



A study on the effects of various *Nigella sativa* dosages on blood creatinine and urea levels of albino rats: An index to the functionality of the kidneys

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Abstract

This study was conducted to determine the toxic effect of *N. sativa* powder on the kidney function of albino rats, evaluated by serum urea and creatinine levels. Twenty-four male albino rats (*Ratus norvegicus*) randomly divided into four groups of six each and kept in the separate cages were used for the study. The treatment groups were given rat chow pellet containing *N. sativa* dose at 0.20, 0.20, and 2.00 g/kg body weight considered as low, normal, and high dose for five weeks while the control group fed with rat pellet without supplementation with *N. sativa*. The rats were sacrificed, at the end of 35 days, to take the blood sample for toxicity evaluation. Standard analytical techniques were used to determine the parameters. There was a significant reduction ($p < 0.05$) in serum creatinine level (mg/dL) at high dose of NS (0.21 ± 0.15) as compared with the control group (0.40 ± 0.10) and low dose *N. sativa* (0.38 ± 0.02) group. The normal dosage gave a value of 0.29 ± 0.22 mg/dL of creatinine. The normal urea and creatinine levels in the blood for all treatment groups implied that there was no toxic effect of *Nigella sativa* on the kidney function of the rats at different doses for a five-week period and thereby showing that *N. sativa* was save for consumption if only to consider its effect on the kidneys.

Keywords: *Nigella sativa*, albino rats, creatinine, urea, toxicity

Introduction

Plants have been known to be excellent source of therapeutic agents. In the words of Hernandez-Ceruelos *et al.* (2002), "the use of natural products with therapeutic properties is as ancient as human civilization and for a long time, mineral, plant, and animal products were the main sources of drugs for therapeutic purpose". They are also major sources of nutrition and health care for both humans and animals (Dollah *et al.*, 2012). Schwartzmann *et al.* (2002), wrote that "in recent years, there has been a growing interest in alternative therapies and the therapeutic use of natural products, especially those derived from plants". *Nigella sativa*, also known as black seed is one of such plants. *Nigella sativa* Linn is an annual herb that belongs to the family Ranunculaceae and is most extensively investigated for its therapeutic purposes. Its use as medicinal plant has been well documented. A lot of research works had also been carried out on the seed. It has been used as a herbal medicine for more than 2000 years. It is also used as a food additive and flavouring agent in many countries (Dollah *et al.*, 2012). The black seed oil is reported to be beneficial due to its content of over one hundred components such as aromatic oils, trace

elements, and vitamins (Ali and Blunden, 2003).

It had been shown that extract of the black seeds have many therapeutic effects, which include immunomodulative (Boskabady *et al.*, 2011), antibacterial (Rakhshandeh *et al.*, 2011; Zaoui *et al.*, 2000), anti-tumor (Turkdogan *et al.*, 2001), diuretic and hypotensive (Kanter *et al.*, 2003), genoprotective (Babazadeh *et al.*, 2012), hepatoprotective and antidiabetic (Kanter *et al.*, 2003), bronchodilator activity (Boskabady and Sheiravi, 2002; Boskabady *et al.*, 2004; Boskabady *et al.*, 2011), and oestrogenic activity (Parhizkar *et al.*, 2011). Several studies had been carried out on the toxicity of *N. sativa*. Only 3.4% were concerned about its toxicity (Anwar *et al.* 2005).

Kidney is the major organ in metabolising toxic compounds besides liver. It receives about 1200 ml blood per minute (Tortora *et al.*, 2006) containing a lot of chemical compounds and are at high risk to be exposed to toxic compounds. Therefore, high dose of *N. sativa* might cause damage to the kidney tissue which can be determined by measuring the level of urea and creatinine in blood as an indicator of kidney damage.

N. sativa is usually consumed by people without measuring the amount to be consumed. Knowing the therapeutic effect, but not the toxicological effect of any xenobiotic or foreign substance is usually a big problem and harmful. This study was, therefore, carried out to evaluate the toxic effect of *N. sativa* seed consumption at various doses, though small, on the kidney function of albino rat. This type of toxicological study is, aptly, termed renal or kidney function test and in this study it was evaluated by serum urea and creatinine levels. The results would help to determine how the consumption of *N. sativa* seed can affect kidney functions in animals generally.



Figure 1: *Nigella sativa* ((Randhawa and Al-Ghamdi, 2002)

Materials and methods

Area of the study: The experiment was carried out at the Department of Science Laboratory Technology and the Research Laboratory, Oyo State College of Agriculture and Technology, Igboora. Igboora is in derived savannah zone of 7°15' North and 3°30' East of the equator with an average rainfall of 1278mm and an annual temperature of 27°C (Akamigbo, 2010).

Sample and sample preparation Plant sample: Dried *N. sativa* seeds (imported from Saudi Arabia) were purchased from a local herb store in Oyo town, Nigeria. The seeds were cleaned by removing impurities mixed with them. They further cleaned under running tap water for 10 minutes, were rinsed twice with distilled water and air dried in a dust-fry environment in the Chemistry Laboratory for two weeks.

The seeds were ground to a powder form using an electric blender. The powder was later mixed with rat chow pellet powder and water at different concentrations of 0.02, 0.2 and 2 g/kg

of rats' body weights. The dough was later baked in an oven at 40 °C until it received constant weight.

Animals: Twenty-four male albino rats (*Ratus norvegicus*) with 300-350 g body weight were used for the study. The rats were supplied by a local breeder at Apete Ibadan. The animals were reared in the Animal House of the Department of Science Laboratory Technology, Oyo state College of Agriculture and Technology, Igboora. The cages were kept at ambient temperature, 70-80% humidity, and automatic 12 hours' light, 12 hours' dark cycle which are suitable condition for albino rats. The animals were allowed to acclimatize for a week before the start of the experiments. The rats were fed with a standard rat diets and given water *ad libitum*. All animals received human care and their handling was conducted between 7.00am and 8.00am daily, to minimize the effects of experimental stress. The treatments were given to the rats for 5 weeks. The body weight was measured once a week.

Experimental design: Animals were assigned into four treatment groups which are considered as control (0 g/kg bw), low (0.02 g/kg bw), normal (0.20 g/kg bw) and high (2.00 g/kg bw) doses of *N. sativa* for five weeks.

The control group was given normal chow pellets without *N. sativa* which was also used to mix the samples for the feeding of other treatment groups containing different doses of *N. sativa*. The dosages were chosen based on the perceived human *N. sativa* consumption which is equal to 2 g/day and considering conversion rate to rats, 0.2 g/kg was selected as the normal dose. The low dose was ten times less than the normal dose (0.02) while the high dose was one hundred times higher than the low dose (2.00). The treatments were given to the rats for five weeks.

Sacrificing and blood collection: At the end of the feeding, the animals were sacrificed by anaesthetizing and dissecting from the lower abdomen to the upper thoracic region. Blood sample from each was collected from the cut aorta of the rat and kept in separate heparinized sample bottle for the determination of creatinine and urea levels.

Determination of serum creatinine and urea levels: Serum creatinine and urea levels were measured using commercial analytical kits



in accordance with the manufacturer's instructions. Urea was determined using the methods outlined by Weatherburn (1967), and creatinine as described by Bartels and Bohmer (1972). Urea was determined by enzyme-kinetic assay (5 μ L serum) and creatinine concentration was evaluated by the kinetic reaction of Jaffe (60 μ L serum). The methods were calibrated by human multi-calibrator and verified using normal human control serum as well as water-based standard solution of creatinine.

Results and discussion

Table 1: Serum creatinine and urea levels of albino rats at different concentration of *Nigella sativa* powder

Group	Serum creatinine level (mgdL ⁻¹)	Serum urea level (mmolL ⁻¹)
Control	0.40 $\pm$ 0.10 ^a	5.94 $\pm$ 0.30 ^c
0.02	0.38 $\pm$ 0.02 ^a	5.10 $\pm$ 0.10 ^c
0.20	0.29 $\pm$ 0.22 ^b	4.99 $\pm$ 0.09 ^c
2.00	0.21 $\pm$ 0.15 ^b	4.50 $\pm$ 0.44 ^d

abcd: Different superscripts indicated mean difference between groups significant at $p < 0.05$.

The results of serum creatinine and urea analyses are shown in Table 1. There was a significant reduction ($p < 0.05$) in serum creatinine level (mg/dL) at high dose of *NS* (0.21 $\pm$ 0.15) as compared with the control group (0.40 $\pm$ 0.10) and low dose *N. sativa* (0.38 $\pm$ 0.02) group. The normal dosage gave a value of 0.29 $\pm$ 0.22 mg/dL of creatinine.

The results of serum urea analysis revealed a reduction in serum urea concentration in normal dose *N. sativa* group in comparison with the control, low and high concentration groups. The present study was designed to investigate the toxicity effect of *N. sativa* on renal function by evaluation of the creatinine and blood urea. Urea is a by-product from protein breakdown. About 90% of urea produced is excreted through the kidney (Walmsley *et al.*, 2010). Creatinine, on the other hand, is a waste product from a muscle creatinine, which is used during muscle contraction. Creatinine is commonly measured as an index of glomerular function (Treasure, 2003). Weber *et al.* (2002), gave the normal range of serum creatinine for rats 0.2–0.8 mg/dl. It is excreted exclusively through the kidney. Any damage to the kidney will make the kidney inefficient to excrete both urea and creatinine and causes their accumulation in the blood. Therefore, the high level of blood urea and creatinine will indicate kidney damage. In our study, there was a

Statistical analysis: Data were expressed as means $\pm$ standard error of mean (SEM). The data were analysed using SPSS windows program version 15 (SPSS Institute, Inc., Chicago, IL, USA). The one-way analysis of variance (ANOVA) and general linear model (GLM) followed by Duncan's Multiple Range Test (DMRT) were used for analysis of data. A p -value less than 0.05 ($p < 0.05$) was considered to be significant.

significant reduction ($p < 0.05$) in serum creatinine in the rats treated with high dose *N. sativa*.

The results of the present study showed that the supplementation of *N. sativa* to the diets of rats for five weeks did not change the biochemical parameters of kidney function which illustrated normal state of the kidneys. The same observations have been made by (Le *et al.*, 2004) in normal rats treated with the petroleum ether extract of *N. sativa* for four weeks. Therefore, the rats maintained healthy and the results were not influenced by the health status of the rats.

Ali and Blundes (2003), in a study, had previously concluded that oral administration of aqueous extract of *N. sativa* seeds showed no significant changes in kidney function. In the same manner, Al-ghamdi (2003), showed that no toxic effect was observed for mice administered with *N. sativa* fixed oil.

This study showed that *Nigella sativa*, when administered orally, has no toxicity by different *N. sativa* doses used. These results are in agreement with previous data reporting that *N. sativa* has a wide margin of safety (Parhizka, 2011; Al-Ameen *et al.*, 2011). With this, normal urea and creatinine levels in blood suggested that there was no toxic effect on kidney



function when *N. sativa* was administered at different doses for a five-week period.

Conclusion: In conclusion, the results of this study showed that there was no of toxic effect of *N. sativa* on rat kidney and suggested that the consumption of *N. sativa* powder by human may not have any toxic effect on the kidney function. So, *N. sativa* may be considered as a veritable therapeutic agent for both animals and human beings

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