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Research Article
Effect of *Datura stramonium* on Cyclophosphamide-induced Oxidative Stress in Albino Rats: Study on Kidney Markers

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Abstract

Background: Cyclophosphamide is a chemotherapeutic agent, whose activation induces oxidative stress, causing nephrotoxicity. The present study ascertained the effect of the aqueous extracts of *Datura stramonium* seeds and leaves on cyclophosphamide-induced oxidative stress in rats. **Materials and Methods:** Twenty-four Wistar albino rats were divided into 6 groups of 4 rats each. All groups, except group 1, were induced with cyclophosphamide at a dose of 150 mg kg^{-1} b.wt. Group 1 was the normal control, group 2 (positive control) was left untreated, group 3 and 4 were administered 200 and 400 mg kg^{-1} b.wt., of the seed extract respectively, while group 5 and 6 were administered 200 and 400 mg kg^{-1} b.wt., of the leaf extract respectively, after cyclophosphamide induction. **Results:** Administration of the extracts caused significant ($p<0.05$) decreases in catalase and glutathione peroxidase, glutathione, malondialdehyde and urea concentrations compared to group 2. The extract also led to significant ($p<0.05$) increases in albumin, globulin, total protein, vitamins C and E concentrations compared to group 2. However, there was no significant difference in vitamin A concentration after extract administration. **Conclusion:** This study showed the ability of *Datura stramonium* in restoring oxidative stress induced by cyclophosphamide, though not all indices considered were significantly reversed.

Key words: *Datura stramonium*, cyclophosphamide, malondialdehyde, nephrotoxicity, glutathione peroxidase, oxidative stress

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Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Oxidative stress is the disproportion between oxidants and antioxidants in favour of oxidants, potentially leading to the generation of highly reactive free radicals[1,2]. It is created when these excessive free radicals react with proteins, cell walls and nucleic acids, causing damage to cell structures and thereby leading to degenerative diseases[3]. Oxidation to macromolecules due to reactive oxygen species (ROS) has been implicated in diseases such as cancer, myocardial infarction, rheumatoid arthritis and degeneration process of aging[4,5]. The ROS are generated internally as by-products of normal aerobic metabolism in the mitochondrion or externally through tobacco smoke, UV radiation and other environmental pollutants[6]. The physiologically important intracellular levels of ROS are maintained at low levels, under normal conditions by enzyme systems participating in the *in vivo* redox process of homeostasis, or by non-enzymatic compounds. However, during oxidative stress, the level of ROS increases and overwhelms the antioxidant systems resulting in damage to tissues[7,8].

Plants have always been used in the treatment of human traumas and diseases worldwide. They represent important source of antioxidants due to their inherent phytochemical constituents[2]. *Datura stramonium*, known as Jimson weed, belonging to the family *Solanaceae*, is a plant native to the tropics[9]. It is also commonly found growing as a weed by roadsides, undisturbed sites[10], grass fields and brushwood. It has small, ovate to sub ovate leaves which have long, stout petioles, coarsely serrate margin, 5-20 cm (2-8 inches) long and acuminate at their tips. The leaves have an unpleasant scent when crushed or bruised[11]. It has medium-sized fruits with several slender spines. Its seed capsules are located at the forks between branches and split open into four segments when matured. They are ovoid in shape, 3-5 cm (1-2 inches) long and are covered in prickles; containing dark, wrinkled seeds[12]. The toxicity of alkaloids isolated from the seeds has been demonstrated[13]. The plant has anti-microbial, antifungal and anti-inflammatory properties[14-16]. Previous studies have also demonstrated the *in vitro* anti-oxidant activity of *Datura stramonium* leaves and seeds[17-20]. Since induction of cyclophosphamide causes oxidative stress, affecting the kidneys[21-23] and owing to the antioxidative capacity of the plant, the present study determined the ability of the plant to reverse oxidative stress induced by cyclophosphamide, by determining its effect on some antioxidant indices and kidney function markers.

MATERIALS AND METHODS

This study was carried out at the Department of Biochemistry, University of Nigeria, Nsukka from April to June, 2017.

Instruments/equipment: Rotary evaporator (Model Modulyo 4K, Edward, England), water bath (Gallenkamp, England), chemical balance (Gallenkamp, England), conical flasks (Pyrex, England), hotbox (Gallenkamp, England), centrifuge (PIC, England), digital photo calorimeter (EI 312 model, Japan), adjustable micropipette (Perfect, USA), pH meter (Pye, Unicam 293, England), multi-well microtiter plate reader (Tecan, Austria), refrigerator (Kelvinator, Germany) and electrical grinder.

Identification and extraction of plant materials: Fresh leaves and seeds of *Datura stramonium* were obtained from Calabar, Nigeria and were identified by a taxonomist-Mr. Alfred Ozioko of International Centre for Ethno medicine and Drug Development (InterCEDD), Nsukka, Enugu State, Nigeria. The leaves and seeds were air-dried at room temperature and ground separately to powdery form using an electrical grinder. The ground samples were extracted with water by maceration and subsequently filtered. The filtrates were concentrated using rotary evaporator, producing the aqueous leaf and seed extracts which were used for analysis.

Animals: Adult male Wistar rats with average weight of 160±15 g were obtained from the Animal House of the Faculty of Veterinary Medicine, University of Nigeria, Nsukka. The animals were acclimatised for one week under standard environmental conditions and maintained on a regular feed (vital feed) with water *ad libitum*. Ethical approval on the use of these animals was obtained from the University of Nigeria committee on the care and use of laboratory animals, in accordance to the revised National Institute of Health Guide for Care and Use of Laboratory Animal (Pub No. 85-23, revised 1985).

Experimental design: Twenty-four male wistar rats were divided into six (6) groups of four (4) rats each and treated as shown below:

Group 1 : Normal control (No induction, no treatment)
Group 2 : Positive control (Cyclophosphamide-induced, untreated rats)

Group 3 : Cyclophosphamide+200 mg kg^{-1} b.wt., aqueous seed extract of *Datura stramonium*
Group 4 : Cyclophosphamide+400 mg kg^{-1} b.wt., aqueous seed extract of *Datura stramonium*
Group 5 : Cyclophosphamide+200 mg kg^{-1} b.wt., aqueous leaf extract of *Datura stramonium*
Group 6 : Cyclophosphamide+400 mg kg^{-1} b.wt., aqueous leaf extract of *Datura stramonium*

Lipid peroxidation assay: Lipid peroxidation assay was done by determining the concentration of malondialdehyde (MDA) formed using the method of Wallin *et al.*[24].

Assay of superoxide dismutase activity: Superoxide dismutase (SOD) activity was assayed using the method of Woolliams *et al.*[25] as contained in the Randox commercial kit.

Assay of catalase activity: Catalase (CAT) activity was assayed using the method of Aebi[26].

Assay of glutathione peroxidase activity: This was done according to the method of Paglia and Valentine[27].

Estimation of reduced glutathione: This was achieved by following the method of Exner *et al.*[28].

Estimation of vitamin A: The method of Subramanyam and Parrish[29] was used to determine the vitamin A concentration.

Estimation of vitamin C: The method of Baker *et al.*[30] was used to determine vitamin C (ascorbic acid) concentration.

Estimation of vitamin E: Vitamin E content was estimated by the method of Pearson[31].

Determination of urea concentration: The concentration of urea was determined using the modified diacetyl monoxime method as described by Wybenga *et al.*[32].

Determination of creatinine concentration: The concentration of creatinine was determined by means of the alkaline picrate method as described by Henry *et al.*[33].

Determination of total protein concentration: Total protein concentration was determined through the Biuret's reaction as described by Henry *et al.*[33].

Determination of albumin concentration: Albumin concentration was determined using the bromocresol green (BCG)-dye binding reaction as described by Doumas *et al.*[34].

Determination of globulin concentration: Serum globulin was estimated indirectly by subtracting the albumin concentration from the total protein concentration.

Albumin/globulin ratio: This was obtained by dividing the concentration of albumin by that of globulin.

Statistical analysis: Data from the experimental study were analysed using one-way analysis of variance (one-way ANOVA) in statistical products and service solutions (SPSS), version 20. Results were expressed as Mean±Standard deviation. Values with different superscripts were considered statically significant at $p<0.05$.

RESULTS

Effect of *D. stramonium* seed and leaf extracts on antioxidant Indices in cyclophosphamide-induced rats: There were significant ($p<0.05$) decreases in the catalase, gluthathione, gluthathione peroxidase and MDA concentrations of group 3-6 rats compared to group 2. SOD activity showed a significant ($p<0.05$) decrease in only group 4 rats compared to group 2 (Table 1).

Effect of *D. stramonium* seed and leaf extracts on antioxidant vitamins concentration in cyclophosphamide-induced rats: Results in Table 2 shows significant ($p<0.05$)

Table 1: Effect of seed and leaf extracts of *D. stramonium* on antioxidant status of experimental rats

Groups	SOD (IU L^{-1})	CAT (IU L^{-1})	GPx (IU L^{-1})	GSH (mg dL^{-1})	MDA (mg dL^{-1})
1	10.93±0.70^{b}	1.47±0.24^{a}	10.14±0.44^{a}	2.72±0.45^{a}	1.45±0.30^{a}
2	11.36±0.11^{b}	3.65±0.70^{b}	14.01±1.78^{b}	3.61±0.57^{b}	2.98±0.59^{b}
3	10.99±0.26^{b}	1.53±0.35^{a}	10.56±1.09^{a}	2.72±0.29^{a}	1.72±0.18^{a}
4	10.38±0.12^{a}	1.84±0.13^{a}	10.56±1.30^{a}	2.57±0.32^{a}	1.53±0.29^{a}
5	11.05±0.20^{b}	1.38±0.23^{a}	10.56±0.83^{a}	2.97±0.57^{ab}	1.36±0.36^{a}
6	10.91±0.27^{b}	1.69±0.29^{a}	10.34±0.71^{a}	2.47±0.26^{a}	1.45±0.35^{a}

Results are Mean±SD (n = 4), values with different superscripts ($^{a, b}$) down the column are significant at $p<0.05$, Group 1: No induction, no treatment (normal control), Group 2: Cyclophosphamide-induced untreated rats (positive control), Group 3: Cyclophosphamide-induced+200 mg kg^{-1} b.wt., aqueous seed extract, Group 4: Cyclophosphamide-induced+400 mg kg^{-1} b.wt., aqueous seed extract, Group 5: Cyclophosphamide-induced+200 mg kg^{-1} b.wt., aqueous leaf extract, Group 6: Cyclophosphamide-induced+400 mg kg^{-1} b.wt., aqueous leaf extract

increases in vitamin C and E concentrations of group 3-6 rats compared to group 2. However, there was a non-significant ($p>0.05$) increase in vitamin A concentration of groups 3, 4 and 6 rats compared to group 2.

Effect of leaf and seed extracts of *D. stramonium* on kidney markers in cyclophosphamide- induced rats: There was a significant ($p<0.05$) increase in albumin, globulin and total protein concentration and a non-significant ($p>0.05$) increase in albumin/globulin ratio and creatinine concentration of treated rats compared to group 2. However, there was a significant ($p<0.05$) decrease in the urea concentration of treated groups compared to group 2 (Table 3).

DISCUSSION

The protective effect of the aqueous extracts of *Datura stramonium* seeds and leaves against cyclophosphamide-induced oxidative stress in rats was ascertained in this study. Induction with cyclophosphamide led to an increase in GSH concentration, SOD, CAT and GPx activities, which could be a protective response of the body to the oxidative stress induced by cyclophosphamide[35]. This was however not in consonance with the findings of Mahmoud[36] and Germoush[37] as they demonstrated increase in the activity of antioxidant enzymes after cyclophosphamide induction. Administration of the extracts restored the altered anti-oxidant defence system to normal. One of the major alterations that also occur in the components of a cell after cyclophosphamide administration is lipid peroxidation[38,39], leading to an increase in the lipid peroxidation product, MDA as observed in this research. The administration of the aqueous extracts of *Datura stramonium* seeds and leaves significantly ($p<0.05$) restored the concentrations of MDA, signifying inhibition of peroxidation of membrane lipids. Cyclophosphamide induction led to decrease in anti-oxidant vitamins due to the increased levels of free radicals generated as a result of acrolein-induced lipid peroxidation. The decrease in vitamin C concentration is corroborated by the report of Olayinka *et al.*[23]. Compared to cyclophosphamide group, the extract increased the concentration of antioxidant vitamins, which could scavenge free radicals generated due to cyclophosphamide induction.

Anticancer drugs, such as cyclophosphamide have been associated with renal dysfunction due to damage to the structure of the kidneys[40]. In this study, this was shown by the significant ($p<0.05$) increase in serum concentration of urea, although there was no significant ($p>0.05$) difference in serum creatinine concentration. The significant increase in the level of urea after cyclophosphamide induction is in tandem with the result of an earlier study[41]. Urea and creatinine are waste products eliminated from the blood, through the kidney by a blood filtration process. Injury in the renal tubule due to the effect of cyclophosphamide on the kidney, affecting tubular transport and increasing oxidative stress[42,43] reduced the glomerular filtration rate, hence, increasing serum urea concentration. The non-significant changes in serum creatinine level after cyclophosphamide induction could be because the level of serum creatinine does not rise until at least half of the kidney's nephrons are damaged or destroyed[44] by cyclophosphamide. This is substantiated by the report of Sugumar *et al.*[45]. The extract restored the observed changes in urea concentration, shown by the significant ($p<0.05$) decrease in the treated groups compared to the cyclophosphamide group. Also, the non-significant difference in serum creatinine observed for the extract group is supported by the study of Benouadah *et al.*[46] which showed that long-term administration of alkaloids from *D. stramonium* does not have effect on urea and creatinine concentration. However, these findings are contrary to those

Table 2: Effect of seed and leaf extracts of *D. stramonium* on antioxidant vitamins status of experimental rats

Groups	Vitamin A	Vitamin C	Vitamin E
	---------- (mg dL^{-1}) ----------		
1	4.29±1.17^{b}	2.65±0.50^{b}	1.65±0.10^{c}
2	2.32±0.79^{ab}	1.32±0.33^{a}	0.95±0.04^{a}
3	2.94±1.39^{ab}	2.93±0.29^{bc}	1.49±0.07^{b}
4	3.01±1.90^{ab}	3.18±0.10^{cd}	1.52±0.09^{bc}
5	2.03±1.19^{a}	2.72±0.33^{bc}	1.52±0.11^{bc}
6	3.37±0.21^{ab}	3.52±0.26^{d}	1.47±0.15^{b}

Results are Mean±SD (n = 4), values with different superscripts ($^{a, b, c}$) down the column are significant at $p<0.05$

Table 3: Effect of seed and leaf extracts of *D. stramonium* on kidney markers in cyclophosphamide-induced rats

Groups	Albumin (g dL^{-1})	Globulin (g dL^{-1})	Alb/glob ratio	Urea (g dL^{-1})	Creatinine (g dL^{-1})	Total protein (g dL^{-1})
1	4.54±0.48^{b}	1.96±0.45^{ab}	2.44±0.77^{a}	26.18±5.91^{a}	0.50±0.22^{a}	6.50±0.57^{b}
2	2.21±0.71^{a}	1.34±0.36^{a}	1.85±1.01^{a}	45.67±5.65^{b}	0.33±0.13^{a}	3.55±0.35^{a}
3	4.49±0.34^{b}	2.17±0.61^{ab}	2.28±0.97^{a}	27.61±5.09^{a}	0.45±0.26^{a}	6.66±0.53^{b}
4	4.48±0.48^{b}	2.69±0.83^{b}	1.89±0.96^{a}	25.18±5.68^{a}	0.61±0.28^{a}	7.17±0.59^{b}
5	4.68±0.12^{b}	1.89±0.69^{ab}	2.95±1.75^{a}	32.05±5.64^{a}	0.61±0.52^{a}	6.57±0.57^{b}
6	4.12±0.50^{b}	2.60±1.11^{b}	1.85±0.84^{a}	24.20±5.28^{a}	0.72±0.34^{a}	6.72±1.06^{b}

Results are Mean±SD (n = 4), values with different superscripts ($^{a, b}$) down the column are significant at $p<0.05$, Alb/glob: Albumin/globulin

of Gidado *et al.*[47] and Hamidu *et al.*[48], who revealed significant changes in creatinine concentration and non significant difference in urea concentration after extract administration.

The significant ($p<0.05$) reduction in serum concentrations of albumin observed in this research after cyclophosphamide induction was due to the fact that albumin trapped the released free radicals, as more than 70% of the free radical-trapping activity of serum was due to serum albumin[49]. Induction of cyclophosphamide also suppresses the humoral immune system[50], leading to the decrease in immunoglobulins and subsequently, globulin in the serum. Administration of different doses of the seed and leaf extracts significantly ($p<0.05$) restored total protein concentrations in the serum. Total protein level is also indicative of kidney disease, as albumin/globulin ratio is an index of disease state. Study showed that serum albumin is lower in malnutrition, inflammation, renal and hepatic disorders[51].

CONCLUSION

The present study demonstrated the anti-oxidative potentials of the aqueous extracts of *Datura stramonium* seeds and leaves against cyclophosphamide-induced oxidative stress and gave credibility to the traditional claim of the use of the plant in managing kidney disorders. The plant restored the enzymatic antioxidant defense systems, decreased MDA and urea concentration, increased antioxidant vitamins and total protein concentration.

SIGNIFICANCE STATEMENT

This research established the antioxidant activity of *Datura stramonium* during cyclophosphamide-induced oxidative stress. Antioxidant principles in *Datura stramonium* scavenged the reactive oxygen species released during the activation of cyclophosphamide. This research will therefore enable further studies on characterizing the antioxidant principles in *Datura stramonium*, which could serve as successful drug leads. In the long run, the side effect of cyclophosphamide-a chemotherapeutic agent-on the kidneys could be ameliorated.

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