Effect of Hexane Seed Extract of *Nigella Sativa* on Cadmium Induced Renal Dysfunction in Rats

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Abstract

This study investigated the effect of hexane seed extract of *Nigella sativa* on cadmium induced renal dysfunction in rats. Twenty four young adult male rats randomly divided into six groups of four animals each were used. Group one was normal control group without treatment of any type. Group 2 was treated with cadmium, Group 3 was treated with extract only, Group 4 was administered cadmium and extract, Group 5 was treated with extract followed by cadmium and Group 6 was treated with cadmium followed by extract. The levels of creatinine, urea, ALT and AST were measured. The result showed that there was significant increase ($P<0.05$) in serum levels of creatinine, Urea, ALT and AST in the cadmium treated group compared with the normal control group. These biochemical changes were alleviated in the extract treated groups as evidenced by significant decrease in serum levels of the assayed biochemical parameters when compared to the cadmium control group. The effect was better in the post-treatment group. This study therefore suggests that cadmium-induced renal dysfunction can be attenuated by administration of hexane seed extract of *Nigella sativa* as co, pre and post-treatment. This alleviation might be attributed to the presence of flavonoids, alkaloids, and steroids which are powerful antioxidants and anti- inflammatory agents.

Key Words: Cadmium, Renal Dysfunction, *Nigella Sativa*, Hexane, Anti-Oxidants, Anti-inflammatory

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INTRODUCTION

Cadmium is a known hazardous environmental and industrial contaminant that is posing a serious threat to human and other animals (EA, 2009). Exposure to cadmium arises from the use of phosphate fertilizers, production of Nicd batteries, and removal of cadmium paints by scraping or blasting, cigarette smoking, waste incineration, fossil fuel production, the production of steel, iron and non-ferrous metals, air and soil, by atmospheric deposition and by the ingestion of vegetables such as lettuce, spinach, celery and cabbage that accumulate cadmium (ATSDR 2008). Recent report indicates that house dust may also be a source of exposure to cadmium (EA, 2009).

The kidney remains the primary target site of cadmium toxicity. A recent report suggests that cadmium exposure causes 7% of the renal dysfunction in the general population (Charles, 2000). The Renal dysfunction arises from chronic oral exposure to cadmium, and is characterized by proteinuria following renal tubular dysfunction. The accumulation of cadmium in the kidney also affects renal vitamin D metabolism, which subsequently disturbs calcium balance and lead to osteomalacia and osteoporosis (EA, 2009). Dyspnea, chest pain and muscle weakness are also other possible consequences. Pulmonary oedema, bronchitis, chemical pneumonitis, respiratory failure and death may occur within days of exposure. In the long-term following exposure, progressive pulmonary fibrosis and impaired lung function may occur (Bull, 2010). Chronic inhalation of cadmium causes loss of renal tubular function and impairs lung function by causing bronchitis, obstructive lung

disease and in some cases interstitial fibrosis (Bull, 2010). Studies indicate that cadmium compounds have mutagenic potential. They have been shown to induce chromosome damage and DNA strand breaks (EA, 2009). The reaction mechanism for the pathogenesis induced by cadmium exposure is generally ascribed to oxidative damage (EL-Demerdash *et al.*, 2004). Lipid peroxidation has also been observed in cadmium toxicity (Naovarat *et al.*, 2011).

In the last two decades a number of research works has been performed to evaluate the effect of *Nigella sativa* in rats. The plant is known in Arabic and Islamic countries with various names. It is known generally by the names HabbatAlbarakah, AlhabahatAlsawda and AlkamounAlaswad. In some countries it is known by the names Shuniz and Khodhira. Its English name is Black Cumin or Black Caraway. There are many reports on the pharmacological significance of the plant including Anticancer (Worthen *et al.*, 1998), diuretic and hypotensive effect (Zaoui *et al.*, 2000), antipyretic analgestics and anti-inflammatory (Al-Ghamdi, 2001), hypoglycemic (El-dakhakhny *et al.*, 2002), Marked antioxidant (Ali and Blunden, 2003), anti-ulcergenics (Rifat-uz-Zaman *eta.l*, 2004), protective effect on pancreatic β-cells (Mansi, 2006), improved fertility (Bashandy, 2007), improved chicken performance (Al-Beitawi, 2008) and has hepatoprotective effect (Abuelgastin *et al.*, 2008).

The interest towards medicinal plants and their active ingredients continue to soar high. Whereas most synthetic drugs have been known to exert dangerous side effect over time, medicinal plants have enjoyed centuries-long use with little known side effects (Al-Shebani, 2009). It is against this backdrop therefore, this study was designed to investigate the effect of *Nigella sativa* seed extract on cadmium-induced renal dysfunction in rats.

MATERIALS AND METHODS

Procurement and identification of seed

Dry *Nigella Sativa* seed were purchased from Jimeta modern market, identified and authenticated at the department of plant sciences, Modibbo Adama University of Technology, Yola.

Animals

Twenty four young adult male albino rats weighing approximately 100-120g were used in this experimental study. The animals were obtained from the animal house of Benue State University. All experiments were carried out in accordance with the guidelines of the Institutional Animal Ethics Committee. They were kept in well ventilated room with adequate access to food and water.

Chemicals and Reagents

Cadmium chloride was obtained from department of Chemistry, Modibbo Adama University of Technology Yola. Urea, Creatinine, ALT and AST kits were products of Randox laboratories Ltd, United Kingdom.

Preparation of Extract

The seeds of *Nigella sativa* about 200g were soaked in 500mls of hexane for 48hr hours. The mixture was then filtered and the filtrate concentrated in a rotary evaporator. A brown oily crude suspension of the extract was obtained.

Experimental design

The rats were randomly divided into six equal treatment groups containing four animals each. The treatment schedules were as follows;

Normal control: Normal diet and water

Cadmium control: Cadmium Chloride only.

Extract control: Extract only.

Co-treatment: Cadmium Chloride with Extract

Pre-treatment: Extract followed by Cadmium Chloride.

Post-treatment: Cadmium Chloride followed by Extract.

Cadmium chloride and extract were administered at a dose of 2mg/kg for seven days and 250mg/kg for fourteen days in the treatment groups respectively. At the end of the experimental period, the animals were anaesthetised in chloroform vapour, dissected and blood samples collected by cardiac puncture into sample bottles. The blood was allowed to clot for few minutes. Serum was obtained by centrifugation at 10,000 RPM for five minutes using a bench top centrifuge.

Determination of body weight

The body weights of each rat before and after the experimental period were obtained using a weighing balance. The average weight of each group was taken and recorded carefully.

Biochemical analysis

Creatinine and Urea measurements: Serum creatinine measurement was carried out using Jaffe's reaction (Barthels and Bohmer, 1971) while the quantitative determination of serum urea was carried out using Berthelot's reaction with the urea enzymatic colometric kit (Young, 1997).

ALT and AST measurements: The method of Reitman and Frankel (1957) modified by Schmidt and Schmidt (1963) was used for assaying the activity of ALT and AST, using Randox Kit.

Statistical Analysis

Results were expressed as means ± SEM. Statistical significance of difference between means was carried out using one-way ANOVA. Level of significance was considered at $P< 0.05$.

RESULTS

The effect of cadmium and various treatment of *Nigella sativa* on serum creatinine and urea are presented in table 1. The result is also depicted graphically in Fig 1 and 2. There were significant elevation in creatinine and urea levels in the cadmium treated groups when compared with the normal control group. In all the *Nigella sativa* seed extract treatment groups, the levels of creatinine and urea were however, decreased when compared with the cadmium control groups. The level of decrease was higher in the post-treatment and pre-treatment groups.

Table 2 shows the effect of cadmium and *Nigella sativa* seed extract on liver enzymes activity. The levels of ALT and AST were significantly elevated in the cadmium treated groups when compared with normal control. The groups treated with extract showed a significant decrease ($p<0.05$) in their respective ALT and AST values compared with the cadmium treated groups. The result is also demonstrated in Fig 3 below.

Table 1: Effect of *Nigella sativa* seed extract on serum level of creatinine and urea

Treatment	Creatinine (mg/dl)	Urea (mg/dl)
Normal Control	0.29±0.00	31.3±1.16
Cadmium Control	1.84±0.42	116.6±7.95
Extract Control	0.66±0.18*	51.2±10.5
Co-Treatment	0.59±0.12*	79.6±0.40*
Pre-treatment	1.02±0.20*	33.2±0.10*
Post-Treatment	0.68±0.17*	35.2±1.10*

Results are expressed as mean± SEM. n=4

*significantly decrease when compared to cadmium control at P<0.005.

Table 2: Effect of *Nigella sativa*seed extract on serum level of ALT and AST activity

Groups	ALT(U/L)	AST (U/L)
Normal Control	21.0±0.00	29.7±1.33
Cadmium Control	44.5±6.11	56.8±6.33
Hexane Control	25.3±3.07*	30.0±1.00*
Co-Treatment	33.0±4.53*	22.0±3.00*
Pre-Treatment	23.0±1.15*	12.3±2.56*
Post-Treatment	22.3±7.62*	23.0±2.31*

Results are expressed as mean± SEM. n=4

*significantly decrease when compared to cadmium control at P<0.005.

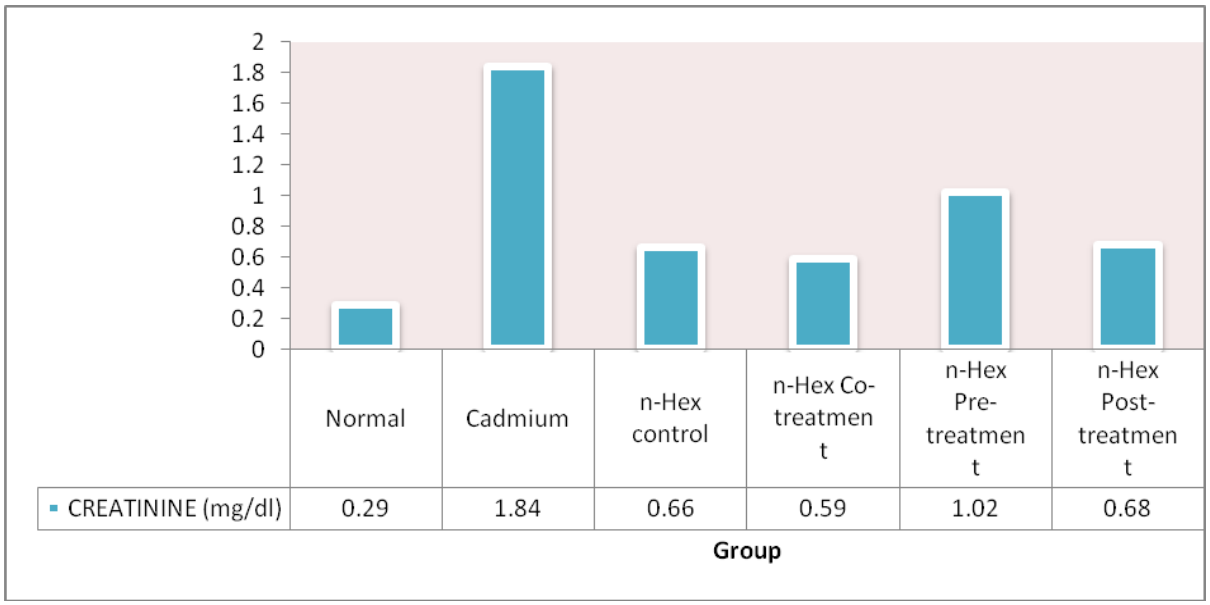

Fig 1: Effect of *Nigella Sativa* Seed Extract on Serum Creatinine Level.

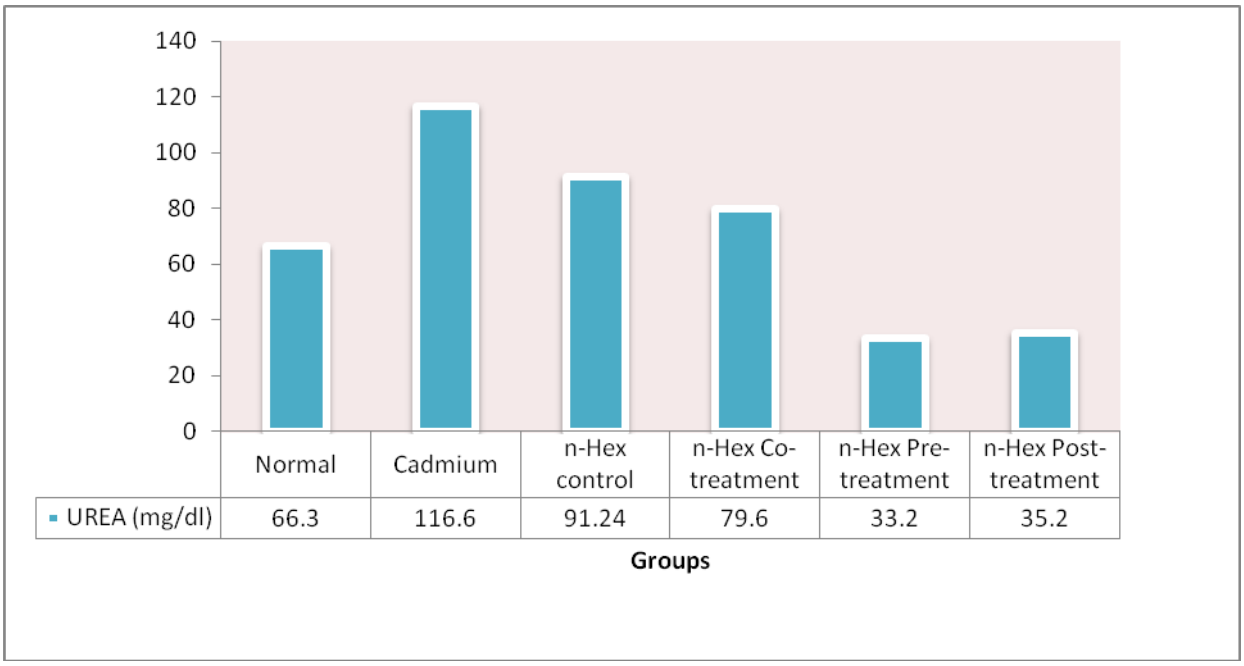

Fig 2: Effect of *Nigella sativa* seed extract on serum urea level.

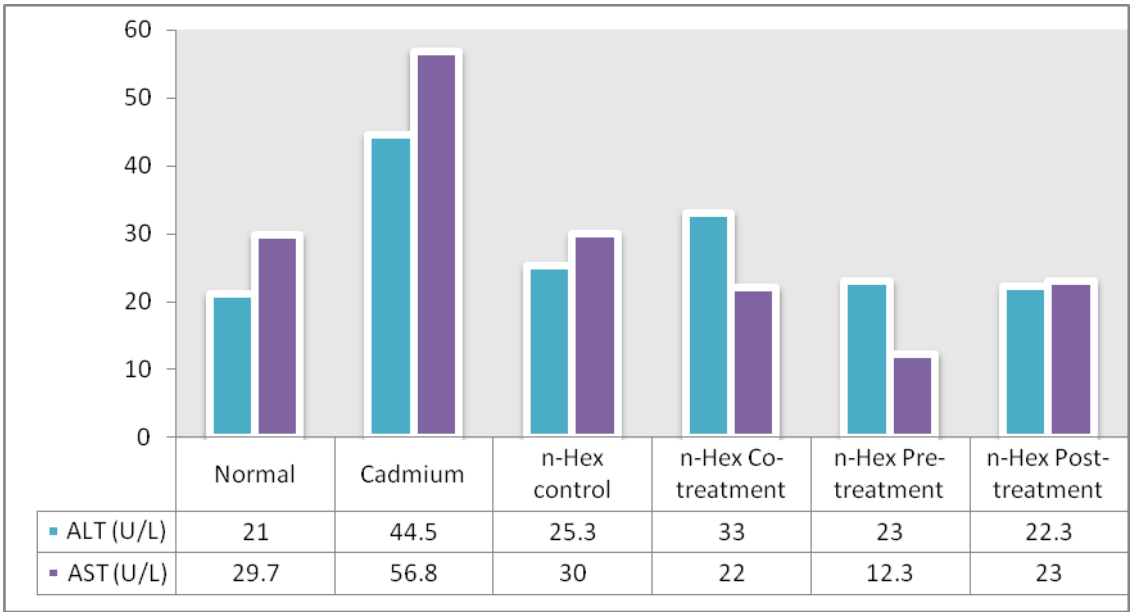

Fig 3: Effect of *Nigella sativa* seed extract on serum ALT and AST level.

The mean body weight of cadmium induced experimental control group showed a significant decrease when compared with normal as is presented in Fig 4 below. The mean body weight of the n-hexane post-treatment indicated an initial decrease in weight during cadmium administration; this was however reversed during extract administration. The result for pre-treated groups indicated an initial rapid increase in weight which later stabilized with time.

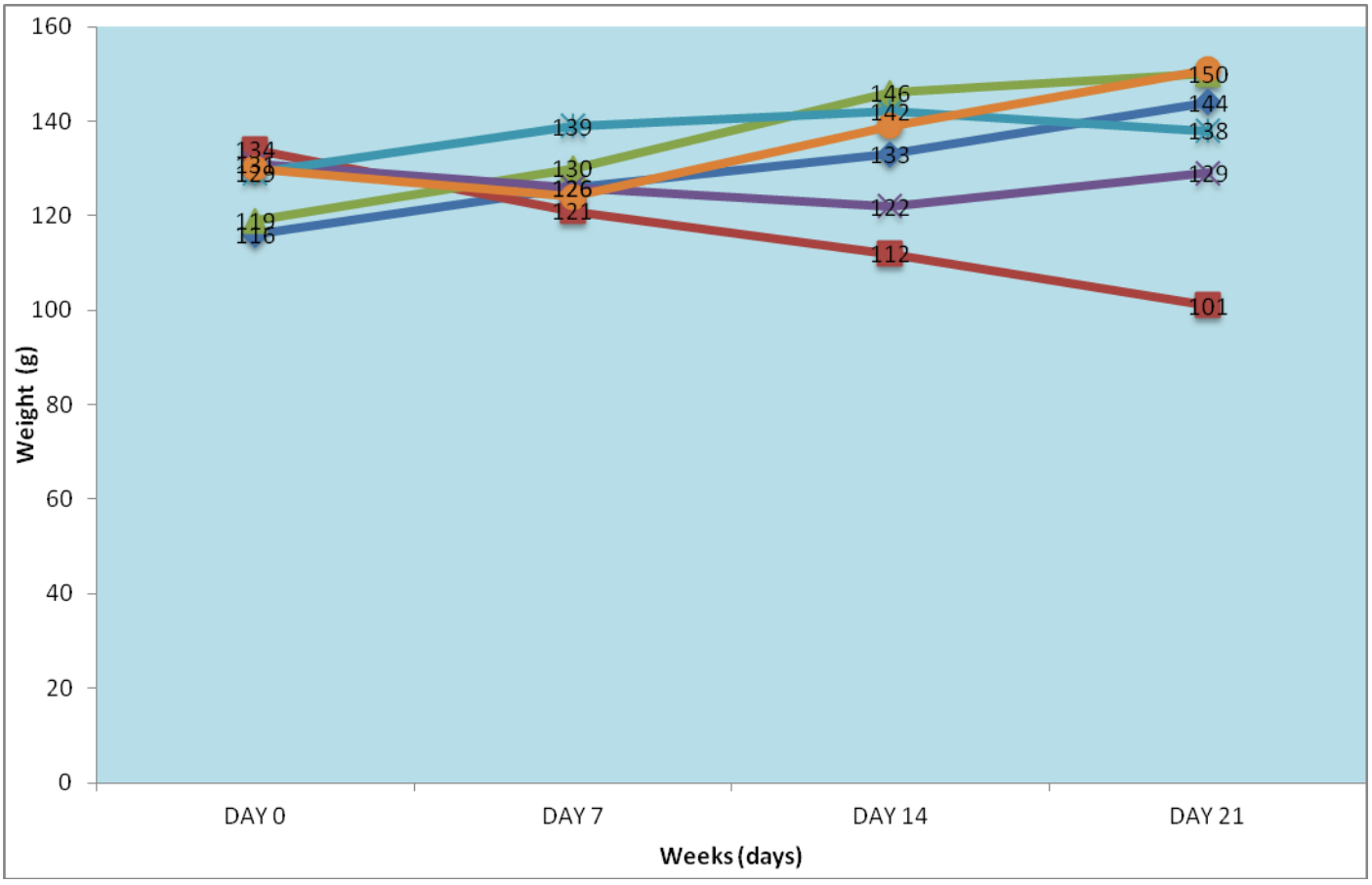

Fig 4: Effect of *Nigella sativa* seed extracts on body weight of rats.

DISCUSSION

The result of this study showed that renal dysfunction was induced by oral exposure of rats to cadmium as evidenced by a significant increase in serum urea, creatinine, ALT and AST. These biochemical alterations were similar to those reported by El-Demerdash *et al*., 2004; and Mohamed, 2009. Cadmium exposure causes formation of metallothionein complexes which are released from necrotic hepatocytes and are delivered via systemic circulation to the kidneys where they are taken up and induce proximal tubular injury and death; hence the renal dysfunction observed in this study (Naovarat *et al.*, 2011).Cadmium toxicity has also been attributed to lipid peroxidation. Although the reasons for lipid peroxidation are not completely known, but it is believe that disturbances in GSH levels may allow free radicals to be free such that HO and O_2 radicals can attach double bonds in membrane lipids and result in elevated lipid peroxidation. Moreover, mitochondrial respiration as the chief source of reactive oxygen species is promoted by lipid peroxidation and therefore enhances oxidative stress induced by cadmium toxicity (Nuran *et al.*, 2001).

Our findings demonstrated that the administration of the seed extract attenuated cadmium induced renal dysfunction as evidenced by lowered creatinine, urea, ALT and AST levels. Report of phytochemical screening of the seed of *Nigella sativa* suggests that the seeds are rich in flavonoids and alkaloids (Kamal El-Din *et al.*, 2006). Several studies have reported that flavonoids especially qurecetin and kaempferol have anti-inflammatory and antioxidant effects (Merfort, 2007). Aglichon and glyceride flavonoles which are present in *Nigella sativa* have strong scavenging and antioxidant effects. Antioxidants are the frontline of defense against free radicals (Osawa and Kato, 2005). Three types of alkaloids identified as the indazolenigelicine, the isoquinolinenigellimine and it's N-oxide and the indazole alkaloid nigellidine isolated from the defatted seeds of *N. sativa* has been known to resist the damaging effect of free radical (Kamal El-Din *et al.*, 2006).It can be speculated that of the role of the seeds in ameliorating cadmium-induced renal dysfunction, as seen in the present study, is in part due to the anti-inflammatory and antioxidant effects of the different compounds of the seeds.

Our result also revealed that cadmium administration caused a significant loss in body weight of rats. This may be attributed to loss in kidney weight, anemia and interference in metabolism of vitamin D leading to bone diseases the consequences which can be loss in body weights ((Staessen *et al.;* 2003).The significant increase in weight of rats in the various treatment groups may be due to the presence of relatively high carbohydrate (34%), fat (35.5), and protein (19.9) found in the seeds (Bahram *et al.;* 2009).

In conclusion, administration of cadmium has been demonstrated to induce renal dysfunction in rats altering the activities of ALT and AST and increasing both urea and creatinine levels. Thus, treatment of cadmium intoxicated rats with a dose of 250 mg/kg of hexane extract of *Nigella sativa* as co, pre and post has the ability to alleviate the deleterious effects of exposure to cadmium. In addition, it also seems that the pre and post treatment effect of the extract is more effective than its co-treatment. Further studies on larger animal model are required to draw final conclusions.

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