Histological comparison of natural lung injury in *Rattus norvegicus* induced by a natural herb (*Nerium oleander*) and a known carcinogen (thioacetamide)

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ABSTRACT

Plant extracts have extensively been studied to look for the treatment of various diseases but their poisoning effects have also been reported. The current study was undertaken with the objective of comparing acute toxic effect of Thioacetamide (TAA), a proven toxicant and *Nerium oleander* (*N. oleander*), a herb reported to have multiple therapeutic properties including anti-inflammatory, antifungal, antibacterial and antioxidative effects using histological parameters. Animals in group A were treated with 10 ml/kg of aqueous decoction of leaves of the plant while 300mg/kg of TAA was administered in group B and control (Con.) did not receive any treatment (n=3). Histological alterations in the lungs were then studied after 6 hours of respective treatment. There was an onset of more or less similar destruction of the tissue in both the experimental groups. Disruption of bronchus mucosal folds and alveolar cells along with significant nodule like accumulation of macrophages and mononuclear cells around arteriole were noticed. These results indicated that use of ethno-medicines, like *N. oleander* caused similar histological alterations in lungs as that of known carcinogen TAA. Thus, application of *N. oleander* may pose a serious risk for health.  

Keywords. Lung, *Nerium oleander*, Thioacetamide.

INTRODUCTION

Plants are the largest source of herbal medicines worldwide and these have the potential to be both therapeutic and harmful but still masses of population rely on such remedies. *Nerium oleander*, (*N. oleander*), (Apocynaceae) an ornamental shrub commonly known as “Kaner”, has been used as a traditional medicine and is reputed to have wide spectrum of bioactivities including pharmaceutical products (Anvirzel), molluscicide, rodenticide and insecticides (Wang et al., 2000; Adome et al., 2004; Turan et al., 2006; Derwich et al., 2010). But poisoning action of this plant has also been reported with significant time-dependent cytotoxic effects in cattle, goat, man and other experimental animal models (Langford & Boor, 1996; Aslani & Rezakhani, 2000; Hughes et al., 2002; Smith et al., 2003; Barbosa et al., 2008; Abbasi et al., 2013b). Animals exposed to the plant are often found suddenly dead owing to cardiac dysfunction. Oral doses of *N. oleander* proved fatal to animals within 1 to 24 hours with clinical toxicosis (Aslani et al., 2007).

Thioacetamide (TAA) a proven carcinogen has been extensively reported to produce toxic effects in animals and is suitable for studying and comparing the histological alterations (Abbasi et al., 2013a).

The aim of this study was to compare acute toxic effect of TAA and *N. oleander* produced histological variations in lungs of albino rats and to find out whether the injury caused by TAA resembles with *N. oleander* induced injury.

MATERIALS AND METHODS

Animals

Male wistar rats of about 200g body weights, were kept in a well ventilated hygienic animal house under standard conditions with 12-h light/dark cycles and access to fresh water and food pellets *ad libitum*. All the animals were acclimatized for a period of 2 weeks before the experiment.

Plant Material

Fresh leaves of *N. oleander* were collected from Govt. College of Science (GCS), Lahore, Pakistan. The plant material was identified by comparing with the reference collection available in the Herbarium of GCS, Lahore, Pakistan and had been preserved in Department of Botany, GCS for further reference.

Extraction protocol

Air-dried leaves were processed according to the method of Rashan *et al.*, 2011, with modifications. Briefly, the leaves decoction was
prepared by boiling in 0.9 % NaCl solution (1:1, w/v) for 3h by steam distillation. The decoction was filtered and used for the experimental animals.

**Experimental design**

The rats were grouped into three viz. A, B and control (Con.) (n=3); A: was injected intramuscularly (i.m) with 10 ml/kg of *N. oleander* leaves decoction, B: 300mg/kg of TAA was administered intraperitoneally (i.p) and Con. did not receive any treatment. After 6h of administration, all the animals were anesthetized with i.p injection of ketamine–distilled water mixture (1:1).

**Tissue Sampling and Histological Examination**

Lungs of all the animals were excised, immediately after sacrifice, rinsed with physiological sodium saline and portion was fixed in 10% formalin for histological studies. The fixed tissue specimens were sectioned by standard methods and stained with Hematoxylin and Eosin (H & E) for detecting the degree of damage.

**Microscopic Analyses**

To assess organ damage, microscopic images were captured using a trinocular IRMECO-GmbH model 1M-910, 21493 Schwarzenbek/Germany displayed on a computer via a Scope Tek® (scope photo 3.0).

**RESULTS AND DISCUSSION**

The present study investigated the histopathological changes in the lung tissues of Wistar rats that were treated with *N. oleander* leaves extract and TAA separately. Since lung is a target organ of many toxicants, an understanding of pulmonary architecture in health and disease is of paramount importance.

H&E stained lung sections of control group showed normal histological architecture; numerous clear alveoli (A) with thin inter-alveolar septa and alveolar sacs (AS). A bronchus (Br) with mucosal folds in a close proximity to blood vessel can also be seen in the section (Fig., 1).

Alterations in the pulmonary architecture were observed in group A with evident disruption of bronchus mucosal folds. Alveolar cells with extreme widening of lumen of the bronchiole and significant signs of vascular lesions were also noticed (Fig., 2).

The damage observed might be due to an oxidative stress produced by toxicants, oleanderin and nerine, cardiac glycosides (cardenolides) which can be isolated from all parts of the *N.oleander* and are similar to the toxin in foxglove (Digitalis) (Al-Farwachi et al., 2008). These toxins may lead to an imbalance in the production/consumption level of reactive oxygen species (ROS).

There was an onset of more or less similar destruction of the tissue in group B with the damage of alveolar cells along with significant nodule like
accumulation of macrophages and mononuclear cells around arteriole (Fig., 2B). It may be explained that during inflammatory response, accumulation of macrophages occurs which ultimately release some chemotactic factors that attract neutrophils which is evident in the sections. Influx of inflammatory cells plays a major role in increasing the permeability of alveolar capillary barrier providing the cellular toxicity. Neutrophils together with other inflammatory cells must frequently found in the broncho-alveolar region in the current study. Sheikh & Javed, 2009 observed similar changes along with pulmonary edema in lung sections of Swiss albino mice, exposed to 0.5% dilution of cypermethrin in an inhalation chamber. Similar histopathological changes in the sections of lungs were observed during Fenvalerate (Fen), a synthetic pyrethroid exposed rats with influx of mononuclear cells admixed with a few giant cells in alveolar lumen (Mani et al., 2001). More pronounced histological changes were noted in pesticide mixture inhaled rats for consecutive 28 days (Noaishi et al., 2013).

CONCLUSION

Taking into consideration these observations, it could be concluded that indiscriminate use of natural herbs for therapeutic purposes could lead to the tissue destruction with subsequent abnormality in function.

REFERENCES


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