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RESEARCH ARTICLE

PROTECTIVE EFFECT OF SOLANUM NIGRUM ON NEPHROTOXICITY IN ANIMALS TREATED WITH ANTITUMOR DRUG (CISPLATIN).

Ayatullah Mohamed Salama, Khalid Bassiouny, Ibrahim Helmy El-Sayed, Mahmoud Emam Nasr.
Department of Molecular Biology, Genetic Engineering and Biotechnology Research Institute, Sadat City University, Sadat City, Menoufia, Egypt.

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Corresponding Author

Ayatullah Mohamed Salama.

Abstract

Objectives. The aim of this study is to evaluate the effect of *Solanum nigrum* on nephrotoxicity and oxidative stress induced by cisplatin. Methods. Male mice were divided into 4 groups: control (Group A), cisplatin (Group B), *Solanum nigrum* (Group C), and cisplatin plus *Solanum nigrum* (Group D). Creatinine and urea were evaluated for nephrotoxicity. Catalase (CAT), glutathione-S-transferase (GST), nitric oxide (NO), and malondialdehyde (MDA) were measured colorimetrically and heme oxygenase-1 (HO-1) was determined by real-time PCR for evaluation of oxidative stress. Results. CAT significantly decreased while creatinine, urea, GST, NO, MDA, and HO-1 significantly increased only in cisplatin-treated group. Discussion. *Solanum nigrum* ameliorates nephrotoxicity and oxidative stress induced by cisplatin.

Introduction:-

Cisplatin is widely used as a potent chemotherapeutic agent available to treat a variety of solid tumors and other malignancies[1-3]. However, nephrotoxicity or acute kidney injury is one of the major side effects of cisplatin[4-6]. The precise mechanism is not determined but there are studies that suggest the role of reactive oxygen species (ROS) in this side effect[7-11], such as superoxide anion and hydroxyl radical which inhibit the activity of antioxidant enzymes in renal tissue[12].

Plant-derived natural products have received considerable attention in recent years due to diverse pharmacological properties, including antioxidant and antitumor activity[13-14]. *Solanum nigrum* L., belonging to the nightshade of the Solanaceae family, is antiseptic and antidysentric agent and is used in the treatment of heart, skin disease, psoriasis, and inflammation of kidney[15-16]. Also, extracts of the plant are used as analgesic, antispasmodic, anti-inflammatory, antidiabetic, vasodilator, emollient, and laxative agents[17-18]. It was also found that *Solanum nigrum* leaves and fruits are potential sources of cytotoxic and antioxidant agents[19-21]. The aim of this study is to detect the antioxidant effect of *Solanum nigrum* on the nephrotoxicity and oxidative stress caused by cisplatin.

Material and Methods:-

Plant:-

Solanum nigrum was received as a gift from Dr. Mamdouh Salem Serag, Department of Botany, Faculty of Science, Damietta University, New Damietta, Egypt. The plant was washed, shade-dried, and ground. An aqueous extract was prepared by immersing the whole plant in boiling distilled water. Then the extract was filtered and evaporated under vacuum.

Animals:-
Thirty-two male mice were divided into 4 groups, each containing 8 mice. The animals were kept in a special room at a constant temperature of 22 ± 1°C with 12 h light/dark cycles and had free access to diet and tap water.

Experimental Design:-
Animals were divided into 4 groups, each of 8 animals. Group A was treated with vehicle (distilled water). Group B was injected with a single dose of cisplatin (10 mg/kg body weight; i.p.) (Mylan, USA). Group C was treated with *Solanum nigrum* aqueous extract (SNE) alone at a daily dose of 1 gm/kg body weight. Group D was treated with *Solanum nigrum* aqueous extract at a daily dose of 1 gm/kg body weight along with a single dose of cisplatin (10 mg/kg body weight). The plant extract was administered via oral gavage for 3 days starting 1 hr after the administration of cisplatin.

Biochemical Analysis:-
Seventy-two hours after cisplatin injection, blood was collected from mice retroorbitally in Eppendorf tubes containing an anticoagulant and then mice were sacrificed and the kidneys were removed.
The blood samples were centrifuged at 3,000 rpm for 10 min at 4°C to separate plasma, which was used for analysis of urea, creatinine, catalase, and glutathione-S-transferase.

One of the two kidneys of each mouse was washed with phosphate-buffered saline and homogenized in cold buffer and the supernatant was collected by centrifugation and used for analysis of MDA and NO (by colorimetry). The other kidney was used for real-time PCR analysis.

Assessment of Nephrotoxicity:-
Creatinine and urea were measured using standard laboratory techniques and employing the commercially available diagnostic kits.

Determination of Lipid Peroxidation and Antioxidant Enzymes:-
Antioxidant markers GST, NO, and CAT and an oxidative damage biomarker (MDA) were measured by a colorimetric method using commercial kits (Biodiagnostic, Dokki, Cairo, Egypt) according to the manufacturer procedures. Catalase has been determined by its ability to react with H_2O_2; it was measured according to the method described by Aebi[22]. NO in the kidney was determined by the method of Montgomery et al.[23]. GST was determined by the method of Habig et al. using 1-chloro-2,4-dinitrobenzene (CDNB) as a substrate[24]. MDA is measured by its ability to react with thiobarbituric acid, according to the method of Ohkawa et al.[25].

Determination of HO-1 by Real-Time PCR:-
Kidney tissues of all studied groups were homogenized and total RNA was isolated with TRIzol reagent (Invitrogen Life Technologies, Carlsbad, CA) and further analyzed for quantity and quality with Beckman dual spectrophotometer (USA). The mRNA expression level was quantified by qRT-PCR (real-time PCR). 1000 ng of the total RNA from each sample was used for cDNA synthesis by reverse transcription using High Capacity cDNA Reverse Transcriptase Kit (Applied Biosystem, USA). The cDNA was subsequently amplified with the SYBRGreen I PCR Master Kit (Thermo Fisher Scientific, USA) in a 48-well plate using the Step One Instrument (Applied Biosystem, USA) as follows: 10 minutes at 95 ºC for enzyme activation followed by 40 cycles of 15 seconds at 95ºC, 20 seconds at 55 ºC, and 30 seconds at 72 ºC for the amplification step. Changes in the expression of target gene were normalized relative to the mean critical threshold (CT) values of 18SrRNA housekeeping gene by the ΔΔCt method. 1 μM of each of the primers specific for target gene was used. Primers sequence and annealing temperature specific for gene are demonstrated in Table 1.

Table 1: Primers sequence and annealing temperature specific for each gene:-

Target gene	**Primer sequence: 5`- 3`**	**Gene Bank accession number**
HO-1	Forward: GGGTGACAGAAGAGGCTAAG Reverse: GTGTCTGGGATGAGCTAGTG	NM010442.2
18SrRNA	Forward : TCAAGAACGAAAGTCGGAGG Reverse: GGACAT CTAAGGGCATCAC	KR054733.1

Statistical Analysis:-

Data was coded and entered using the statistical package SPSS version 22. Data was summarized using mean plus or minus standard deviation. Comparisons between groups were done using analysis of variance (ANOVA) with multiple comparisons post hoc test in normally distributed quantitative variables while nonparametric Kruskal-Wallis test and Mann-Whitney test were used for nonnormally distributed variables. *P* values less than 0.05 were considered as statistically significant.

Results and Discussion:-

It was found that urea and creatinine increased significantly in the cisplatin-treated group but not in the group treated with both cisplatin and *Solanum nigrum* (Table 2; Figures 1 and 2), which indicates that cisplatin caused a nephrotoxicity that was ameliorated with *Solanum nigrum*. HO-1, GST, NO, and MDA were also significantly increased and CAT was significantly decreased in cisplatin-treated group compared to the other three groups (Table 3; Figures 3–7). All of this indicates that cisplatin caused an oxidative stress while *Solanum nigrum* had an antioxidant effect that protected against cisplatin-induced oxidative stress.

Table 2: The difference in values of creatinine and urea among the 4 groups:-

	Group			
	A	**B**	**C**	**D**
Creatinine (mg/dl)	**0.60± 0.28**	**1.05± 0.19 ***	**0.62± 0.23 #**	**0.48± 0.24 #**
Urea (mg/dl)	**20.00± 6.16**	**41.60± 8.85 ***	**21.20± 7.19 #**	**21.80± 7.16 #**

Table 3: The difference in values of HO-1, NO, MDA, CAT, and GST among the 4 groups:-

	Group			
	A	**B**	**C**	**D**
HO-1	**0.30± 0.21**	**1.15± 0.11 ***	**0.27± 0.16 #**	**0.26± 0.14 #**
Nitric oxide (µmol/l)	**2.01± 1.29**	**4.03± 1.12 ***	**1.52± 0.64 #**	**1.34± 0.74 #**
Lipid peroxide (nmol/g tissue)	**2.04± 0.86**	**3.39± 0.95 ***	**1.55± 0.54 #**	**1.85± 0.67 #**
CAT (U/L)	**319.09± 132.08**	**102.90± 85.42 ***	**341.80± 135.64**	**236.51± 89.07 #**
GST (U/L)	**318.32± 153.83**	**1048.08± 453.35 ***	**244.64± 134.41 #**	**254.45± 130.01 #**

Values are represented as mean ± SD

*Statistically significant compared to corresponding value in group A (P<0.05).

#Statistically significant compared to corresponding value in group B (P<0.05).

$Statistically significant compared to corresponding value in group C (P<0.05).

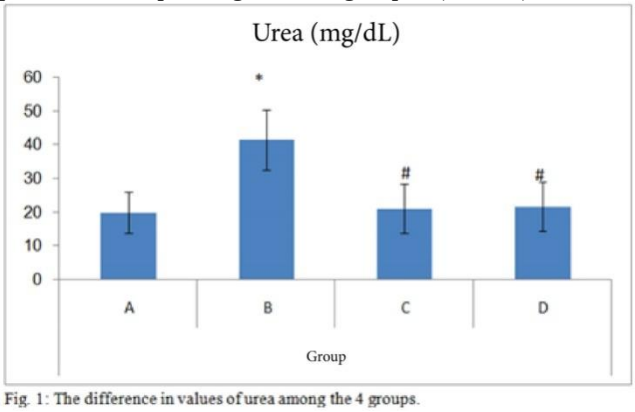

Fig. 1: The difference in values of urea among the 4 groups.

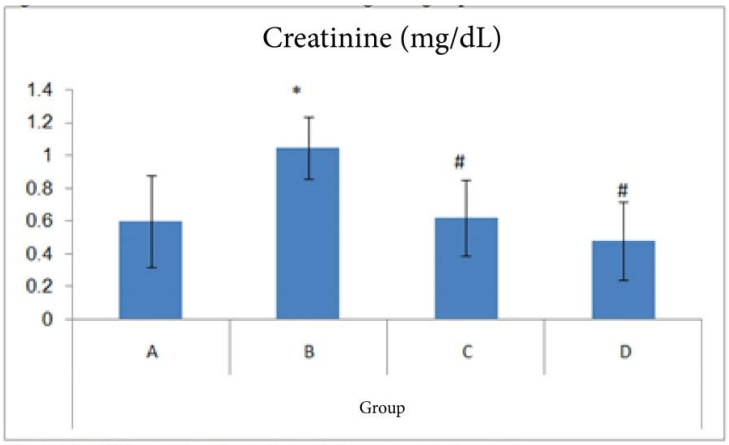

Fig. 2: The difference in values of creatinine among the 4 groups.

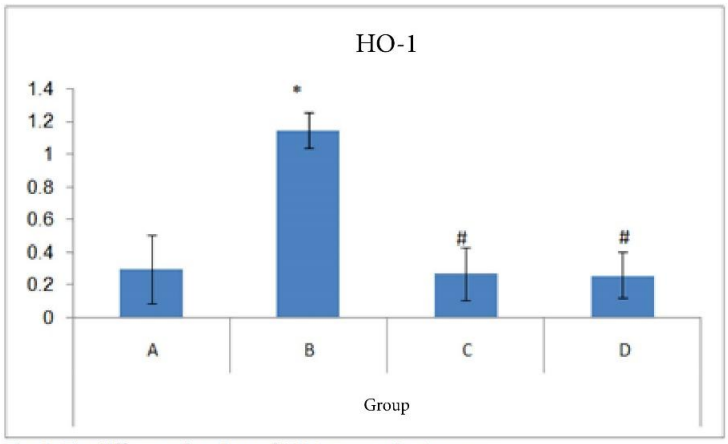

Fig. 3: The difference in values of HO-1 among the 4 groups.

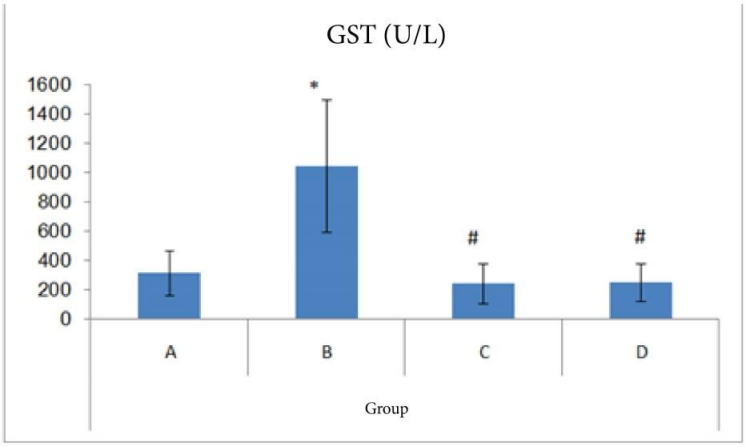

Fig. 4: The difference in values of GST among the 4 groups.

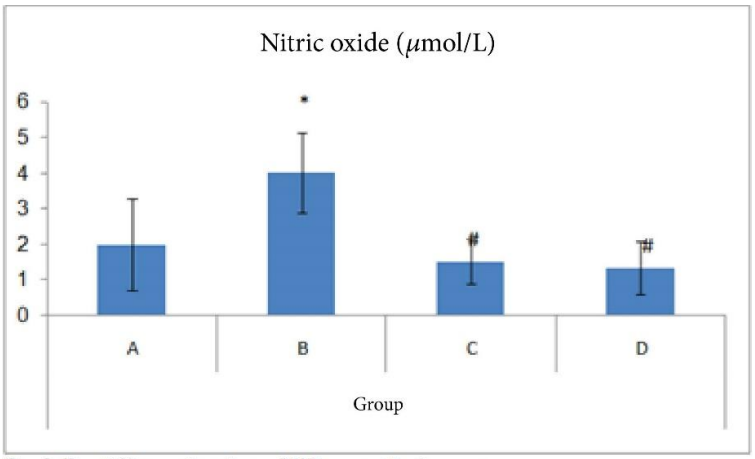

Fig. 5: The difference in values of NO among the 4 groups.

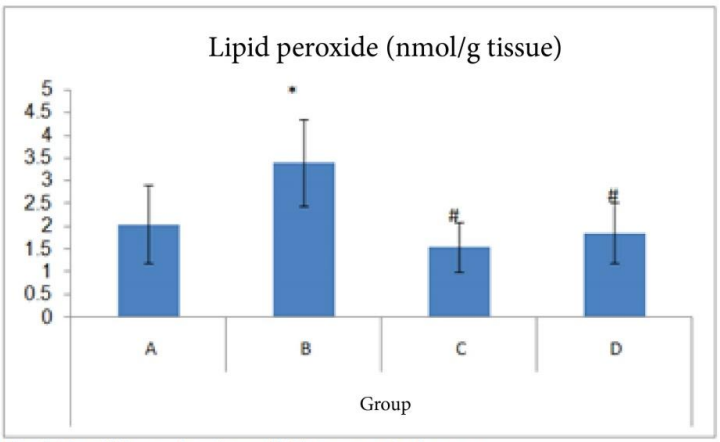

Fig. 6: The difference in values of MDA among the 4 groups.

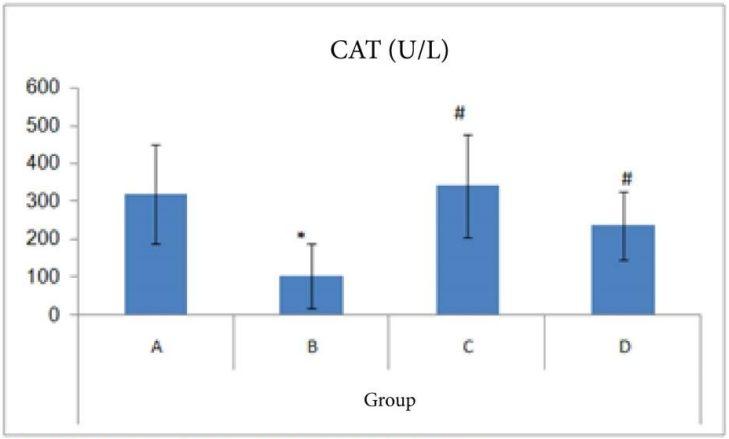

Fig. 7: The difference in values of CAT among the 4 groups.

Conclusion:-

From the findings of the present study, we can conclude that *Solanum nigrum* ameliorates cisplatin-induced oxidative stress and renal damage through its antioxidant properties.

Conflict of Interests:-

The authors declare that there is no conflict of interests regarding the publication of this paper.

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