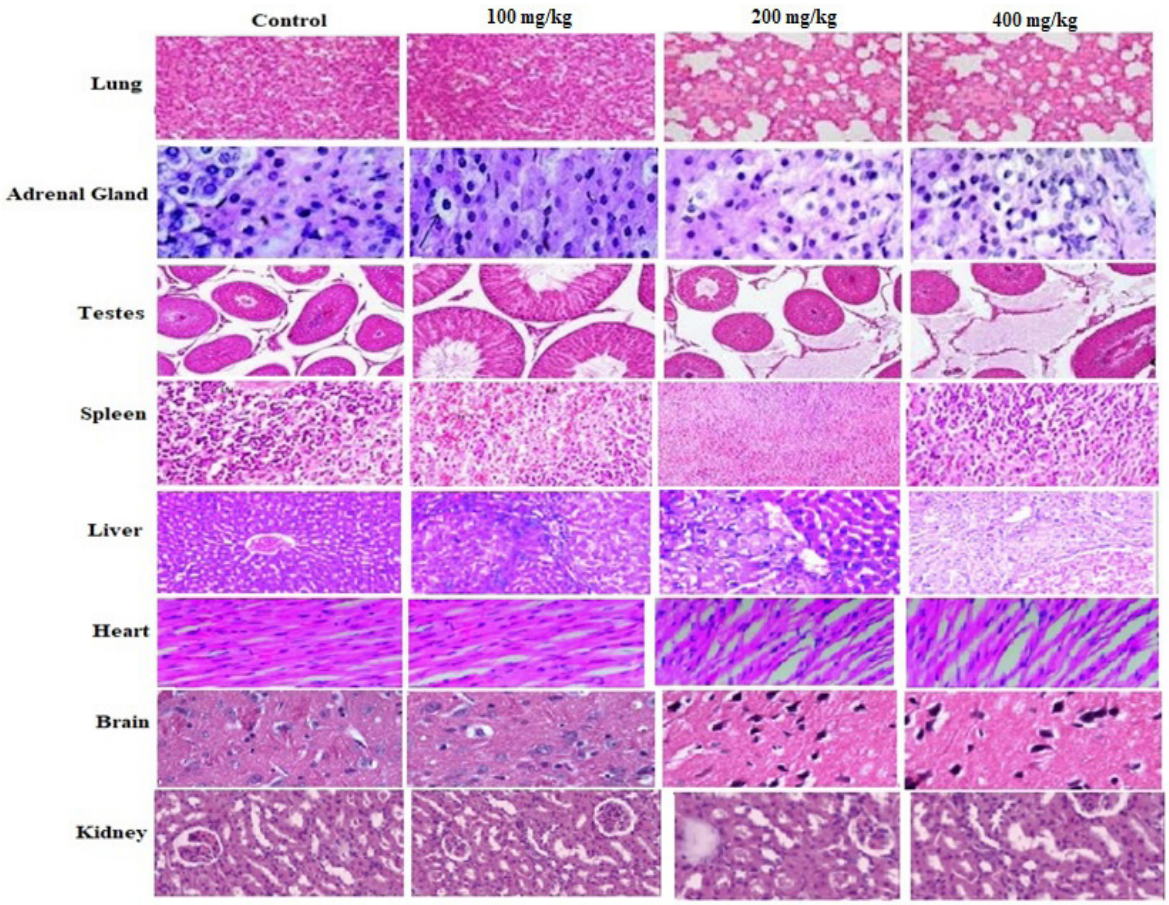


**Figure 3:** The histopathology of various organs of different groups organs treated in male rats with NVK orally for 90 days

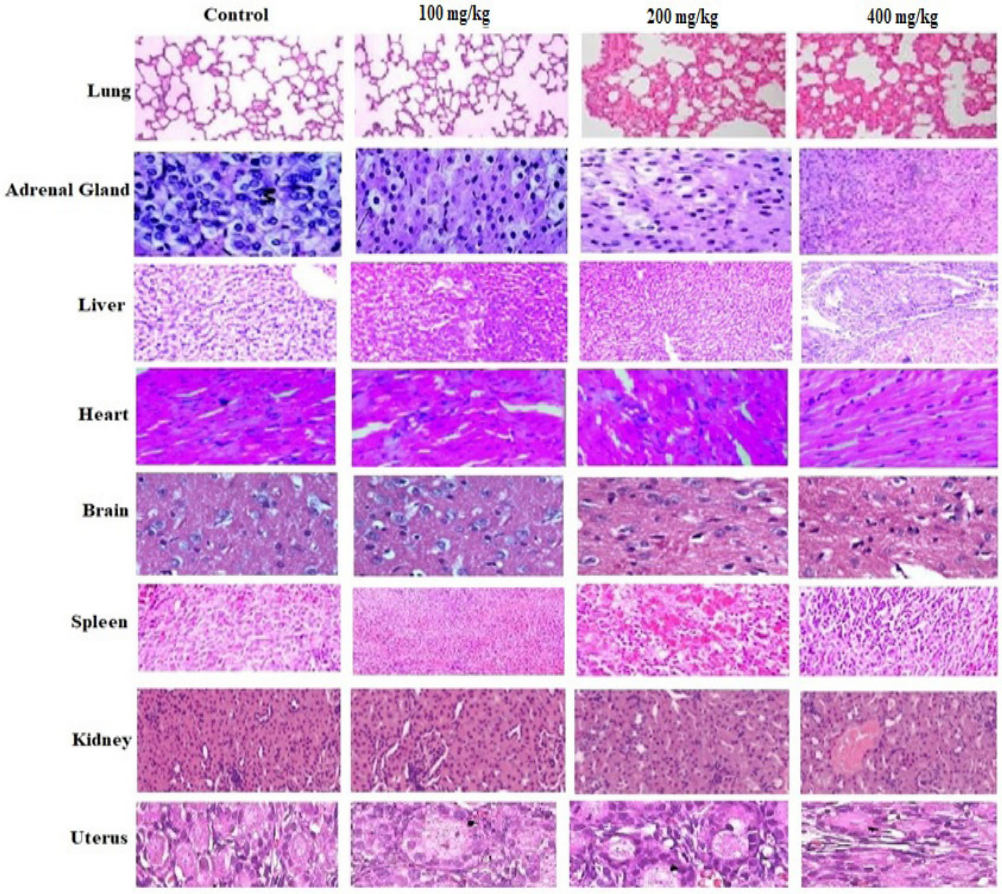


**Figure 4:** The histopathology of various organs of different groups organs treated in female rats with NVK orally for 90 days

eloquently illustrate the histopathological transformations observed in the liver sections. Upon meticulous examination, it becomes evident that the oral administration of NVK extracts did not precipitate any deleterious or anomalous conditions within various tissues.[34-36]

## DISCUSSION

Herbal therapies and medicinal plants have long been integral to human and animal healthcare, offering a rich repository of wellness-enhancing potential. As we delve into the realm of natural remedies, the significance of understanding the long-term impact of substance exposure gains prominence. Studying the possible health concerns of long-term use, such as target organ toxicity, cumulative effects, and non-toxic dosage levels, falls squarely within the purview of repeated oral dose toxicity research.[21] The purpose of this 14-day research was to determine an appropriate dose range in accordance with stringent regulatory standards for subchronic toxicity evaluation. In accordance with accepted standards in the field of sub-chronic toxicity assessment, this preliminary research allowed for careful dosage selection in the following 90-day oral toxicity study. Subchronic oral toxicity research in rats was stratified into three dosage levels based on careful results from the dose range finding investigation: 100, 200 and 400 mg/kg b.w. The lack of deaths or toxic symptoms during administration and subsequent recovery periods after oral administration of NVK at dosages up to 400 mg/kg b.w. for 90 days is intriguing.

Further insights emerged from functional observation and ophthalmoscopic examinations, where the physiological responses of treated animals mirrored those of the control group. This harmony underscores the non-disruptive nature of NVK administration within the studied domains. Interpreting weight variation is a crucial aspect of toxicity testing that is impacted by established standards for toxicity testing.[19,21] For chronic exposure safety characterization, a 10% decrease in body weight or progress rate is used as a benchmark.[37-39] In particular, male rats in similar groups, as well as both sexes in the treatment and recovery groups, demonstrated consistent growth trends whereas female rats in the mid- and high-dose groups showed minor weight decrease. In contrast to obvious toxicological implications, the observed changes in body weight are consistent with normal physiological dynamics, as shown by this discrepancy.

The endpoint values for the measured hematological variables (including MCH, MCV, HCT, MCHC, total WBC, and total RBC) were slightly different from the control group. However, no histological abnormalities were found despite these variances

**Table 3:** Male rats were given NVK orally for 90 days, and the results in clinical biochemistry were analysed (mean ± SD)

| *Parameters* | *Control* | *100 mg/kg* | *200 mg/kg* | *400 mg/kg* | *Control recovery* | *400 mg/kg recovery* |
|---|---|---|---|---|---|---|
| Total protein (g%) | 7.75 ± 0.5 | 7.63 ± 0.5 | 7.76 ± 0.6 | 7.58 ± 0.5 | 7.49 ± 0.5 | 7.47 ± 0.4 |
| Bilirubin (mg%) | 0.69 ± 0.2 | 0.67 ± 0.2 | 0.73 ± 0.1 | 0.69 ± 0.1 | 0.72 ± 0.2 | 0.66 ± 0.2 |
| Albumin (g%) | 3.42 ± 0.5 | 3.29 ± 0.4 | 3.49 ± 0.4 | 3.57 ± 0.4 | 3.65 ± 0.2 | 3.58 ± 0.3 |
| Triglycerides (mg%) | 106.30 ± 8.7 | 102.10 ± 7.6 | 107.50 ± 7.2 | 108.20 ± 3.4 | 106.00 ± 4.5 | 107.20 ± 1.3 |
| Blood sugar (mg%) | 89.60 ± 10.3 | 92.40 ± 10.6 | 91.67 ± 9.2 | 88.87 ± 8.3 | 96.56 ± 7.1 | 97.67 ± 12.3 |
| BUN (mg%) | 36.20 ± 6.1 | 37.10 ± 8.1 | 41.20 ± 4.6 | 37.60 ± 6.4 | 40.50 ± 2.3 | 39.25 ± 4.4 |
| Cholesterol (mg%) | 62.30 ± 5.6 | 58.60 ± 2.8 | 63.30 ± 6.3 | 64.30 ± 4.9 | 63.70 ± 4.1 | 62.28 ± 6.4 |
| Creatinine (mg%) | 0.97 ± 0.1 | 0.98 ± 0.2 | 0.99 ± 0.1 | 0.98 ± 0.2 | 1.02 ± 0.2 | 1.04 ± 0.2 |
| AST (IU/L) | 62.00 ± 4.1 | 61.40 ± 4.7 | 60.87 ± 3.3 | 64.26 ± 4.8 | 61.85 ± 8.1 | 60.34 ± 6.2 |
| ALP (IU/L) | 69.50 ± 6.8 | 69.92 ± 5.9 | 70.86 ± 6.7 | 115.38 ± 14.4 | 73.20 ± 8.5 | 66.30 ± 5.1 |
| ALT (IU/L) | 43.80 ± 6.6 | 38.87 ± 7.9 | 41.45 ± 9.0 | 42.50 ± 6.1 | 38.89 ± 3.2 | 37.68 ± 6.5 |
| LDH (IU/L) | 348.30 ± 31.8 | 364.00 ± 27.0 | 346.30 ± 28.8 | 361.60 ± 29.5 | 352.40 ± 32.3 | 338.00 ± 34.6 |
| Sodium (mmol/L) | 141.35 ± 5.6 | 148.20 ± 6.6 | 141.20 ± 6.7 | 136.50 ± 5.7 | 138.60 ± 1.1 | 140.40 ± 6.2 |
| γGT (U/L) | 14.50 ± 2.7 | 16.70 ± 4.5 | 14.80 ± 2.9 | 14.90 ± 3.3 | 16.80 ± 4.2 | 17.00 ± 1.6 |
| CPK (IU/L) | 63.60 ± 5.0 | 62.30 ± 5.0 | 66.0 ± 4.1 | 64.60 ± 3.6 | 63.50 ± 3.8 | 67.40 ± 3.0 |
| Chloride (mmol/L) | 102.66 ± 3.5 | 101.50 ± 2.9 | 102.70 ± 3.2 | 101.40 ± 2.9 | 102.20 ± 2.7 | 101.80 ± 2.8 |
| Potassium (mmol/L) | 3.70 ± 0.4 | 3.59 ± 0.3 | 3.56 ± 0.2 | 3.75 ± 0.3 | 3.81 ± 0.2 | 3.84 ± 0.2 |
| Phosphorus (mg%) | 4.56 ± 0.3 | 4.34 ± 0.5 | 4.26 ± 0.8 | 4.09 ± 0.8 | 4.13 ± 0.8 | 4.44 ± 0.3 |
| Calcium (mg%) | 9.74 ± 0.6 | 9.55 ± 0.5 | 9.63 ± 0.6 | 9.68 ± 0.7 | 9.96 ± 0.5 | 10.04 ± 0.7” |

**Table 4:** Clinical biochemistry information gathered from 90-day-long NVK-oral treatment studies in female rats (mean ± SD)

| *Parameters* | *Control* | *100 mg/kg* | *200 mg/kg* | *400 mg/kg* | *Control Recovery* | *400 mg/kg recovery* |
|---|---|---|---|---|---|---|
| Total protein (g%) | 7.64 ± 0.3 | 7.67 ± 0.5 | 7.93 ± 0.6 | 7.75 ± 0.5 | 7.78 ± 0.3 | 7.54 ± 0.3 |
| Bilirubin (mg%) | 0.58 ± 0.1 | 0.69 ± 0.1 | 0.83 ± 0.0 | 0.74 ± 0.1 | 0.66 ± 0.1 | 0.72 ± 0.1 |
| Albumin (g%) | 3.70 ± 0.3 | 3.66 ± 0.4 | 3.48 ± 0.4 | 3.64 ± 0.5 | 3.39 ± 0.4 | 3.69 ± 0.3 |
| Triglycerides (mg%) | 106.70 ± 9.1 | 105.30 ± 9.0 | 103.50 ± 9.4 | 106.30 ± 7.8 | 104.40 ± 3.7 | 105.87 ± 3.2 |
| Blood sugar (mg%) | 93.18 ± 11.8 | 88.87 ± 13.9 | 93.80 ± 12.5 | 85.60 ± 8.4 | 91.70 ± 10.9 | 91.20 ± 7.3 |
| BUN (mg%) | 39.64 ± 4.8 | 41.78 ± 4.6 | 38.40 ± 5.0 | 36.92 ± 5.7 | 37.40 ± 7.4 | 44.40 ± 4.3 |
| Cholesterol (mg%) | 64.43 ± 5.5 | 61.80 ± 4.6 | 64.50 ± 6.5 | 64.20 ± 6.0 | 65.20 ± 4.0 | 66.40 ± 5.7 |
| Creatinine (mg%) | 0.99 ± 0.1 | 0.98 ± 0.1 | 0.98 ± 0.1 | 0.96 ± 0.1 | 0.97 ± 0.1 | 1.03 ± 0.1 |
| AST (IU/L) | 58.90 ± 5.4 | 61.20 ± 5.6 | 62.50 ± 4.6 | 63.70 ± 4.4 | 62.20 ± 8.3 | 65.80 ± 6.0 |
| ALP (IU/L) | 64.90 ± 6.1 | 66.70 ± 5.3 | 68.60 ± 8.3 | 108.80** ± 8.5 | 72.00 ± 9.1 | 70.60 ± 6.3 |
| LDH (IU/L) | 359.50 ± 33.6 | 358.50 ± 31.0 | 372.50 ± 16.6 | 360.60 ± 34.9 | 344.60 ± 26.7 | 367.80 ± 34.5 |
| ALT (IU/L) | 41.30 ± 6.7 | 42.40 ± 7.4 | 38.40 ± 8.0 | 39.60 ± 9.4 | 39.60 ± 6.9 | 36.60 ± 7.5 |
| γGT(U/L) | 15.60 ± 2.9 | 14.60 ± 4.3 | 15.90 ± 2.8 | 15.60 ± 3.5 | 15.90 ± 2.4 | 16.60 ± 3.5 |
| CPK (IU/L) | 63.70 ± 4.6 | 63.20 ± 5.1 | 64.50 ± 4.6 | 62.60 ± 4.7 | 66.40 ± 3.2 | 65.00 ± 4.7 |
| Sodium (mmol/L) | 144.10 ± 5.3 | 137.80 ± 5.7 | 147.80 ± 6.2 | 136.70 ± 6.3 | 138.40 ± 3.4 | 137.00 ± 5.1 |
| Potassium (mmol/L) | 3.73 ± 0.3 | 2.96* ± 0.1 | 3.93 ± 0.2 | 3.68 ± 0.3 | 3.77 ± 0.2 | 3.79 ± 0.1 |
| Chloride (mmol/L) | 102.90 ± 3.5 | 103.80 ± 1.4 | 102.67 ± 3.5 | 101.20 ± 2.9 | 102.50 ± 1.7 | 103.18 ± 1.8 |
| Phosphorus (mg%) | 4.83 ± 0.3 | 4.54 ± 0.3 | 4.51 ± 0.4 | 4.33 ± 0.4 | 4.57 ± 0.4 | 4.09 ± 0.7 |
| Calcium (mg%) | 9.54 ± 0.6 | 9.42 ± 0.7 | 9.49 ± 0.7 | 9.47 ± 0.7 | 9.54 ± 0.5 | 9.78 ± 0.7” |

**Table 5:** Male rat organ weights (mean SD) following subchronic oral treatment of NVK for 90 days

| *Organ* | *Control* | *100 mg/kg* | *200 mg/kg* | *400 mg/kg* | *Control recovery* | *400 mg/kg recovery* |
|---|---|---|---|---|---|---|
| Terminal body weight | 358.9 ± 18.3 | 342.7 ± 28.11 | 332.90 ± 24.41 | 337.11 ± 32.93 | 367.32 ± 29.61 | 346.08 ± 35.86 |
| Liver | 11.62 ± 1.22 | 10.544 ± 1.26 | 10.307 ± 2.07 | 10.314 ± 1.80 | 8.769 ± 1.68 | 9.520 ± 1.10 |
| Brain | 1.998 ± 0.1 | 1.968 ± 0.06 | 1.878 ± 0.12 | 1.971 ± 0.06 | 1.975 ± 0.17 | 2.013 ± 0.05 |
| Adrenals | 0.0516 ± 0.009 | 0.0593 ± 0.009 | 0.056 ± 0.01 | 0.0524 ± 0.01 | 0.0568 ± 0.01 | 0.0526 ± 0.01 |
| Kidneys | 2.567 ± 0.22 | 2.488 ± 0.225 | 2.367 ± 0.30 | 2.507 ± 0.30 | 2.472 ± 0.34 | 2.570 ± 0.24 |
| Heart | 1.138 ± 0.100 | 1.046 ± 0.09 | 0.996 ± 0.14 | 1.008 ± 0.12 | 0.994 ± 0.11 | 1.057 ± 0.06 |
| Testes | 2.883 ± 0.27 | 2.844 ± 0.27 | 3.000 ± 0.34 | 2.859 ± 0.24 | 2.637 ± 0.26 | 2.858 ± 0.40 |
| Lungs | 1.616 ± 0.24 | 1.769 ± 0.22 | 1.567 ± 0.34 | 1.623 ± 0.15 | 1.489 ± 0.26 | 1.321 ± 0.57 |
| Spleen | 1.058 ± 0.22 | 1.126 ± 0.34 | 1.203 ± 0.35 | 1.176 ± 0.18 | 1.086 ± 0.30 | 1.190 ± 0.24 |

**Table 6:** Organ weights in female rats given NVK orally subchronically for 90 days (mean ± SD, g)

| *Organ* | *Control* | *100 mg/kg* | *200 mg/kg* | *400 mg/kg* | *Control recovery* | *400 mg/kg recovery* |
|---|---|---|---|---|---|---|
| Terminal body weight | 235.66 ± 12.4 | 228.66 ± 15.67 | 216.17 ± 13.90 | 214.87 ± 6.75 | 268.88 ± 20.45 | 256.00 ± 12.63 |
| Liver | 6.870 ± 1.06 | 8.069 ± 0.49 | 7.072 ± 0.88 | 6.121 ± 0.57 | 8.379 ± 1.16 | 7.535 ± 0.71 |
| Brain | 1.843 ± 0.08 | 1.909 ± 0.08 | 1.805 ± 0.08 | 1.719 ± 0.08 | 1.837 ± 0.06 | 1.824 ± 0.06 |
| Adrenals | 0.0676 ± 0.02 | 0.0757 ± 0.08 | 0.0646 ± 0.08 | 0.0638 ± 0.01 | 0.0608 ± 0.02 | 0.0712 ± 0.02 |
| Kidneys | 1.620 ± 0.15 | 1.665 ± 0.19 | 1.641 ± 0.18 | 1.449 ± 0.09 | 1.586 ± 0.27 | 1.615 ± 0.23 |
| Heart | 0.764 ± 0.07 | 0.797 ± 0.05 | 0.750 ± 0.08 | 0.736 ± 0.09 | 0.828 ± 0.14 | 0.833 ± 0.010 |
| Ovaries | 0.0956 ± 0.01 | 0.1077 ± 0.03 | 0.0942 ± 0.03 | 0.0956 ± 0.01 | 0.146 ± 0.04 | 0.1488 ± 0.03 |
| Lungs | 1.307 ± 0.16 | 1.588 ± 0.43 | 1.534 ± 0.41 | 1.337 ± 0.17 | 1.007 ± 0.12 | 1.02 ± 0.14 |
| Spleen | 0.754 ± 0.17 | 0.832 ± 0.01 | 0.935 ± 0.19 | 0.725 ± 0.09 | 1.010 ± 0.07 | 0.868 ± 0.11 |
| Uterus | 0.545 ± 0.16 | 0.589 ± 0.13 | 0.563 ± 0.19 | 0.487 ± 0.09 | 0.488 ± 0.12 | 0.582 ± 0.06 |

**Table 7:** Relative organ weights, as a percentage of fasting body weight, were calculated for male rats that were orally administered NVK for 90 days

| *Organ* | *Control* | *100 mg/kg* | *200 mg/kg* | *400 mg/kg* | *Control recovery* | *400 mg/kg recovery* |
|---|---|---|---|---|---|---|
| Terminal body weight (g) | 367.9 ± 18.41 | 352.77 ± 27.01 | 332.90 ± 24.71 | 349.11 ± 32.83 | 367.37 ± 28.61 | 347.08 ± 38.86 |
| Liver | 3.33 ± 0.30 | 3.08 ± 0.28 | 3.08 ± 0.54 | 3.03 ± 0.38 | 2.41 ± 0.57 | 2.79 ± 0.57 |
| Brain | .56 ± .03 | .57 ± .05 | .57 ± .04 | .59 ± .06 | .54 ± .04 | .58 ± .06 |
| Kidneys | .74 ± .08 | .73 ± .06 | .71 ± .06 | .74 ± .04 | .68 ± .11 | .75 ± .14 |
| Adrenals | .01 ± .01 | .02 ± .01 | .02 ± .01 | .02 ± .01 | .02 ± .04 | .02 ± .04 |
| Lungs | .46 ± .07 | .52 ± .07 | .48 ± .10 | .48 ± .06 | .41 ± .10 | .39 ± .18 |
| Heart | .32 ± .02 | .31 ± .02 | .30 ± .03 | .30 ± .03 | .27 ± .03 | .39 ± .04 |
| Spleen | .30 ± .06 | .33 ± .11 | .36 ± .10 | .35 ± .06 | .30 ± .09 | .34 ± .04 |
| Testes | .83 ± .06 | .83 ± .10 | .97 ± .12 | .85 ± .12 | .73 ± .11 | .84 ± .18 |

being clearly visible. These modifications do not represent substantial pathogenic changes, as shown by the fact that gross necropsy and histology both confirm the overall hemopoietic organs' integrity. The 400 mg/kg group's elevated alkaline phosphatase levels demand attention when analyzing blood biochemistry. However, alkaline phosphatase is ubiquitous in various tissues, including the liver and bone, the lack of corroborating macroscopic, microscopic evidence, or notable elevation in other organ damage indicators negates pronounced biological significance. Moreover, isolated instances of

**Table 8:** Organ weight distributions (mean SD) in female rats given oral NVK for 90 days compared to controls

| *Organ* | *Control* | *100 mg/kg* | *200 mg/kg* | *400 mg/kg* | *Control recovery* | *400 mg/kg recovery* |
|---|---|---|---|---|---|---|
| Terminal body weight (g) | 242.66 ± 11.33 | 238.66 ± 14.67 | 216.817 ± 10.90 | 244.97 ± 6.75 | 238.78 ± 20.25 | 235.00 ± 13.8 |
| Liver | 2.89 ± 0.52 | 3.73 ± 0.23 | 3.27 ± 0.33 | 2.88 ± 0.20 | 3.12 ± 0.30 | 3.60 ± 0.33 |
| Brain | 0.78 ± 0.07 | 0.82 ± 0.030** | 0.74 ± 0.04 | 0.77 ± 0.02 | 0.68 ± 0.01 | 0.79 ± 0.03 |
| Adrenals | .029 ± .04 | .033 ± .004 | .031 ± .01 | .031 ± .01 | .023 ± .01 | .028 ± .005 |
| Kidneys | .693 ± .07 | .763 ± .07 | .715 ± .08 | .712 ± .05 | .573 ± .08 | .634 ± .06 |
| Heart | .33 ± .06 | .35 ± .04 | .35 ± .05 | .36 ± .04 | .31 ± .06 | .33 ± .07 |
| Ovaries | .0421 ± .01 | .0492 ± .01 | .0437 ± .01 | .0426 ± .01 | .0521 ± .01 | .0587 ± .01 |
| Lungs | .559 ± .08 | .678 ± .17 | .647 ± .16 | .653 ± .07 | .365 ± .04 | .389 ± .03 |
| Spleen | .334 ± .07 | .361 ± .04 | .425** ± .11 | .348 ± .05 | .329 ± .03 | .334 ± .05 |
| Uterus | .246 ± .06 | .260 ± .05 | .273 ± .08 | .260 ± .04 | .182 ± .03 | .215 ± .04 |

serum potassium level reduction in the low-dose male group further exemplify the need for comprehensive interpretation, particularly when devoid of broader toxicological context. In parallel, organ weight analysis established the harmonious correlation of treated animals' absolute and relative organ weights with control groups. Occasional minor increases in relative weights of select organs were observed, deemed non-dosage-dependent and devoid of toxicological pertinence. Histological congruence between control and high dosage treatment groups reinforces the contextual understanding of these observations.

As the tapestry of chronic toxicity assessment unfolds, histopathology studies emerge as a vital thread. This microscopic exploration of tissues and organs unveils structural insights that illuminate the impact of NVK. This intricate exploration paints a clearer canvas of potential health implications, enhancing our grasp of herbal formulations' safety landscape**.**

## CONCLUSION

In conclusion, there were no appreciable treatment-related clinical symptoms of toxicity observed after NVK extract was regularly consumed orally for 90 days at different dose levels. Both male and female rats benefited equally from this thorough examination. The no observable effect level (NOEL) was successfully established within the confines of the long-term oral toxicity research. Interestingly, this NOEL was found to be 200 mg/kg for both male and female Sprague-Dawley rats**.**

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