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Evaluation of In-vitro Antiproliferative Potency and In-vivo Induced Biochemical Parameters of Curcin from *Jatropha curcas* seed in Comparison to Doxorubicin and Cisplatin

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ABSTRACT

Plants are promising sources of new bioactive compounds that mostly showed less side effects. The aim of this study is to evaluate in vitro antiproliferative Potency and in vivo induced biochemical parameters of curcin extracted from *Jatropha curcas* seeds in comparison to Cisplatin and Doxorubicin. In vitro antiproliferative potency of curcin towards human promyelocytic leukemia (HL-60), lung (A549), breast (T-47D, MCF-7), colon (LoVo) and liver (HepG2) cancer cell lines were investigated. In vivo studies were carried on albino rats to investigate the effect of curcin on different biochemical parameters. Results showed that the highest *in vitro* cytotoxic activity of curcin was towards HL-60, MCF-7 and HepG2 cell lines (IC_{50} 3.67, 3.12 and 4.9 µg/ml respectively). On the other hand, results of the biochemical investigations indicated that Cisplatin and Doxorubicin caused significant changes in the level of all parameters tested while treatment with curcin showed slight, moderate or no significant changes.

Keywords: *Jatropha curcas*, Curcin, Doxorubicin, Cisplatin, Antiproliferative Potency, Cancer cell lines, Biochemical Parameters.

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INTRODUCTION

Jatropha curcas L., family (*Euphorbiaceae*) grows in many tropical and sub-tropical regions at Africa, Asia and South America [1]. It is reported as a multipurpose plant with several industrial and medicinal applications [2-6]. Different parts of *J. curcas* are considered toxic particularly the seeds; their toxicity were attributed to a protein component. This protein was extracted from the seeds of *J. curcas* and designated as "curcin" [7]. Barbieri et al. [8] reported that curcin is type I ribosome inactivating protein (RIPs) a single-chain protein; the molecular mass was 28.1kD, and its yield is 1.4mg=100 g. Many studies showed curcin exhibited various pharmacological and biological activities such as anti-tumor [9], pesticidal [10], and antifungal [11]. On the other hand, chemotherapeutic treatment of tumors involves the use of chemical agents to stop the growth and eliminate cancer cells even at distant sites from the origin of primary tumor. However, it does not distinguish between a cancer and normal cells, and eliminates not only the fast-growing cancer cells but also other fast-growing cells in the body, including, hair and blood cells [12]. More than half of all people diagnosed with cancer receive chemotherapy regimen, that usually include drugs to treat cancer as well as drugs to help support the completion of the cancer treatment at the full dose on schedule [13]. Cisplatin and doxorubicin have been extensively used for chemotherapy of various cancers, including that of the liver [14]. However, while they generate acceptable outcome in chemotherapy of some cancers, they also exhibit severe toxicity and undesirable side effects [15]. Extensive investigations have been conducted on the hepatotoxicity as well as general organ toxicity of these anticancer drugs [16].

The purpose of this study is to investigate *in vitro* antiproliferative potency of curcin extracted from *Jatropha curcas* seeds towards a variety of cultured cancer cells in comparison to traditional anticancer drugs : cisplatin (CIS) and Doxorubicin (DOX). Moreover, *in vivo* studies will be carried on albino rats to investigate the effect of curcin on different biochemical parameters in comparison to CIS and DOX in order to explore the application of curcin as a possible plant origin anticancer drug that may have the advantages of cost effective, less toxic with fewer side effects.

MATERIALS AND METHODS

Chemicals

Fetal bovine serum (FBS) and L-glutamine, were obtained from Gibco Invitrogen Company (Scotland, UK). Dulbecco's modified Eagle's (DMEM) medium was provided from Cambrex (New Jersey, USA). Dimethyl sulfoxide (DMSO), doxorubicin, penicillin, streptomycin and Sulfo-Rhodamine-B stain (SRB) (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) were obtained from Sigma Chemical Company (St. Louis, MO, USA). Sigma-Aldrich chemical Co. (St. Louis, MO, USA). All kits are the products of Sigma Chemical Company (St. Louis, MO,USA). All other chemicals and reagents used in this study were of analytical grade and purchased from Sigma - Aldrich and Merck.

Collection of Plant Material and Sample Preparation

The matured seeds of *Jatropha curcas* were harvested in autumn from the Aromatic and Medicinal Plant Department farm, Agriculture Research Centre, Giza, Egypt. The plant was authenticated by Agricultural engineering Therese Labib, El Orman Botanical Garden, Egypt (http://wikimapia.org/9432/Orman-Botanical-Gardens-Giza).

Extraction and isolation of Curcin [17]

Two hundred and fifty grams of *J. curcas* seeds were homogenized with a blender using 50 mmol/L sodium phosphate buffer (containing 0.2 mol/L NaCl, pH 7.2), and then extracted for 24 h at 4 °C. The homogenate was subjected to centrifugation at 10 000 r/min. Solid ammonium sulphate $(NH_3)_2 SO_4$ was added to the supernatant till 60% saturation and leave overnight. The precipitate was collected by centrifugation at 12 000 r/min for 20 min, and then redissolved in 5 mmol/L sodium phosphate buffer (containing 0.2 mol/L NaCl, pH 7.2). The solution was dialyzed with the same buffer. At the end of dialysis, a

brown precipitate was present in the bags, that was removed by centrifugation. The brownish supernatant referred to as crude curcin.

Evaluation of *in vitro* anti-proliferative potency

Crude curcin was tested on the human promyelocytic leukemia (HL-60), lung (A549), breast (T-47D, MCF-7), colon (LoVo) and liver (HepG2) cancer cell lines. Reference compounds were CIS and DOX, whereas control of the dissolvent was DMSO, tested in the same concentration. Test solutions of curcin (1 mg/ml) were prepared by dissolving the substances in 100 µl of DMSO completed with 900 µl of tissue culture medium. Afterwards, they were diluted in culture medium to reach the final concentrations of 100, 10, 1, 0.1, 0.01 and 0.001 µg/ml.

Cell lines

Established *in vitro,* human cell lines: HL-60 (human promyelocytic leukemia), A549 (lung cancer), T-47D, MCF-7 (breast adenocarcinoma), HepG2 (liver hepatocellular carcinoma) and LoVo (human colon cancer cells) were used. All lines were obtained from American Type Culture Collection (Rockville, Maryland, USA) with exception of LoVo by courtesy of Prof. E. Borowski (Technical University of Gdańsk, Poland). These lines were maintained at the Institute of Immunology and Experimental Therapy (IIET), Wroclaw, Poland.

HL-60 cell line was cultured in RPMI 1640 medium (Gibco, Scotland, UK) with 2 mM L-glutamine adjusted to contain 1.5 g/L sodium bicarbonate, 4.5 g/L glucose, and 1.0 mM sodium pyruvate, 10% fetal bovine serum (all from Sigma-Aldrich Chemie GmbH, Steinheim, Germany). LoVo, A549, T47D and HepG2 cells were cultured in RPMI 1640+Opti-MEM (1:1) (both from IITD, Wroclaw, Poland), supplemented with 2 mM L-glutamine (Sigma-Aldrich Chemie GmbH, Steinheim, Germany), 5% fetal bovine serum (Thermo-Fisher Scientific Oy, Vataa, Finland) and with 1.0 mM of sodium pyruvate (Sigma-Aldrich Chemie GmbH, Steinheim, Germany). MCF-7 cells in Eagle medium (IIET, Wroclaw, Poland) supplemented with 2 mM L-glutamine and 1% MEM non-essential amino acid solution, 10% fetal bovine serum (all from Sigma-Aldrich Chemie GmbH, Steinheim, Germany). The culture of T47D and MCF-7 cells was supplemented with 0.8 mg/L of insulin (Sigma-Aldrich Chemie GmbH, Steinheim, Germany). All culture media were supplemented with 100 units/ml penicillin, and 100 µg/ml streptomycin (both from Polfa Tarchomin S.A., Warsaw, Poland). All cell lines were grown at 37°C with 5% CO_2 humidified atmosphere.

Anti-proliferative assay in vitro

Twenty four hours prior to the addition of the tested compounds (curcin, DOX and CIS), the cells were plated in 96-well plates (Sarstedt, Germany) at a density of 1×10^4 or 0.5×10^4 cells per well. The assay was performed after 72 h of exposure to varying concentrations of the tested agents. The *in vitro* cytotoxic effect of all agents was examined using the MTT (HL-60) or SRB (A549, T-47D, LoVo, HepG2 and MCF-7) assay. The results were calculated as an IC_{50} (inhibitory concentration 50) – the dose of tested agent which inhibits proliferation of 50% of the cancer cell population. Each compound in each concentration was tested in triplicate in a single experiment, which was repeated at least 3 times.

MTT assay [18]

This technique was applied for the cytotoxicity screening against leukemia cells growing in suspension culture. An assay was performed after 72-hours exposure to varying concentrations (from 0.01 to 100 µg/ml) of the tested agents . For the last 3-4 hours of incubation 20 µl of MTT solution were added to each well (MTT: 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide; stock solution: 5 mg/ml, Sigma-Aldrich, Germany). The mitochondria of viable cells reduce the pale yellow MTT to a navy blue formazan. When incubation time was completed, 80 µl of the lysing mixture were added to each well (lysing mixture: 225 ml dimethylformamide, POCh, Gliwice, Poland, 67.5 g sodium dodecyl sulphate, Sigma-Aldrich, Germany, and 275 ml of distilled water). After 24 h, when formazan crystals had been dissolved, the optical densities of the samples were read on Synergy H4 photometer (BioTek Instruments, USA) at 570 nm wavelength. Each compound in given concentration was tested in triplicates in each experiment, which was repeated at least 3 times.

SRB assay [19]

This technique was applied for the cytotoxicity screening against cells growing in adherent culture. The cytotoxicity assay was performed after 72-hour exposure of the cultured cells to varying concentrations (from 0.01 to 100 μg/ml) of the tested agents as described by Sekhan etal [14]. The cells attached to the plastic were fixed by gently layering cold 50% TCA (trichloroacetic acid, Aldrich-Chemie, Germany) on the top of the culture medium in each well. The plates were incubated at 4°C for 1h and then washed five times with tap water. The background optical density was measured in the wells filled with culture medium, without the cells. The cellular material fixed with TCA was stained with 0.1% sulforhodamine B (SRB, Sigma, Germany) dissolved in 1% acetic acid (POCh, Gliwice, Poland) for 30 minutes. Unbound dye was removed by rinsing (4x) with 1% acetic acid. The protein-bound dye was extracted with 10 mM unbuffered Tris base (Sigma, Germany) for determination of optical density (at 540 nm) on Synergy H4 photometer (BioTek Instruments, USA).

***In vivo* Biochemical studies in rats**

Animals

Male albino rats (Sprague-Dawley strain) weighing between 200 - 250 g were used for the study. The animals were fed *ad labium* with standard pellet diet and had free access to water. All experiments and protocols described in present study were in accordance with guidelines as per "Guide for the care and use of laboratory animals" published by NIH publication (NO 85-23 revised 1996).

Experimental Protocol

The animals were divided into four groups each consisting of six rats and received the following treatment:

Group I (Control): each rat was given intraperitoneal injection of 0.1ml DMSO on days 1, 7, 14, 21, 28.
Group II (crude curicin): each rat was given a single intraperitoneal injection of 0.1ml containing 3 mg/kg body weight of crude curcin dissolved in DMSO on days 1, 7, 14, 21, 28.
Group III (DOX): each rat was given a single intraperitoneal injection of 0.1ml containing 3 mg/kg body weight of DOX dissolved in DMSO on days 1, 7, 14, 21, 28.
Group IV (CIS): each rat was given a single intraperitoneal injection of 0.1ml containing 3 mg/kg body weight of CIS dissolved in DMSO on days 1, 7, 14, 21, 28.

Biochemical Analysis

The effects of crude curcin from *Jatropha caracus* on different biochemical parameters in sera and testes of rats were evaluated in comparison to CIS and DOX.

Serological analyses: After 72 hours of the last injection of all groups of rats, blood was collected using heparinized capillary tubes for serological analyses. Some liver enzymes such as aspartate and alanine aminotransferases (AST and ALT) and alkaline phosphatase (ALP) , were done using blood auto analyzer (Olympus AV 400, Japan) [20], lactate dehydrogenase (LDH) was assayed using commercial kits of Transaminase (Sigma-Aldrich), Blood urea nitrogen were assayed using commercial kits (Sigma-Aldrich). Moreover, glucose [21], albumin [22], globulins [23], creatinine [24], total lipids [25], cholesterol [26], triglycerides [27], bilirubin [28] , Glutathione reductase [29] and Isocitrate dehydrogenase [30] levels in sera of rats were estimated. Testosterone level was estimated by direct chemiluminescent assay (ADVIA CENTAUR) [31].

Biochemical analyses of different parameters in testes of rats

All groups of rats were sacrificed and testes were removed. The excised testes were then homogenized in cold Tris buffer (10 mM, pH 7.4) at a concentration of 10% (w/v). The homogenates were centrifuged at 10,000×*g* at 0◦ C for 20 min using high speed cooling centrifuge. The supernatant was used for the assays of biomarkers of the oxidative stress such as lipid peroxidation (LP) [32], superoxide dismutase

(SOD) [33], catalase (CAT) [34] and reduced glutathione (GSH) [35]. Tissue homogenate sediment was resuspended in ice-cold Tris buffer (10 mM, pH 7.4) to get a final concentration of 10% and was used for the estimation of different membrane bound enzymes such as Na+K+ATPase [36], Ca2+ATPase [37] and Mg2+ATPase [38].

Statistical analysis of the results was performed using Chi-square values (SPSS computer program).

RESULTS

Evaluation of *in vitro* antiproliferative potency

Results illustrated in table 1 indicated that the highest *in vitro* antiproliferative activity of curicin was observed towards HL-60, MCF-7 and HepG2 cell lines (IC_{50} 3.67, 3.12 and 4.9 µg/ml respectively). It is clear from the data that curcin was found to be more potent towards MCF-7 cell line than the standard drug, DOX with IC_{50} value 3.12 ± 0.60 µg/ml versus 4.70 ± 0.55 µg/ml for DOX, while it had an IC_{50} value near to CIS (IC_{50}: 3.36 ± 0.91. Curcin showed also similar antiproliferative activity towards HepPG2 cell line to the standard drugs (IC_{50}: 4.90 ± 0.40 µg/ml, versus 4.20 ± 0.46 and 5.01±0.70 µg/ml for DOX and CIS respectively). On the other hand, curcin revealed week or no activity towards LoVo, A549 and T-47D cell lines in comparison to DOX and CIS.

Table 1: Antiproliferative activity of Curicin, Dox and CIS against A549, T-47D , LoVo , HepG2 and MCF-7 cancer cell lines.

COMPOUNDS	HL-60	A549	T-47D	LoVo	HepG2	MCF 7
	IC_{50} (µg/ml)	IC_{50} (µg/ml)	IC_{50} (µg/ml)	IC_{50} (µg/ml)	IC_{50} (µg/ml)	IC_{50} (µg/ml)
CURICIN	3.67 ± 0.26	N.A.	N.A.	45.10 ± 9.92	12.90 ± 0.40	4.12 ± 0.60
CISPLATIN	0.27 ± 0.03	2.36 ± 0.53	2.27 ± 0.25	0.88 ± 0.18	5.01 ± 0.70	3.36 ± 0.91
DOXORUBICIN	0.06 ± 0.06	0.20 ± 0.06	0.09 ± 0.03	0.09 ± 0.06	4.20 ± 0.46	4.70 ± 0.55

Data are expressed as Mean ± S.D. N.A. : not active

Evaluation of *in vivo* induced biochemical parameters in rats

Table 2: Biochemical effects of Curicin, Dox and CIS on serum levels of ALT, AST, ALP, LDH, Glutathione reductase and Isocitrate dehydrogenase in rats.

Rat Groups / Biochemical Parameters	Control	Curicin P<	Doxorubcin P<	Cisplatin P<
Alanine amino transferase ALT (IU/ml)	43.5 ± 2.03	40.56 ± 3.7 n.s.	62.26 ± 4.03 0.001	74.3 ± 8.09 0.001
Aspartate amino transferase AST (IU/ml)	108.32 ± 11.09	112.81 ± 9.88 n.s.	147.22 ± 16.34 0.001	158.3 ± 18.92 0.001
Alkaline phosphatase ALP (k.k./dl)	18.70 ± 4.10	21.94 ± 3.4 0.01	30.317 ± 5.14 0.001	42.48 ± 2.03 0.001
LDH Mean ± SD (U/L)	169.83 ± 14.62	185 ± 17.638 n.s.	610.33±77.66 0.0001	254 ±18.42 0.001
Glutathione reductase (U/l)	52.6 ± 7.03	57.3 ± 6.18 0.01	72.9 ± 8.41 0.001	78.9 ± 8.92 0.001
Isocitrate dehydrogenase (U/l)	9.02 ± 1.03	8.74 ± 1.61 n.s.	16.29 ± 3.43 0.001	12.5 ± 3.06 0.01
Testosterone (ng/	0.80 ± 0.045	0.84 ± 0.021 n.s.	0.60 ± 0.073 0.01	0.54 ± 0.08 0.001

Data are expressed as Mean ± S.D. P< 0.01: significant, P< 0.001: highly significant, n.s. : non significant

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Table 3: Biochemical effects of Curicin, Dox and CIS on serum glucose, total lipids, cholesterol, triglycerides, bilirubin, albumin, globulin and creatinine and Blood urea nitrogen in rats.

rat Groups / Biochemical Parameters	Control	Curicin P<	Doxorubcin P<	Cisplatin P<
Glucose (mmol/1)	7.6 ± 0.2	8.03 ± 0.17 n.s.	5.3 ± 0.62 0.001	6.7 ± 0.4 0.01
Total Lipids mg/dl	323.41 ± 27.1	317.4 ± 30.7 n.s.	366.7 ± 6.10	375.2 ± 31.4
Cholestrol mg/dl	94.32 ± 13.5	96.4 ± 10.5 n.s.	109.3 ± 14.2 0.001	107.9 ± 11.7 0.001
Triglycerides mg/dl	108.7 ± 16.8	112.6 ± 19.70 n.s.	137.8 ± 17.10 0.001	129.5 ± 19.4 0.001
Bilirubin mg/dl	0.63 ± 0.04	0.51 ± 0.08 0.01	0.81 ± 0.19 0.001	0.84 ± 0.10 0.001
Albumin mg/dl	5.63 ±0.51	11.43 ± 1.48 0.01	6.37 ± 0.85 0.01	4.73± 0.92 0.001
Globulin mg/dl	4.32 ± 0.9	6.1 ± 0.73 0.01	5.91 ± 0.63 0.01	3.75 ± 0.8 0.01
Creatinine mg/dl	0.69 ± 0.03	0.68 ± 0.08 n.s.	0.78 ± 0.04 0.01	2.8 ± 0.06 0.001
Blood urea nitrogen (mmol/1)	8.2 ± 0.3	13.5 ± 6.3 0.01	54.2 ± 11.4 0.001	66.3 ± 12.6 0.001

Data are expressed as Mean ± S.D. P< 0.01: significant, P< 0.001: highly significant, n.s. : non significant

Data obtained in table 2 presents the effect of curcin on serum levels of ALT, AST, ALP, LDH, Glutathione reductase and Isocitrate dehydrogenase in rats in comparison to DOX and CIS. The results showed that the values recorded for all enzymatic levels tested were significantly higher ($P < 0.001$) in DOX and CIS treated groups of rats than the control except level of Isocitrate dehydrogenase in CIS group and testosterone in DOX group ($P < 0.01$) . On the other hand, treatment with curcin caused inverse effects, where most values recorded for ALT, AST, LDH, testosterone and Isocitrate dehydrogenase were non significant (n.s.) or slightly higher for ALP and Glutathione reductase ($P < 0.01$) in comparison to control.

Data listed in table 3 demonstrates a comparison between the levels of glucose, total lipids, cholesterol, triglycerides, bilirubin, albumin, globulin and creatinine and Blood urea nitrogen in sera of curcin treated rats and DOX and CIS treated groups of rats. It can be deduced from the present data that DOX and CIS treatment caused a high significant increase in the levels ($P < 0.001$) of total lipids, cholesterol, triglycerides, bilirubin, and Blood urea nitrogen except levels of albumin and globulin were slightly high ($P < 0.01$), while Glucose level was decreased compared to untreated rats. On the other hand, curicin treatment gave close biochemical levels to control with slightly high ($P < 0.01$) or no significant changes.

Table 4 showed the effect of Curcin, Dox and CIS on levels of some biomarkers related to the oxidative stress in rats compared to untreated group. It is clear from the table that administration of Dox or CIS significantly increases lipid peroxidation (LP) while there was a significant decrease in superoxide dismutase

(SOD), catalase (CAT) and reduced glutathione (GSH) levels as compared to control rats. On the other hand, administration of curcin indicated no significant changes in these parameters as GSH, SOD, CAT and LP levels were towards control values.

Table 4: Biochemical effects of Curicin, Dox and CIS, on biomarkers of the oxidative stress in testes of rats.

Rat Groups / Biochemical Parameters	Control	Curicin P<	Doxorubcin P<	Cisplatin P<
LP (nmolesof MDA/mg protein)	1.26 ± 0.043	1.19 ± 0.05 n.s.	2.52 ± 0.10 0.001	2.14 ± 0.1 0.001
GSH (µg of GSH/ mg protein)	4.33 ± 0.52	4.22 ± 0.37 n.s.	2.39 ± 0.16 0.001	2.97 ± 0.087 0.001
SOD (Units/mg protein)	4.52 ± 0.68	4.71 ± 0.19 n.s.	2.55 ± 0.15* 0.001	3.32 ± 0.16 0.001
CAT(µmoles of H2O2consumed/min/ mg protein)	7.48 ± 0.57	7.27 ± 0.34 n.s.	3.90 ± 0.27 0.001	4.38 ± 0.36 0.001

Data are expressed as Mean ± S.D.
P< 0.01: significant, P< 0.001: highly significant, n.s. : non significant

Table 5: Biochemical effects of Curicin, Dox and CIS, on membrane bound enzymes in testes of rats.

Rat Groups / Biochemical Parameters	Control	Curicin P<	Doxorubcin P<	Cisplatin P<
Na+K+ATPase (µmoles of inorganic phosphrous liberated /min/mg protein)	8.50 ± 0.34	7.85 ± 0.48 n.s.	5.74 ± 0.61 0.001	6.13 ± 0.42 0.001
Ca 2+ATPase (µmoles of inorganic phosphrous liberated/min/ mg protein)	4.24 ± 0.57	4.16 ± 0.55 n.s.	2.08 ± 0.32 0.001	1.98 ± 0.41 0.001
Mg 2+ATPase (µmoles of inorganic phosphrous liberated /min/mg protein)	6.56 ± 0.40	5.93 ± 0.96 n.s.	3.43 ± 0.21 0.001	4.19 ± 0.3 0.001

Data are expressed as Mean ± S.D. P< 0.01: significant, P< 0.001: highly significant, n.s. : non significant

The effect of Curcin , Dox and CIS on membrane bound enzyme levels in testes of rats in comparison to untreated group was demonstrated in table 5. It was shown that Dox and CIS treatment of rats significantly decreased ($P < 0.001$) the levels of membrane bound enzymes like Na+K+ATPase, Ca2+ATPase and Mg2+ATPase , while in curicin treated group, the membrane bound enzymes levels were non significant compared to control.

DISCUSSION

It is well known that chemotherapy aims to destroy cancer cells with various types of chemicals. However, most drugs used for cancer chemotherapy are known to produce toxic side effects in multiple organ systems [39]. On the other hand, recent studies indicated that more than 30% of the pharmaceutical agents currently available are of natural origins or their derivatives which form the major source of therapeutic options with lower side effects against a wide range of diseases including cancer [40-41]. Moreover, many anticancer drugs have been discovered through random screening of plant materials [42]. Previous works indicated the potential of *J. curcas* plant as a source of bioactive compounds [43]. It has been reported that phenolics, flavonoids, saponins and phorbol esters have been shown to be cytotoxic on different cell lines [44]. Other workers observed that curcin isolated from *J. curcas* showed a potenial cytotoxic activity [45-46].

The present investigation focuses on evaluating in vitro antiproliferative potency and in vivo induced biochemical parameters of curcin extracted from *J. curcas* seeds in comparison to DOX and CIS for searching of a possible plant origin drug that may have the advantages of cost effective, less toxic with fewer side effects. Results showed that the highest *in vitro* cytotoxic activity of curcin was towards HL-60, MCF-7 and HepG2 cell lines (IC_{50} 3.67, 3.12 and 4.9 µg/ml respectively) while there was weak or no activity towards A549, T-47D and LoVo cell lines. These results are in accordance with some researchers [47] who investigated the toxicity of curcin against SGC-7901, Sp2/0, Human hepatoma, Hela cell lines and they reported that different effects of curcin on various cells examined were observed. Moreover, data of present work are consistent with the knowledge of the application of *Jatropha* extracts in traditional medicine, especially to cure/ameliorate cancer [48].

On the other hand, the present work extended to focus on the induced biochemical parameters of curcin *in vivo.* Studies were carried on sera and testes of male albino rats to evaluate toxic effects of curcin on different parameters in comparison to DOX and CIS. It was observed from the present work that Dox and CIS were considerably raised serum levels of different enzymatic biomarkers including ALT and AST, which are associated with degrees of liver damage. It can be suggested that the elevated serum markers were released from the injured liver upon exposure to both drugs. This is in agreement with other works indicated that serum enzymes, ALT and AST, LDH, ALP as well as others, are commonly elevated following cellular damage s a result of enzymes leakage from cells to the blood [49].

Moreover, the present work demonstrated that Dox and CIS elicited increasing effect on metabolites such as serum glucose, total lipids, cholesterol, triglycerides, bilirubin, albumin, globulin in comparison to control group of rats. These results were previously reported by other researchers [50]. Also, it was observed that DOX and CIS treated rats showed increased creatinine and Blood urea nitrogen in rats which is an indicator of kidney damage. These observations were previously remarked [51]. Investigation of the effect of Dox and CIS, on biomarkers of the oxidative stress and membrane bound enzymes in rats testes was performed. Results showed that both drugs elicited a significant decrease in the levels of GSH, SOD and CAT, Na+K+ATPase, Ca2+ATPase and Mg2+ATPase while LP was markedly increased as compared to control. These results demonstrated that both drugs showed pathological changes in serum and biochemical markers indicative of toxicity and increases the free radical production, which can be a remark of induced testicular toxicity. These results were consistent with earlier studies [52].

It can be deduced from the present work that DOX and CIS treatment may causes liver , kidney and testes damage in rats. On the other hand, treatment with curcin showed slight, moderate or no significant changes in the level of all biochemical parameters evaluated.

CONCLUSION

In conclusion, curcin exert cytotoxic activity towards HL-60, MCF-7 and HepG2 cancer cell lines through reducing cell proliferation that resulted in a significant growth inhibition. The present study also, reveals that HL-60 cells are more sensitive to curicin than the MCF-7 and HepG2 cells. Moreover, curcin had important potential advantages over CIS and DOX because of its less toxicity and its ability to induce lower biochemical parameters.

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