Effects of *Nerium oleander* leaves extract against Thioacetamide induced Liver injury

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**ABSTRACT**

Various serum proteins are synthesized in the liver. Their serum levels may reduce or elevate due to liver disorders. Thioacetamide (TAA) is a powerful hepatotoxican, multiple protein systems have been revealed to be injured, counting those present in the mitochondrial respiration, endoplasmic reticulum, and stress response proteins. The study was conducted to evaluate the hepatoprotective potentials of aqueous extracts of *Nerium oleander* leaves against thioacetamide induced liver damage in rats. Densitometric analysis revealed that the protein bands of ceruloplasmin and transferrin in TAA treated group are less dense as compared to respective bands in control sample while the group received additional 7 days *N. oleander* treatment showed more or less similar band densities as compared to control sample. *N. oleander* extract also attempted to reverse or at least minimized the effect of TAA on protein fractions of molecular weight 38, 46 and 110 KDa. Elevated level of C-reactive protein was also observed in all experimental groups as compared to control. The results obtained in the above study suggested that *N. oleander* extract possesses a significant hepatoprotective activity against TAA induced hepatotoxicity. More research is required to derive an optional therapeutic dose.

**Keywords:** Thioacetamide, Hepatoprotection, Antioxidant, Hepatotoxicant, Hypoproteinemia

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**INTRODUCTION**

Liver is the main organ in the body and plays a significant role in a variety of responsibilities mandatory to maintain homeostasis of the body. It has acquired its own significance in the physiological system (Ravichandra et al., 2013). It is concerned in nearly all biochemical pathways including overall growth, fighting against disease, nutrient supply, energy provision and reproduction. Metabolism of ingested substances like carbohydrate, lipids, proteins, storage of vitamins, and immunomodulation are the prime role of the liver (Ahsan et al., 2009; Rajesh & Latha, 2004). Most of the time liver injury is connected with disruption of these metabolic functions and consequences into disturbance in homeostasis of the body (Ramachandra et al., 2007). Liver diseases have turned out to be one of the main reasons of morbidity and death throughout the globe. But till now there is no really acceptable liver protective drug in the contemporary system of medicine, which is effective and safe. Liver injury due to drugs is the foremost contributory aspect that poses a main clinical and regulatory challenge (Russmann et al., 2009). Regardless of marvelous scientific innovation in the area of hepatology in the modern era, liver problems are getting higher (Anbarasu et al., 2012).

Plants play a vital role for the survival of life in the universe. Herbal drugs are contributing a vital role in health care programs throughout the world, primarily owing to the universal faith that they are devoid of any side effects, in addition, being cheap and locally accessible (Singh & Abrar, 1990). The healing advantage of medicinal plants is recognized by almost every society today (Sheeja et al., 2006). Due to lack of trustworthy liver protective drugs in modern system of medicine, a huge quantity of herbal products are suggested for the cure of liver disorders (Chatterjee, 2000) and a few of them are very useful. Efforts are being made worldwide to find scientific confirmations for these traditionally documented herbal preparations (Anbarasu et al., 2012).

*Nerium oleander* Linn. (Family Apocynaceae), commonly known as “Kaner”, is native to Indo-Pak subcontinent (Rashan et al., 2011). This plant has been reported having noxious effects because of its number of components that may show signs of toxicity by inhibiting plasma lemma Na⁺, K⁺-ATPase (Barbosa et al., 2008). Despite its well-recognized toxic potential, all parts of plant are reputed therapeutic agents and have been used in folklore in a variety of ailments, including dermatitis, cardiac disorders, eczema, cancer, skin and heat diseases etc., (Haq et al., 1999; Singhal & Gupta, 2011; Sugimoto et al., 2000). *N. oleander* extract induces apoptosis in different tumor cells and this outcome is accomplished through inhibition of Na-ATPase with consequently increased level of intracellular calcium, release of mitochondrial cytochrome-C, activation of the caspase cascade, and Poly ADP

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Ribose polymerase (PARP) cleavage (McConkey et al., 2000). In addition, the capacity of oleandrin to obstruct activation of NF-kB (Nuclear Factor-KappaB) possibly will also add to the antitumor effect (Manna et al., 2000).

About 5% of all hospital admittance and 50% of all acute liver failure has been associated to chemical and drug induced liver injury. Thioacetamide is an organosulfur compound endowed with liver damaging and carcinogenic activity (Low et al., 2004). TAA is a model hepatotoxicant, consumed to stimulate acute and chronic liver injury due to its effects on protein synthesis, RNA, DNA and Gamma-glutamyl transpeptidase activity (Yang et al., 1998). TAA undergoes a two-step bioactivation to sulfine, and afterward to sulfene, a reactive metabolite (Amali et al., 2006; Chilakapati et al., 2005). Sulfine is accountable for the enlargement of nucleoli, increase in nuclear volume and intracellular concentration of Ca^{2+}, change in cell permeability, and inhibit mitochondrial activity (Gupta and Dixit, 2009; Prabha et al., 2012). At the same time Sulfene is responsible for the release of nitric oxide synthase and NF-jB directing to centrilobular necrosis, protein denaturation and lipid peroxidation (Caballero et al., 2001; Lee et al., 2003; Rahman & Hodgson, 2003).

The current experimental work was carried out to examine the hepatoprotective action of aqueous extracts of *N. oleander* leaves against thioacetamide (TAA) produced liver damage in wistar rats.

**MATERIALS AND METHODS**

**Animals**

Healthy wistar rats (about 175±25g) were acquired from the Department of Zoology, University of the Punjab Lahore (Pakistan), housed in wire-bottomed well-ventilated hygienic cages in an animal room under standard conditions with 12-h light/dark cycles with 25±2°C temperature. All the animals were given *ad libitum* access to standard laboratory pellets and drinking water.

**Dose preparation & administration**

Dose of TAA (Sigma-Aldrich, Switzerland) was prepared by dissolving 200mg of TAA in 1L of distilled water and stirred well until all crystals were dissolved. *N. oleander* leaves extract (distilled water based) was purchased from commercially available source. Rats were alienated into four groups, Con group for control animals and group I, group II and group III for experimental animals. Con group was given with normal drinking water, among the experimental groups, group I was provided with TAA (200mg/L) in drinking water for 18 weeks. Group II was given TAA (200mg/L) in drinking water for 18 weeks plus additional 7 days oral intake of *N. oleander* leaves extract and group III was provided with 7 days oral intake of *N. oleander* leaves extract.

**Blood sampling and processing**

All the animals were anesthetized with intra-peritoneal injection of ketamine – distilled water mixture (1:1), (50 mg/ml of ketamine) and scarified. The dissections were done in aseptic conditions to draw the blood through direct cardiac puncture. 6ml of the blood was transferred to vacutainers (without any clotting factor) to separate the serum. Blood samples were kept for 2-3 hours at room temperature and after that centrifuged for 20 minutes at 4000 rpm. The serum was saved in new marked eppendorf cups and was stored at-20°C until used for Sodium Dodecyl Sulfate Polyacrylamide Gel Electrophoresis (SDS-PAGE).

**Evaluation of serum proteins variations**

Laemmli method was used to prepare Polyacrylamide gel (Laemmli, 1970). 12% gel was used to resolve low molecular weight proteins. Low molecular weight Fermentas PageRuler™ unstained protein ladder # SM0661 was used as standard. The density of bands in a specific well was used to generate the densitometric graph to infer increase or decrease and appearance or disappearance of particular protein fractions as well as new protein fraction in comparison to control group. Gene Genius Bio-imaging Gel Documentation System was used to determine the molecular weight of the protein fractions of the samples.

**RESULTS**

The protein bands on the SDS-PAGE gel were analyzed and their densities were recorded. The densitometric analysis of protein bands revealed that the protein bands of group I are less dense as compared to respective bands in control sample while the group II and group III showed more or less similar band densities as compared to control sample in term of protein density. Different bands present in the gel and variations in the normalized volume of all sera samples against molecular weight were shown in Fig. 1a and b.
The decreased density of the protein band 4 in group I indicated the low level of Ceruloplasmin (Fig., 2a), at the same time the level of ceruloplasmin in group II was higher than group I but low as compared to control group (Fig., 2b). While ceruloplasmin level in group III was roughly similar as in control group (Fig., 2c). Transferrin (protein band 7) revealed low level in group I (Fig., 2a) and elevated level in group III as compared to control animals (Fig., 2c). While group II showed very negligible change in Transferrin level in comparison to Con group (Fig2b). On the other side C-reactive protein (protein band 17) showed elevated level in group I and group III as compared to control animals (Fig., 2a and b). N. oleander extract seems to reverse or at least minimize the effect of TAA on protein fractions of molecular weight 38, 46 and 110 KDa (Fig., 2a and b).

Fig., 1(a): Representation of all the lanes present in the gel, (b) Electropherogram showing the variations in Normalized volume of all sera samples against Molecular weight (kDa).

Fig., 2: Electropherogram showing the comparison of, a; Group I and Control with Vol on the Y-axis and Molecular weight (KDa) on X-Axis, b; Group II and Control with Vol on the Y-axis and Molecular weight (KDa) on X-Axis, c; Group III and Control with Vol on the Y-axis and Molecular weight (KDa) on X-Axis.
DISCUSSION

Hypoproteinemina condition was seen in animals treated with thioacetamide. The decreased serum protein values were usually concurrent with reduction in albumin concentration. The total protein level was down in hepatotoxic situation owing to trouble in the carbohydrate, lipid and protein metabolism or perturbed protein biosynthesis in the cirrhotic liver (Schwartz et al., 1974). Ceruloplasmin (glycoprotein) has ferroxidase activity oxidizing Fe(2+) to Fe(3+) without releasing radical oxygen species. It is involved in iron transport across the cell membrane and provides Cu2+ ions for the ascorbate-mediated deaminase degradation of the heparan sulfate chains of GPC1 (Heijnen et al., 2006). In this study N.oleander enhance the ceruloplasmin level which was initially low as compared to control animals owing to TAA treatment. The decrease in the ceruloplasmin level of TAA group is a consequence of decreased level of transferin level in group one might be due to adverse effect of TAA on iron regulatory genes. Decreased level of transferrin in TAA treated group than N. oleander treated and control groups further indicating the hepatoprotective potential of the N. oleander extract. C-reactive protein exhibits numerous functions linked with host defense: it promotes agglutination, phagocytosis and complement fixation due to its calcium-dependent binding to phosphoryl choline. C-reactive protein also has the ability to interact with cytokines, Leptin Precursor, Endothelin-1 Precursor, Oxidized low-density lipoprotein receptor 1 and tumor necrosis factor-alpha (Nunez et al., 1991). Rise in the level of C-reactive protein in group II and III indicate acute inflammation resulting from N. oleander extract.

CONCLUSION

Taken together these findings, it can be concluded that N. oleander extract has the potential to reverse or at least minimize the liver injury caused by TAA due to its potential anti carcinogenic effect. However, purification of different chemical components from N. oleander extract without having inflammation belongings is required before the extract can be used for any kind of protective treatment.

REFERENCES


