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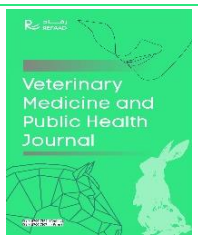


# Studying the Effect of nano-extract of Nerium Oleander Leaves on the Histological Structure of the Liver in Male Laboratory Mice

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**Abstract:**

**Objectives:** The purpose of the research was to see how a nano-extract (zinc oxide) of oleander leaves affected the histological structure of the liver in male laboratory mice aged (7-8) weeks and weighing (28-35 g).

**Methods**: They were split up into three groups, with ten mice in each. The control group made the first group. The second group was treated with an oleander leaf nano-extract at a concentration of (20 mg/kg). The group that was administered a 30 mg/kg dosage of oleander leaf nano-extract is the third group. For 30 to 45 days on a daily basis, a (30 mg/kg) concentration of the oleander leaf nano-extract was used. Half of the rodents were sacrificed at the end of the initial thirty days, and histological sections of the liver were obtained.

**Results:** Histological sections of the liver were obtained from the residual half, which was sacrificed after 45 days when the experiment ended. Animals injected with a Nano-extract of *oleander* leaves at a dose of (20 mg/kg) for 40 days had aberrant liver structure, cells, and histological abnormalities. Animals that received a 45-day injection of a (30 mg/kg) dosage of *oleander* leaf nano-extract also had pathological alterations and aberrant liver and central vein cell morphology.

**Conclusion**s: The results showed the toxic effects of the Nano-extract of the oleander plant and the xistence of histological alterations in the histological structure of the liver in groups treated with varying amounts of oleander leaf nano-extract.

***Keywords:*** *zinc oxide; nanoparticles; Nerium oleander.*

## 1 Introduction

The liver, the body's biggest organ and gland, located in the upper right quadrant of the abdomen, below the diaphragm and right of the stomach. The sickle ligament divides the liver into two larger lobes. The gastro hepatic ligament, which has neurovascular features like the hepatic division of the ambiguous nerve, connects the stomach to the left hepatic lobe and the liver to the digestive system. Furthermore, the liver transports oxygenated and deoxygenated blood from the duodenum and portal structures to the heart via the hepatoduodenal ligament and portal liver. The transverse colon is occasionally in close touch with the right lobe (Abdel-Misih and Bloomston, 2010).

A medicinal plant belonging to the Apocynaceae family, *Nerium oleander* is also referred to as sugar apple, sugar sweet, and sugar pineapple. There are over 2,300 species and 135 genera in the Apocynaceae family. *Annona*, with 166 species, is the most significant genus with the most species. *"Annona"* refers to *"annual production"* in Greek (Kusmardiyani *et al*., 2020).

Apocynaceae plants are characterised by their ability to synthesise secondary metabolites, including congenital acerogenins (ACGs), essential oils, phenols, and alkaloids that are exclusive to this family. In their study, Gonzalez-montelongo et al. (2010) discovered the Uvaricin polyketide. This compound was obtained from the roots of Ovaria acuminate, which represents the first identification of an ACG. This polyketide represented the initial group of ACGs to be described. All of the sections of the *N. oleander* plant, which is a multipurpose plant with edible fruits and the potential to provide industrial and medical goods, have bioactive qualities. Numerous studies have been conducted to determine the phytochemical, pharmacological, and antibacterial qualities of the leaves (Gupta *et al*., 305; Thafar *et al*.,2016). Diabetes, ulcers, heart damage, worms, constipation, bleeding, painful urination, heat, thirst, cancer, and convulsions are treated with the plant (Shirwaikar *et al*., 304; Muna *et al*.,2020). The antioxidant characteristics of the plant species *N. oleander* have earned it recognition as a medicinal plant. The plant also contains alkaloids (Kumar *et al*., 304; Shirwaikar *et al*., 304).

### 1.1 Aim of the Study

The aim of the current investigation is to determine the influence of Nano-extract (zinc oxide) of Oleander leaves on the histological structure for the liver of male laboratory mice.

## 2 Materials and Methods

- Preparing alcoholic extract from *N.oleander* leaves powder: The method was employed for preparing the leaves metabolic extract of the *N.oleander* plant (Chen *et al*., 2012) with certain alternations. Using the process with certain changes, nanoparticles (Zinc Oxide) were synthesised from the metabolic sour N.oleander leaves extract (Upadhyaya *et al*., 2018).
- The research used 30 mature white male Albino mice weighing (28-35 g) and aged (7 - 8 weeks). The animals were housed in plastic cages secured with iron clamps to which water bottles were connected. Sawdust is used to cover the cages, which are then regularly replaced. The animals are given a natural diet of pellets while being kept in a laboratory setting with a temperature range of 20 to 25 degrees Celsius. Before the experiment began, the animals were left alone for 14 days to get used to their new environment. During this time, they were given water and a specially made food (Ward, 1970).
- The total number of rats used in the research was 30, and they were split into three groups (N=3). During a period of one and a half months, administer a subcutaneous injection once daily. There are 10 animals that make up the T1 control group. Throughout the trial, the mice were given 0.5mg injections of normal saline. The second group, T2, 10 rats were given a zinc oxide Nano solution of N.oleander leaves extract at a dosage of 20mg/kg, with a volume of 0.3 ml. In group T3, 10 rats received 0.3 cc zinc oxide Nano solution of N.oleander leaves extract at 30mg/kg.
- After a period of 30 days after the initiation of the first trial, fifty percent of the animals were euthanized. The remaining half were terminated 45 days subsequent to the conclusion of the first experiment. The remaining half were executed 45 days after the first experiment ended. The animals were anaesthetized using a combination of Xylazine (20 mg) and Ketamine (10 mg). Afterwards, they were placed on a cork dissection plate and fastened with staples to make the autopsy process easier. This was done to investigate the impact of Nano-zinc oxide from N.oleander leaf extract on the liver.

## 3 Results

(Figure 1) displayed that the control animals' livers showed normal liver structure and no histological changes in the hepatocytes. As shown in (Figure 2), the livers of rats that received nano-extract of *N. oleander* leaves at a dose of 20 mg/kg for 30 days exhibited histological alterations, regularity of the hepatic cords, and a normal appearance of hepatocytes. As illustrated in (Figure 3), the histological examination of the livers of rats administered the Nano-extract of *N. oleander* leaves at a dosage of 20mg/kg over a period of 45 days demonstrated alterations in the structure of the hepatic cords and hepatocytes. As shown in (Figure 4), liver and cell structure were normal in rats given a forty-day injection of a nano-extract of N. oleander leaves at a dosage of 20 mg/kg. As it is shown in (Figure 5), animals that were given a 30 mg/kg nano-extract of *N. oleander* leaves had changes in their livers that were not normal, along with changes in the shape of the hepatocytes and the central vein.

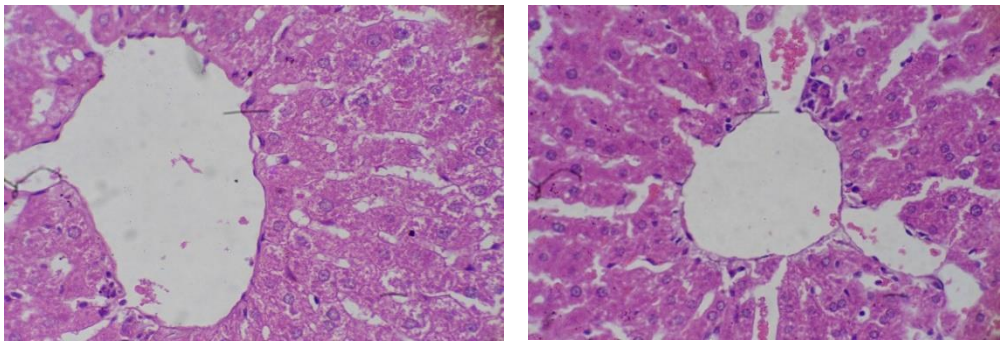

**Figure 1:** A liver tissue cross section from a white rat in the control group over a period of 30 days, illustrating the liver's typical structure. (H & E stain) (300x)

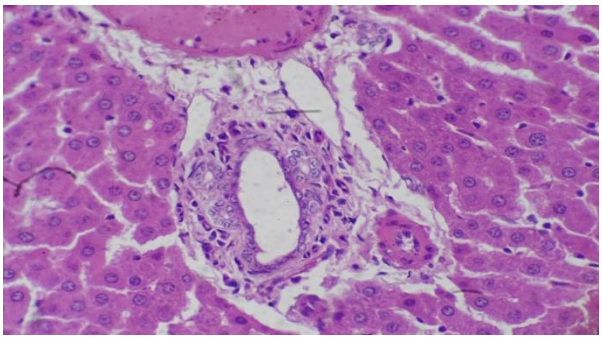

**Figure 2:** A liver tissue cross section of the rat in the group that was subjected to a 30-day treatment with a nano-extract derived from the leaves of the N. oleander plant at a dosage of (20 ml/kg). In addition to the absence of histological alterations and the irregularity of the hepatic cords, it manifests in the hepatocytes as normal. (H & E stain) (300x)

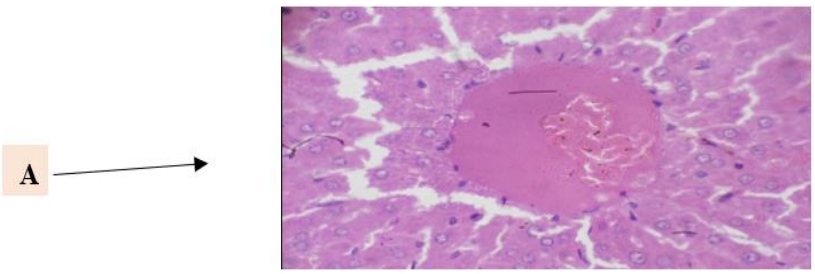


**Figure 3:** A liver tissue cross section of a rodent in the group that was subjected to a 45-day treatment with a nano-extract derived from the leaves of the *N. oleander* plant at a dosage of (20 ml/kg). Hepatocytes and hepatic cords are regular in their abnormal form, and bleeding is evident. (H & E stain) (300x)

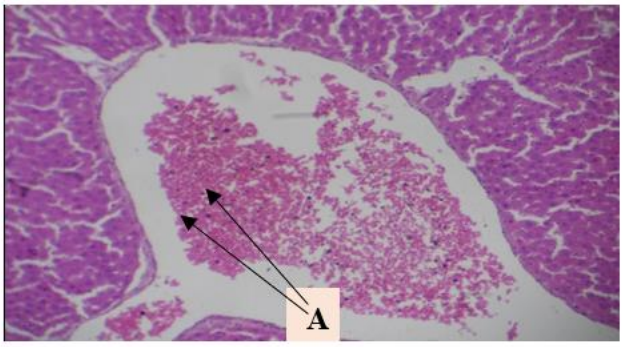


**Figure 4:** A liver tissue crossn section of a white rat in the group that was subjected to a 30-day treatment with a nano-extract derived from the leaves of the *N.oleander* plant at a dosage of 30 ml/kg. The section illustrates the liver's lucid structure and cells, while also noting any histological alterations that may have occurred. (H & E stain) (300x)

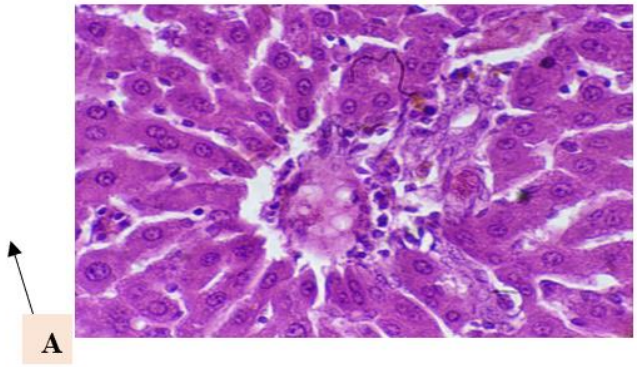


**Figure 5:** A liver tissue cross section was obtained from a white rat in the group that was subjected to a 45-day treatment with a nano-extract derived from the leaves of the N. oleander plant at a dosage of (30 ml/kg). Pathological alterations in liver tissue included hepatocyte and central vein morphological deviations. (H & E stain) (300x)

## 4 Discussion

Microscopic analysis of white rat organ histological sections revealed normal liver histology and histopathological changes after 30 and 45 days of treatment with nano-extract of *N. oleander* plant leaves (20-30 mg/kg). The results also demonstrated a protective role for the kernel extract of *N. oleander* leaves. The findings are in line with those of (Sheikuduman & Karunakaran, 308), in which he discussed the beneficial effects of EEAS and AEAS leaf extracts on the liver. The results were in agreement with those of (Mandal *et al*., 309; Shamkuwar *et al*., 2012), which demonstrated that the alcoholic extract of the Saad plant had no negative impact on the liver functions of mice. This implies that the plant offers a protective effect against free radical production. Due to the defensive effect exhibited by the active compounds present in the plant, particularly flavonoids, which constitute the primary active constituents (Mushattat &Alaridi, 2018).

*N. oleander* extracts enhance hepatic function in mice by elevating the hepatic toxicity of alkaline phosphate, as measured by AST and ALT. Both heart and liver disorders reduce AST and ALT, although heart disease reduces AST more and liver disease more. Bilirubin, which is produced when red blood cells are broken down in the liver, is an excellent indicator of liver function. Increased amounts cause jaundice by indicating damage to the liver and bile ducts (Rajesh *et al*., 305). *N. oleander*

extracts reduced total bilirubin and ALP levels, indicating they protect and improve liver function (Jabbar & Noor, 2018).

The result of the current study is in agreement with the study (Raish *et al*., 2016) in which mice were treated with alcoholic extract of *N.oleander* leaves at a concentration of (150-300mg/kg) and there were no side effects of the extract on liver tissues, given the non-stimulating substances it contains. On the occurrence of oxidative stress or the generation of free radicals as it does not have any toxic effect on tissues. The results of the current study agree with (Jiang *et al*., 304, Ravinder *et al*., 306), where the previous study also showed the same result in the groups treated with isoniazid (Ahmed *et al*., 2018).

## 5 Conclusions

- The results showed the toxic effects of the Nano-extract of the oleander plant.
- The existence of histological alterations in the liver's histological structure in groups treated with varying amounts of oleander leaf nano-extract.

**Recommendations:**

- It is recommended not to grow oleander indoors due to its toxicity.
- The possibility of using nanocomposites of some plant extracts as pesticides.
- Conducting the experiment on female laboratory white mice.

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