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Suaeda vermiculata Extract as Protective Agent Against Doxorubicin Induced Toxicity in Rats

[1]Sarah Samir Othman, [2]Safaa Mohamed Ali and [2]Nehal M. El-Deeb

[1]Pharmaceutical Bioproducts Research Department,
Genetic Engineering and Biotechnology Research Institute, City of Scientific Research and
Technological Applications, New Borg El-Arab City 21934, Alexandria, Egypt
[2]Nucleic Acid Research Department, Genetic Engineering and
Biotechnology Research Institute, City of Scientific Research and
Technological Applications, New Borg El-Arab City 21934, Alexandria, Egypt
[3]Biopharmaceutical Products Research Department, Genetic Engineering and
Biotechnology Research Institute, City of Scientific Research and
Technological Applications, New Borg El-Arab City 21934, Alexandria, Egypt

Abstract: Plants are important sources of natural compounds that can be used in drug industry. *Suaeda vermiculata* is a promising plant as a strong antioxidant. Although this plant can be used as an antioxidant and antimicrobial agent due to its constituents, there is no enough data on its biological effect. This work was performed to evaluate the antioxidant effect of *Suaeda vermiculata* against oxidative stress induced by doxorubicin administration in which 4 groups of male Wistar rats were used as control, plant extract treated (100 mg/ml/Kg, orally), doxorubicin treated (15 mg/kg, i.p.) and plant extract plus doxorubicin treated groups respectively. Data of the present work illustrated the ability of *Suaeda vermiculata* to overcome doxorubicin toxicity on different biochemical and hematological parameters, increasing various antioxidants and decreasing TBARS. It is highly recommended to use *Suaeda vermiculata* as an additional natural supplement by chemotherapeutic treated patients due to its strong antioxidant effect to overcome oxidative stress induced by these drugs that is the main cause of their complications.

Key words: *Suaeda vermiculata* · Rats · Oxidative stress · Gene expression

INTRODUCTION

Doxorubicin is a chemotherapeutic agent used in treatment of various kinds of cancer including breast cancer and acute lymphocytic leukemia. It is injected intravenously. It has numerous side effects including hair loss, bone marrow suppression as well as proved to be a cardio toxic drug [1]. Mai *et al*. [2] illustrated that the main mechanism of action of this medication is by causing oxidative stress.

Suaeda vermiculata which is mainly present in Africa and the Middle East [3] contains high amounts of flavonoids, phenolics and alkaloids. The results of its antioxidant (DPPH assay) activity of *Suaeda vermiculata* was 90.5%. *Suaeda vermiculata* was also proved to have antimicrobial activity. GC–MS analysis was used to detect the fatty acid composition of *Suaeda vermiculata* leaf extracts. This plant was proved to be a novel source for the exploration of new antioxidant and antimicrobial agents that are potentially valued for food and biomedical applications [4].

From the previous research, *Suaeda vermiculata* was not used widely in different uses because it is only available in certain parts of the world and since it is

Corresponding Author: Sarah Samir Othman, Pharmaceutical Bioproducts Research Department, Genetic Engineering and Biotechnology Research Institute, City of Scientific Research and Technological Applications, New Borg El-Arab City 21934, Alexandria, Egypt.

widely present in Egypt so the aim of our work was to use it as a natural mixture of antioxidants to overcome the oxidative stress induced by doxorubicin (chemotherapeutic medication).

MATERIALS AND METHODS

Chemicals: Doxorubicin was purchased from Sigma Chemical Company; St Louis, MO, USA and its dose was used according to the previous work of Mello *et al.* [5].

Safety Assay of Using *Suaeda vermiculata* Extract: Neutral red assay protocols were used to quantify the safety patterns of plant extract on preferal blood mononuclear cells (PBMCs) cells using neutral red assay protocols. $6X10^4$ cells/ml cell suspension was seeded in 96 well plate and incubated at 37°C in 5% CO_2 incubator till semiconfluency. 24 hrs. later, the exhausted media were discarded and replaced with serially diluted plant extract prepared in RPMI media. The inoculated plates were incubated for 48 hrs., the Cytotoxicity percentages of the plant extract were quantified using neutral red.

Preparation of Plant Extract: 100 gm wet plant were dissolved in 100 ml distilled water, boiled for 5 minutes, homogenized and filtered then lyophilized. The doses were prepared in which 1gm was dissolved in each 10 ml.

Experimental Animals: Male Wistar rats were used in this research, thirty six rats were used having weights 150-180 g and were kept on basal diet and tap water which were provided *ad libitum* and kept under standard conditions which conformed to the National Institutes of Health (NIH) guidelines. After 14 days of acclimation, animals were divided into 4 groups 9 rats in each, control, administrated the plant extract (100 mg/kg BW, orally), doxorubicin (15 mg/kg BW, ip), the plant extract plus doxorubicin respectively. The experiment duration was 14 days, doses of the extract were given daily but doxorubicin was given on 14^{th} day.

Blood Collection and Tissue Preparation: Blood samples were collected from the sacrificed animals, placed on ice, then they were centrifuged for 20 min at 3000 rpm, plasma was stored at -80°C. Tissues samples were homogenized in ice-cold sodium and potassium phosphate buffer (0.01 M, pH 7.4). The homogenate was centrifuged for 20 min at 4°C at 3, 000 rpm and the supernatant was used.

Thiobarbituric Acid Reactive Substances and Antioxidants: Plasma and liver supernatant thiobarbituric acid-reactive substances (TBARS) were estimated using Esterbauer and Cheeseman method [6], Total antioxidant capacity was measured by Koracevic *et al.* [7] method while Reduced glutathione content was estimated using Beutler *et al.* [8] method.

Biochemical Parameters: BioSystems (Spain) kits were used to examine total protein, albumin, urea, creatinine, LDH (lactate dehydrogenase), γ-GT (γ- Glutamyl transferase), ALT (alanine transaminase) and AST (aspartate transaminase) in stored plasma samples. Whereas thiobarbituric acid was purchased from Sigma Chemical Company, St Louis, MO, USA.

Haematological Parameters: Blood samples were collected 24 h after the last dose and placed on ice. Red Blood cells (RBC), Hemoglobin (HGB), Mean corpuscular hemoglobin (MCH), Hematocrit (HCT), Mean corpuscular volume (MCV) and platelets (PLT) were examined in non coagulated blood using HA-VET CLINDIAG.

RESULTS

Safety Assays

Biochemical Parameters: No significant change in body weight was noticed. Doxorubicin injection alone significantly decreased total protein and albumin and increased urea, creatinine, LDH, γ-GT. ALT and AST. Suaeda vermiculata extract treatment with doxorubicin was able to alleviate its toxicity on most of the evaluated parameters.

Hematological Parameters: Using *Suaeda vermiculata* extract significantly increased HGB and HCT whereas RBC, HGB, HCT and PLT were decreased in rats treated with doxorubicin while MCH increased. Treatment with *Silybum marianum* extract with doxorubicin minimized its toxic effect on various estimated hematological parameters.

Oxidative Stress and Antioxidants: Oxidative stress was stimulated via doxorubicin administration of a single i.p. dose (15 mg/kg BW, ip) at 14^{th} day which was confirmed by the obtained results in which plasma and liver TBARS were increased and decreased their TAC, GSH, GST and GPx. On the other hand *Suaeda vermiculata* extract administration deteriorated this oxidative stress via decreasing plasma and liver TBARS and increasing TAC and various antioxidants in rats as shown in Table (1).

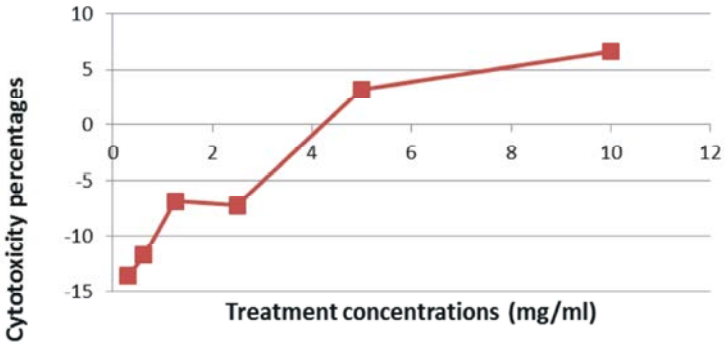

Fig. 1: Cytotoxicity Results of Suaeda vermiculata extract

Table 1: Effect of *Suaeda vermiculata* Extracts administration on various parameters

Parameters	Group 1	Group 2	Group 3	Group 4
Body weight (gm)	140±3.73^{ab}	146±6.76^{ab}	120±4.45^{ab}	142±2.65^{ab}
Biochemical				
Total protein (g/L)	74.3±1.19^{a}	73.2±1.03^{a}	57.3±0.92^{c}	62.9±1.67^{b}
Albumin (g/L)	39.3±0.47^{b}	50.5±1.31^{a}	20.4±0.09^{d}	27.5±0.74^{c}
Urea (mg/dl)	38.9±1.22^{d}	39.3±2.50^{c}	60.3±3.23^{a}	44.7±0.23^{bc}
Creatinine (mg/dl)	0.81±0.03^{c}	0.82±0.02^{c}	2.18±0.06^{a}	1.75±0.12^{b}
LDH (U/L)	150±1.31^{c}	149±3.12^{c}	354±16.8^{a}	266±12.4^{b}
γ-GT (U/L)	21.0±0.36^{c}	19.9±0.40^{c}	31.2±0.48^{a}	26.6±1.25^{b}
ALT (U/L)	31.2±2.33^{c}	28.6±1.33^{c}	60.7±4.47^{a}	46.8±1.26^{b}
AST (U/L)	25.2±1.27^{b}	22.0±0.47^{c}	31.4±2.30^{a}	30.4±2.19^{a}
Antioxidants				
Plasma				
TAC (mM/L)	1.43±0.14^{b}	2.24±0.13^{a}	0.91±0.02^{d}	1.23±0.14^{c}
TBARS (nmol/ml)	0.80±0.01^{c}	0.41±0.02^{d}	2.11±0.21^{a}	2.00±0.14^{b}
GSH (µmole/ml)	4.53±0.11^{b}	5.04±0.15^{a}	3.50±0.15^{d}	4.29±0.19^{c}
Catalase (U/ml)	38.1±1.41^{b}	40.2±1.11^{a}	36.1±1.18^{c}	36.9±0.97^{c}
GPx (U/ml)	8.29±0.10^{ab}	8.60±0.18^{a}	7.64±0.08^{c}	7.93±0.16^{bc}
GST (µmol/hr/ml)	1.11±0.12^{ab}	1.33±0.10^{a}	0.79±0.05^{d}	0.97±0.05^{bc}
Liver				
TAC (mM/L)	0.80±0.01^{b}	0.95±0.01^{a}	0.49±0.01^{d}	0.58±0.02^{c}
TBARS (nmol/gm wet tissue)	19.0±0.27^{c}	18.0±0.47^{d}	28.0±0.87^{a}	26.0±0.95^{b}
GSH (µmole/gm wet tissue)	4.29±0.05^{b}	4.57±0.12^{a}	3.48±0.08^{c}	3.70±0.11^{c}
Catalase (U/mg protein)	48.1±1.23^{b}	49.8±1.42^{a}	36.2±0.87^{d}	41.4±0.94^{c}
GPx (U/mg protein)	26.6±1.16^{a}	27.6±0.86^{a}	23.0±0.31^{b}	24.0±0.93^{a}
GST (µmol /hr/mg protein)	0.77±0.01^{bc}	0.81±0.01^{a}	0.57±0.01^{d}	0.68±0.02^{c}
Hematological				
Plasma RBC's (10^6 /µL)	7.82±0.53^{ab}	7.71±0.16^{b}	6.87±0.32^{c}	7.87±0.19^{ab}
HGB (g/dL)	9.24±0.58^{d}	11.7±0.19^{b}	10.4±0.56^{c}	12.4±0.13^{a}
HCT (%)	33.1±2.56^{d}	39.5±0.84^{b}	36.1±1.58^{c}	42.1±0.66^{a}
MCV (fL)	46.4±1.00^{d}	50.0±0.64^{ab}	51.0±1.67^{c}	53.8±0.59^{a}
MCH (Pg)	14.3±0.10^{c}	15.4±0.25^{ab}	16.0±0.24^{ab}	16.1±0.27^{a}
PLT (10^3/µL)	329±16.4^{a}	84.6±4.34^{d}	188±16.6^{c}	280±11.4^{b}

Data are presented as Mean ± S.E, S.E: Standard Error.

Mean values within a row not sharing a common superscript letters (a, b, c, d) were significantly different, $p<0.05$.Group 1: control group, Group 2: *Suaeda vermiculata* extract group, Group 3: doxorubicin group, Group 4: *Suaeda vermiculata* extract + doxorubicin group.

DISCUSSION

The results of our experiment are in consistence with Elsharabasy *et al.* [9] who found that *Suaeda* extract had successfully reduced the elevated levels of ALT, AST and ALP suggesting that these biochemical restorations could be due to the extract ability to inhibit the cytochrome P450 or/and ability to promote the PCM glucuronidation.

They added that *Suaeda* extract contain cysteine and Methionine with excessive amount which is considered as -SH donner for GSH synthesis. They also proved that this extract has hepatoprotective effect.

Also they suggested that this process can possibly be achieved via the antioxidant activity of Suaeda extracts due to the presence of high amount of á linolenic and linoleic fatty acid.

CONCLUSION

Suaeda vermiculata can be used as a promising source of natural strong antioxidants and antimicrobial compounds.

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