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Ameliorative Effects of Amla (*Emblica officinalis*) Fruit Pulp Extract and Selenium on Dimethoate Induced Changes in Biochemical Parameters of Kidney in Rats

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Abstract: 120 Wistar rats were randomly divided into 6 numerically groups (A, B, C, D, E, F). These groups of rats were treated as-- group A: control; group B: dimethoate (20 mg/ kg b wt); group C: dimethoate (20 mg/kg b wt) + selenium (0.5 mg/ kg b wt); group D: dimethoate (20 mg/ kg b wt) + amla (200 mg/kg b wt); group E: selenium (0.5 mg/kg b wt); and group F: amla fruit pulp extract (200 mg/kg b wt). Blood was collected from rats of all groups on day 7 and 14. Sera were separated by centrifugation and analyzed for uric acid, urea and creatinine. Dimethoate exposure to rats caused an increase in serum uric acid on day 7 and day 14 as compared to group A (control). The serum uric acid levels decreased in group C (dimethoate + selenium) and group D (dimethoate + amla fruit pulp extract) at day 7 and day 14 when compared with group B (dimethoate only). The uric acid level remain unaffected in group E (selenium) and group F (amla fruit pulp extract) on day 7 and day 14. After 7 and 14 days treatment with dimethoate (group B) there was increase in serum urea level as compared to group A (control). Serum urea levels was decreased in group C and group D on day 7 and 14 as compared to group B. There was no alteration in urea level of group E (selenium) and group F (amla fruit pulp extract) on days 7 and 14 as compared to group A (control). Dimethoate (group B) provoked an increase in serum creatinine levels on day 7 and day 14 as compared to group A (control). The serum creatinine levels was decreased in group C and group D on day 7 and 14 as compared to group B (dimethoate only). In group E and F group F the creatinine levels remained unaffected as compared to group A (control).

Keywords: Dimethoate, Kidney, Selenium, Amla fruit pulp extract, Creatinine, Urea, Uric acid

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Introduction

The usage of pesticide has changed considerably nowadays. The hazards of using such chemical compounds have been accentuated by the sharp rise of their use in agriculture, industry and in households. Exposure to organophosphorus (OP) insecticides in agriculture is one of the

occupational hazards (Sivapiriya *et al.*, 2006). Numerous studies indicate that dimethoate intoxication can cause oxidation stress by the generation of free radicals and induce hepatic lipid peroxidation in mice (Attia *et al*., 2009). Dimethoate cause oxidative stress and DNA damage (Dogon *et al*., 2011). Dimethoate an Organophosphate insecticide, inhibits the enzyme acetylecholinestrase and cause damage to nervous tissue and provoke neurophysiological abnormalties (Khogali *et al.*, 2005; Apaydin *et al.*, 2016). Excessive use of pesticides in large quantities is of concern as it cause environmental pollution. Remnants of these pesticides have been noticed in the soil, water reservoirs, vegetables, grains and other food products. Dimethoate can potentially enter the bodies of mammals through various routes, including ingestion, dermal contact, inhalation, accidental exposure (Attia *et al.*, 2009). Dimethoate causes oxidative stress in kidney of rat (Mahjoubi-Samet *et al*., 2008). It also effect bone (Mahjoubi-Samet *et al*., 2005). Kidney is one of the target organ of the animals exposed to dimethoate (Oncu *et al*., 2002; Sulak *et al*., 2005; Sivapriya *et al*., 2006; Suna *et al*., 2007). Prolonged exposure or high-level exposure to dimethoate may leads to kidney damage or dysfunction of nephrons (Baothman *et al.*, 2023).

Emblica officinalis Gaertn (also called *Phylanthus emblica*), known as Amla or Indian gooseberry, belongs to the family Euphorbiaceae. Ayurveda believed that the fruit of iincrease defence against numerous disease such as cancer, ulcer, anemia, heart diseases, liver, kidney and diabetes (Ali *et al*., 2021); Vasant and Narsimhacharya, 2012; Majeed *et al*., 2023). It also acts as antioxidant, antitussive, gastroprotective, immunomodulatory, analgesic, and antipyretic. Besides, it is useful to control the cholesterol level and also boost memory in ophthalmic disorders. It is also beneficial in snake venom neutralization and as an antimicrobial (Ali *et al*., 2021). The pulp of amla fruit has been used for various ayurvedic treatment and prevention of various maladies for centuries. Various animal and clinical studies have provided a scientific basis for the rejuvenating and curative properties of amla (Rao *et al*., 2013). The amla fruit includes numerous bioactive components including isostrictinin, ellagic acid, apigenin, chebulinic acid, quercertin, gallic acid and chebulagic acid. The tannins are also found in the fruit extract of amla which are pedunculagin, emblicanin A, phyllaemblicin B, emblicanin B and punigluconine. 100 g of edible fruit have been reported to contain 470-680 mg of vitamin C (El-Desouky *et al*., 2008). Amla pulp extract has been reported to protect kidney damage and improve kidney function (Ajmera, 2021). Supplementation of amla reduces oxidative stress in uremic patient and may increases kidney and hepatic function (Chen *et al*., 2009).

Selenium (Se) is a trace mineral, ubiquitously occurring in the environment that is of importance to human health. Its impact on the human body and the mechanisms involved have been thoroughly investigated. Selenium is important for controlling many natural body functions which is related to its incorporation through selenocystein (Kieliszek *et al*., 2022). The most important metabolic roles of Se in mammalian cells are due to its function in the active site of many antioxidant enzymes, for example thioredoxin reductase and glutathione peroxidase (GPx), the strongest enzyme in the body. Due to its antioxidant properties administration of selenium in diet protect kidney from diseases (Xie *et al*., 2022).

The present study was undertaken to evaluate the protective efficacy of amla fruit pulp extracts (APE) and selenium (Se) against nephrotoxicity in terms of kidney biomarkers induced by dimethoate in rat. To the best of our knowledge protective effects of amla fruit pulp extracts and selenium on nephrotoxicity induced by dimethoate has not been reported prior to this study.

Materials and Methods

Wistar rat (50-60 g b wt; n=120) were purchased from Asia Scientific Emporium, Varanasi, India and were kept for 15 days for acclimatization under

standard laboratory conditions in polypropylene cages at 20 to 25℃. They were fed with standard food and water *ad libitium* throughout experiments.

The doses of Dimethoate, selenium and amla fruit pulp extract given to rats are based on the reports of previous investigators- Dimethoate [20 mg/kg b wt (Sayim, 2007); 30 mg/kg b wt (Sharma *et al*., 2005); 15, 28 mg/kg b wt (Farag *et al*., 2007); 25 mg/kg b wt (Barski and Spodniewska, 2012)]; Selenium [0.5 mg/kg b wt (Hadrup *et al.*, 2016)]; and Amla pulp extract (200 mg/kg b wt (Elobeid and Elham, 2015; Yadav *et al*., 2017).

Dimethoate 30% EC organophosphate insecticide was purchased from Land Agriculture Store, Gorakhpur, India

The fruit of Amla were purchased locally. These were washed thoroughly with water, then semi-dried in oven at 40℃. After this the pulp of the fruit was taken and cut into small pieces and dried at 40℃. After drying the pulp was grinded into powder. Soxhlet extraction was not used in this extraction process to prevent denaturation of heat-labile several compounds present in amla fruit pulp (AP). The powder was mixed with 90% ethyl alcohol (1:20 ratio) for use. Then it was filtered with Whatman no.1 filter paper. The residue was dried at 40℃ and stored at -20℃ for further use. For use, the residue was reconstituted with ethanol to make the required dose for administration to rats.

Purified Sodium selenite was purchased from Eastern Scientific Emporium Gorakhpur. For further use it was dissolved in distilled water. The study was approved by Research Degree Committee of DDU Gorakhpur University, Gorakhpur, India.

For the experiment the rats were randomly divided into six numerically (for each group n=20) equal groups A, B, C, D, E and F. These groups were treated as follow at 8:00 a.m. daily throughout the experiment:

Group A- Control: No treatment was given

Group B- (Dimethoate treated rats): Received daily only Dimethoate (20 mg/kg b wt)

Group C- (Dimethoate + Selenium): These rats were given daily Dimethoate (20 mg/kg b wt) and Selenium (0.5 mg/kg b wt) simultaneously

Group D- (Dimethoate + Amla fruit pulp pxtract): These rats were given daily Dimethoate (20 mg/kg b wt) and Amla fruit pulp pxtract (200 mg/kg b wt) simultaneously

Group E- (Selenium): Rats received daily Selenium (0.5 mg/kg b wt).

Group F- (Amla fruit pulp pxtract): Rats received daily Amla fruit pulp pxtract (200 mg/kg b wt)

Rats (n=10 from each group at each interval) were sacrificed at 7 and 14 days after treatment under light ether anesthesia. Animals were fasted over night before the day of sacrifice. Blood samples were collected by cardiac puncture and centrifuged at 3000 rpm for 5 minutes. The serum was separated and stored at -20℃. Uric Acid, Urea and Creatinine levels were analysed by using kits (Beacon Diagnostics Private Ltd, India). Each sample was analysed in duplicate.

Statistical analysis:

ANOVA (One Way Analysis of Variance) was used to determine the statistically significant differences between various exposure times and treatments. The Student's t test was used to evaluate the statistical significance. The significance level was set at $P<0.05$.

Results

Dimethoate exposure to rats caused an increase in serum uric acid on day 7 ($P<0.0001$) and day 14 $P<0.0002$) as compared to group A (control) (Fig. 1). The serum uric acid levels decreased in group C (dimethoate + selenium) and group D (dimethoate + amla fruit pulp extract) at day 7 and day 14 when compared with group B (dimethoate only). This shows that amla fruit pulp extract and selenium is effective in decreasing the uric acid levels which was increased by dimethoate.

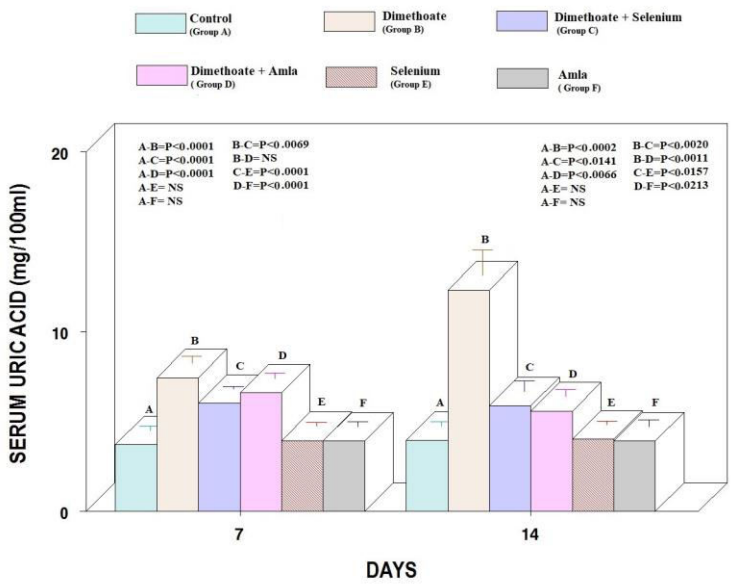

Fig. 1: Serum uric acid levels (mg/100 ml) of rats after various treatments. All values indicate mean ± SE of six specimens.

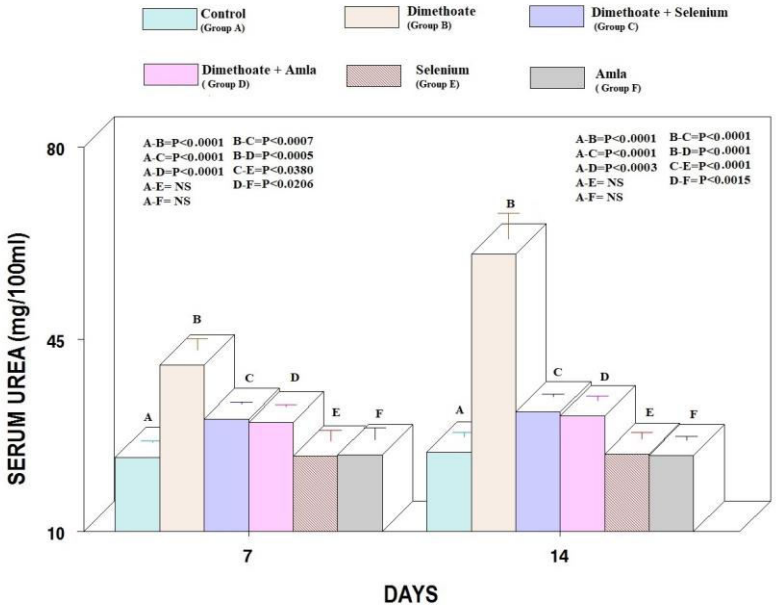

Fig. 2: Serum urea levels (mg/100 ml) of rats after various treatments. All values indicate mean ± SE of six specimens.

The uric acid level remained unaffected in group E (selenium) and group F (amla fruit pulp extract) on day 7 and day 14. The ANOVA indicated that treatments are significant (day 7- F= 34.986, P<0.0001; day 14 - F= 21.377, P<0.0001).

After 7 and 14 days treatment with dimethoate (group B) there was an increase in serum urea level as compared to group A (control) (Fig. 2). Serum urea level was decreased in group C (dimethoate + selenium) and group D

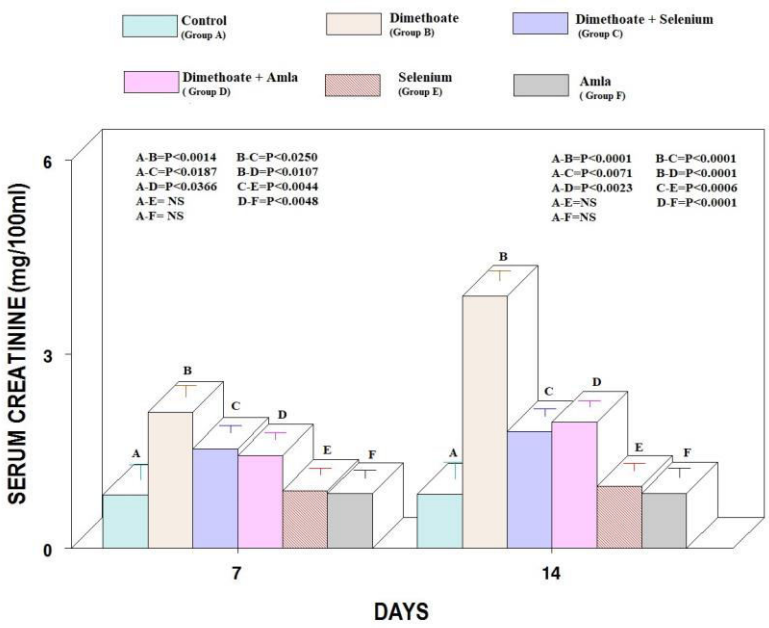

Fig. 3: Serum creatinine levels (mg/100 ml) of rats after various treatments. All values indicate mean ± SE of six specimens.

(dimethoate + amla fruit pulp extract) on day 7 and 14 as compared to group B (dimethoate exposed) (Fig. 2). This shows that selenium and amla fruit pulp extract are effective in restoring the urea level which was increased by dimethoate treatment. There was no alteration in urea level of group E (selenium) and group F (amla fruit pulp extract) on days 7 and 14 as compared to group A (control) (Fig. 2). The ANOVA indicated that treatments are significant (day 7- F= 20.06, P<0.0001; day 14- F= 43.829, P<0.0001).

Dimethoate (group B) provoked an increase in serum creatinine levels (Fig. 3) on day 7 and day 14 as compared to group A (control). The serum creatinine levels was decreased in group C (dimethoate + selenium) and group D (dimethoate + amla fruit pulp extract) on day 7 and 14 as compared to group B (dimethoate only). This shows that selenium and amla fruit pulp extract can protect the kidney biomarkers which was affected by dimethoate. In group E (selenium) and group F (amla fruit pulp extract) the creatinine levels remained unaffected as compared to group A (control) (Fig. 3). The ANOVA indicated that treatments are significant (day 7- F= 10.994, P<0.0001; day 14- F=56.503, P<0.0001).

Discussion

In mammals dimethoate provoked toxic effects such as teratogenicity (Javed *et al*., 2023), genotoxicity (Nazam *et al*., 2020), hepatotoxicity (Sayim, 2007), and nephrotoxicity (Mahjoubi-Samet *et al*., 2008; Al-Awthan *et al.,* 2014). In the present study uric acid levels were elevated after exposure to dimethoate (group B) as compared to the levels of control (group A). The findings of Abbas *et al*. (2016), Abungabal *et al.* (2020) and Yusuf *et al.* (2023) lend support to the present study as they have also noticed elevation of uric acid levels after exposure to toxicants. In the forgoing study there is a decrease in the uric acid levels when the rats were supplemented with selenium or amla fruit pulp extract in combination with dimethoate. This clearly shows the protective role of selenium and amla fruit pulp extract on nephrotoxicity induced by dimethoate.

Serum urea level of rats was increased after Treatment with dimethoate after 7 and 14 days.

Increased serum urea levels in rats after exposure to toxicants have been noticed by other workers ---- Arsenic –Yousuf *et al.* (2023); Ammonia- Peygham and Takamy (2002); and Arsenic and Nicotine- Jain *et al*. (2015). Administration of selenium and amla fruit pulp extract in combination with dimethoate significantly decreased the urea level as compared to group B (dimethoate exposed rats only). Increased urea level in mice after treatment with chromium has been noticed by Abbas *et al.* (2016). They have also noticed restoration of urea levels to near control levels after administration of jamun pulp extract. The present study derives support from the studies of Abbas *et al*. (2016) as they have also noticed restoration of urea levels after jamun pulp extract. Urea levels of rat remained unaffected in group E (Selenium) and group F (amla fruit pulp extract).

In this study dimethoate elevated the creatinine levels in rats which was decreased when the rats were given selenium or amla fruit pulp extract in combination with dimethoate. This indicates that the nephrotoxicity in terms of elevated creatinine levels induced by dimethoate can be protected with administration of selenium and amla fruit pulp extract. Elevated creatinine levels has been reported by other investigators after exposure to toxicants----Arsenic (Cardenas Gonzalez *et al*., 2016), Diazinon (Al-Attar and Abu Zeid, 2013), Aflatoxin and cypermethrin (Hussain *et al*., 2009), Mercury (Desai *et al*., 2021), Indoxacarb (Ali *et al*., 2018), Chromium (Abbas *et al*., 2016), Cadmium (Ivan *et al*., 2010), Sodium nitrite (Sanjari and Seyyed, 2021). In non-mammalian vertebrates also, toxicants elevate the creatinine levels-- (i) Amphibians- Oxyfluorfen (Ghada *et al*., 2019), Atrazine (Sena *et al*., 2021); and (ii) Fishes – Bisphenol A (Akram *et al*., 2022), Malathion (Bharti and Rasool *et al*., 2021), Imidacloprid (Priya *et al*., 2012). No changes was noticed in cretinine levels after exposure of selenium and amla fruit pulp extract. There exist no report regarding the protective effects of selenium and amla fruit pulp extract on creatinine levels of rat.

Conclusion

It can be concluded from the present study that dimethoate provokes changes in the uric acid, urea and creatinine levels. Administration of selenium and amla fruit pulp extracts could protect alterations in these kidney biomarkers. It is advisable that the organisms exposed to organophosphates should be given dietary supplement of selenium or amla fruit pulp extract which would ease the toxic symptoms.

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