

The Effect of Seeds and Fruit Pulp of *Adansonia digitata* L. (Baobab) on Ehrlich Ascites Carcinoma

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ABSTRACT

The anti-tumor effect of *Adansonia digitata* on Ehrlich ascites carcinoma cells (EAC) is still novel talk. This study is focusing on the role of the extracts of seeds and the fruit pulp of *Adansonia* on the antioxidants activity and the molecular changes of pro-apoptic and anti-apoptic genes expression before and after the treatment of EAC cells bearing mice. Adult female BALB/C mice were used in this study; subgrouped randomly into four groups: control group (non-tumorized); EAC tumorized group, mice was i.p. inoculated with 2.5×10^6 of EAC cells; EAC+ extract of seeds group, tumorized mice was inoculated with 2.5×10^6 of EAC cells and i.p. administered with the extract of *Adansonia* seeds (300 mg/kg b. wt.); EAC+ fruit pulp group, tumorized mice was inoculated with 2.5×10^6 of EAC cells and i.p. administered with the extract of *Adansonia* fruit pulp (300 mg/kg b. wt.). The antioxidant enzymes were inhibited in EAC cells and in ascetic fluid of tumorized mice. Also the oxidative stress was increased significantly in EAC cells bearing mice. The liver was affected with the transplantation of EAC cells as reflected by the imbalance in the antioxidants and oxidants in the EAC cells bearing mice. Moreover, the molecular changes in *p53* and B-cell lymphoma (*Bcl-2*) genes expression were recorded in EAC cells bearing mice. The extracts of *adansonia* have a promising role as antioxidant action due to their antioxidant effect as they ameliorate the imbalance in antioxidants and oxidants balance. The plant extract has anti-apoptosis role by restoring the P53 and Bcl-2 genes expression. Also the plant has antitumor action as they restore tumor markers levels such as α -l-fucosidase and arginase to the normal levels.

Keywords: Ehrlich Ascites Carcinoma; Tumor Markers; Antioxidants; Oxidative Stress; Gene Expression; *Adansonia digitata*; Baobab

1. Introduction

Ehrlich ascites carcinoma (EAC) is transplantable tumors and they were taken into concern in the last 2 to 3 decades. Experimental tumors have vital significance for the purposes of modeling, and EAC is one of the top and famed cases. EAC is indicated to as an undifferentiated carcinoma, and has high transplantable capability, no-regression, rapid proliferation, shorter lifespan and 100% malignancy [1]. EAC is used as ascites or a solid form due to these purposes, that is, if ascites fluid contains the tumor cell that injects intraperitoneal, the ascites form is obtained, but if it contains subcutaneously, a solid form is obtained [2]. In 4 or 6 days after inoculation, the ascites fluid is formed and a total of 5 or 12 mL ascites fluid is accumulated [3]. Following the inoculation into the peritoneal cavity of mice, EAC cells proliferating by which the number of cells increases exponentially,

and a plateau phase followed by are sting period, in which a number of cells stay almost constant [4]. Bulan [5] reported that the number of EAC cells increased exponentially in the 9th day after intraperitoneal transplantation of 3×10^6 EAC cells. After inoculation of EAC, the host animal died due to the pressure exerted by the tumor volume and/or the damage that resulted from the tumor [6]. For the accumulation of ascites fluid, whether or not the tumor cells secrete a vascular permeability factor that stimulated the accumulation of ascites fluid was investigated that damage changed the vascular permeability [7]. Altun [8] in another study investigated the liver regeneration in mice with EAC and reported that tumor growth stimulated the regenerative growth.

Oxidative stress exerts toxic effects on cells and is potent in inducing apoptosis [9]. The inhibition of superoxide dismutase (SOD) and catalase (CAT) activities as a

result of EAC growth was reported in liver [10]. Aktas [6] and Lobo [11] showed that the antioxidant level such as glutathione (GSH) concentration decreased in EAC cells. Similar decrease in the activity of SOD, CAT, GSH content and increase in lipid peroxidation was observed in the EAC and the liver of EAC-bearing mice [12]. Overproduction of reactive oxidative stress in SOD antisense-transfected cells was reported to increase pro-apoptotic proteins Bax and Bcl-2 and to decrease anti-apoptotic proteins Bcl-2 and Bcl-xL by regulating their phosphorylation and ubiquitination [13]. However, Xu [14] found that the expression of some antioxidant enzymes such as SOD presented a more complex pattern, which differed from the expression of Bcl-2. Among the positive and negative regulators of apoptosis, p53, the tumor suppressor gene, has an important role against cancer as it suppresses tumor growth through two mechanisms, cell cycle arrest and apoptosis [15]. Cell growth and its proliferation are two tightly linked processes. Both are characterized by increasing protein synthesis which are achieved by changes in the activity of ribosomal biogenesis and p53 expression [16]. Bax, the pro-apoptotic member of Bcl-2 family, is a p53 target and is transactivated in a number of systems during p53-mediated apoptosis [17]. The up-regulation of Bax expression and down-regulation of Bcl-2 have been well demonstrated during apoptosis [18]. Also Cho [19] showed that H₂O₂-induced apoptosis was accompanied by changes in apoptosis-related factors such as the Bcl-2 family of regulatory proteins. The intracellular concentration of the anti-apoptotic protein Bcl-2 acts as a molecular indicator that influences whether a cell lives or dies [20]. All these studies demonstrated that pro- and anti-apoptotic proteins are the important factors deciding the fate of neoplastic cells.

Some researchers reported that some plant extracts were effective against EAC [21]. Although there are a lot of floristic studies, approximately 10% of the 250,000 complex plant species only were investigated at their chemical and pharmacological sites [7]. The exploring of new toxic agents from natural products has been concerned in collaboration with scientists worldwide [22]. Kamatou [23] reported that it was an excellent antioxidant due to the vitamin C content which was seven to ten times higher than the vitamin C content of oranges. Kamatou [23] suggested that consumption of *A. digitata* fruit might play an important role in resistance to liver damage in areas where baobab was consumed [24]. Phytochemical investigation showed the presence of flavonoids, phytosterols, amino acids, fatty acids, vitamins and minerals. The seeds are a source of significant quantities of lysine, thiamine, calcium and iron [23]. Recently, the European Commission authorized the import of baobab fruit pulp as a novel food [25] and it was approved in

2009 by the Food and Drug Administration as a food ingredient in the United States of America [26]. Due to the high demand for commercial baobab products in EU and United States, this tree with its edible fruits needs to be conserved and treasured [27].

Upon of these, the study aimed to investigate the role of *Adansonia digitata* on the biochemical changes associated with the EAC transplantation in mice. Moreover, this is may be the first topic that deals with the vital role of methanolic extracts of seeds and dry powder fruitpulp of *Adansonia digitata* in regulating the role of pro- and anti-apoptotic genes in tumor cells.

2. Material and Methods

2.1. About Plant

Baobab (*Adansonia digitata* L.; Bombacaceae) is a large tree with an unusual shape growing in the drier parts of Africa (Sudan). The plant has a wide range of uses, not only as a food and beverage, but also medicinally to treat fevers and dysentery [28]. In recent years, there has been an upsurge of interest in the development of baobab fruits as a botanical dietary supplement in the United States. The fruit pulp affords high levels of vitamin C (range 2.8 - 3 g/kg) and has also been documented as having high antioxidant potency [29]. An aqueous extract of *A. digitata* fruit pulp has shown anti-inflammatory and analgesic effects in rat models, but at quite a high dose range (400 - 800 mg/kg, p.o.) [30]. The various plant parts of baobab have been subjected to relatively few studies phytochemically, and, for example, a number of proanthocyanidins of previously known structure were reported as major constituents from an 80% methanol extract of the fruit pulp [31]. The acute toxicity of baobab fruit pulp extract was tested *in vivo* on rats and the results showed that the LD₅₀ was 8000 mg/kg following parenteral administration suggesting low toxicity [30].

2.2. Plant Extract

The air-dried and powdered of seeds and fruit pulp (10 g) were extracted with 200 ml methanol by Soxhlet extraction for 18 hrs. The residue was dried overnight and then extracted with 250 ml water by using a shaking water bath at 70°C for 2 hrs. The obtained methanolic and water extracts were filtered and evaporated by using a rotator evaporator. The dried extracts of seeds and fruits pulp were stored at -80°C until use. The method of extraction is used herein with a little modification as Mothana [32].

2.3. Tumor Cell Line

A line of Ehrlich Ascites Carcinoma (EAC) obtained from Egyptian National Cancer Institute, Cairo, Egypt.

The tumor line was maintained in female BALB/C mice by weekly intraperitoneal injection of 2.5×10^6 cells/mouse/0.25 ml of phosphate buffer saline (PBS). EAC cells counts were done in a Neubauer hemocytometer using the trypan blue dye. Cell viability was always found to be 95% or more. Tumor cell suspensions were prepared in PBS. EAC cell line is characterized by its reasonable rapid growth which could not kill the animal due to the accumulation of Ascites.

2.4. Animal Grouping

The experiment has been used three month old female BALB/c mice 40 ± 5 g. The local committee approved the design of the experiments and the protocol conforms to the guidelines of the National Institutes of Health (NIH). All measures were taken to minimize the number of mice used and their suffering. Animals were kept in vivarium, housed in polycarbonate cages, 10 mice per cage, at a constant room temperature of $23^\circ\text{C} \pm 1^\circ\text{C}$ with a 12-h light/dark cycle (light on from 7:00 AM till 7:00 PM). Standard rat chow diet and water were available *ad libitum*. Animals were divided into four groups (Ten/group); Control group: mice were received no treatment; Ehrlich Ascites Carcinoma (EAC) group: mice were inoculated intraperitoneally with of 2.5×10^6 cells/mouse/0.25 ml of PBS; EAC+ seed extract group: Tumorized mice were daily intraperitoneally administered with seed extract of *Adansonia* at 300 mg/kg b. wt. for 14 days; EAC+ fruits extract group: Tumorized mice were daily intraperitoneally administered with fruits extract of *Adansonia* at 300 mg/kg b. wt. for 14 days. At the end of the experimental period mice were sacrificed by decapitation then blood, ascites fluid, EAC cells, and liver tissue were collected from each group. EAC cells from EAC bearing-mouse were homogenized then centrifuged at 2000 rpm for 10 min at 4°C . The ascetic fluid obtained was used for the estimation of the antioxidant enzymes such as SOD, GSH-Px in different animal groups. Also the EAC cells pellet and liver tissues were immediately homogenized in a phosphate buffer solution pH 7.4 and centrifuged at 2000 rpm for 10 min at 4°C . The liver samples were then rinsed in ice-cold normal saline followed by cold 0.15 M Tris-HCl (pH 7.4), blotted dry, and weighed. A 10% w/v homogenate was prepared in 0.15 M Tris-HCl buffer; of which a portion was utilized for the estimation of lipid peroxidation and H_2O_2 and a second portion was used for the estimation of GSH after precipitating proteins with TCA. The supernatant of liver tissues, EAC cells, ascites fluid and sera were stored at -80°C until be used in the biochemical assay. The EAC-cells that be used in the molecular study was stored at -80°C until be further analyzed.

2.5. Enzymatic Assay

2.5.1. Superoxide Dismutase (SOD) Activity

This assay relied on the ability of the enzyme to inhibit the phenazinemetosulphate-mediated reduction of nitrobluetetrazolium dye [33].

2.5.2. Glutathione Peroxidase (GSH-Px) Activity

The activity was measured by the method described by Ellman [34]. Briefly, the reaction mixture contained 0.2 ml of 0.4 M phosphate buffer (pH 7.0), 0.1 ml of 10 mM sodium azide, 0.2 ml of supernatant (homogenate on 0.4 M phosphate buffer, pH 7.0), and 0.2 ml glutathione, 0.1 of 0.2 mM H_2O_2 . The reaction content was incubated at 37°C for 10 min. The reaction was arrested by adding 0.4 ml of 10% TCA and then centrifuged. The molar extinction coefficient for NADPH is $6220 \text{ mM}^{-1}\cdot\text{cm}^{-1}$ at 340 nm.

2.5.3. GSH (GSH) Content

GSH served as an antioxidant, reacting with free radicals and organic peroxides, in amino acid transport, and as a substrate for the GSH-Px and glutathione-S-transferase in the detoxification of organic peroxide and metabolism of xenobiotics, respectively. A tissue was homogenized in 10 ml cold buffer (50 mM potassium phosphate, pH 7.5, 1mM EDTA) per gram tissue. Centrifuge at 100,000 x g for 15min at 4°C was performed. The supernatant was transferred into a new sterile tube for assay and the rest of the samples stored at -80°C for further work. The method based on the reduction of 5,5'-Dithiobis(2-nitrobenzoic acid) (DTNB) with GSH to produce a yellow compound. The reduced chromogen directly proportional to GSH concentration and its absorbance can be measured at 405 nm [35].

2.5.4. Malondialdehyde (MDA) Level

The thiobarbituric acid reactive substances (TBARS) as malondialdehyde were estimated by the method of Ohkawa [36]. Briefly, to 0.2 ml of homogenate, 0.2 ml of 40% sodium dodecylsulphate, 1.5 ml of 20% acetic acid (prepared in 0.27 M of HCl) and 1.5 ml of 0.5% thiobarbituric acid were mixed together. The mixture was heated for 60 min at 95°C in a water bath to give a pink color. The mixture was then centrifuged at 3500 rpm for 10 min. Finally absorbance of the supernatant layer was read spectrophotometrically at 532 nm, the molar extinction coefficient factor equal $1.56 \times 10^5 \text{ M}^{-1}\cdot\text{cm}^{-1}$.

2.5.5. Hydrogen Peroxide (H_2O_2) Level

Its concentration was measured according to the method of Aebi [37]. The principle of this method based on that, in the presence of peroxidase, H_2O_2 reacts with 3,5-dichloro-2-hydroxy benzene sulfonic acid (DHBS) and 4-aminophenazone (AAP) to form a chromophore. The

absorbance was read spectrophotometrically at 510 nm.

2.5.6. Bilirubin Content

The reaction between bilirubin and the diazonium salt of sulphanic acid produced azobilirubin which shows a maximum absorption at 535 nm in an acid medium [38].

2.5.6. Statistical Analysis of Biochemical Data

The biochemical data recorded were expressed as mean \pm SD and statistical and correlation analyses were undertaken using the One-way ANOVA followed by a post-hoc LSD (Least Significant Difference) test. A *P* value $<$ 0.05 was statistically significant. A Statistical analysis was performed with the Statistical Package for the Social Sciences for Windows (SPSS, version 10.0, Chicago, IL, USA).

2.5.7. Extraction of Total RNA from EAC

Homogenates

Total RNA was isolated from EAC pellets using RNeasy Mini Kit according to manufacturer's instructions (QIAGEN, Germany). About 100 μ l of homogenate was subjected to RNA extraction and the resultant RNA was dissolved in DEPC-treated water, quantified spectrophotometrically and analyzed on 1.2% agarose gel. RNAs inhibitors were added to the samples during the RNA extraction process.

2.5.8. Real Time PCR and Gene Expression

1) For Pro-Apoptotic and Anti-Apoptotic Genes (*P53* and *Bcl2*)

The extracted RNA was subjected to Real Time PCR reaction to examine the expression *p53* and *Bcl2* genes in EAC, EAC+ Seeds extract and EAC+ fruits extract of *Adansonia* using specific primers in the presence of glyceraldehyde-3-phosphate dehydrogenase (GPDH) as a housekeeping gene. The Real time reaction consists of 12.5 μ l of 2X Quantitech SYBR[®] Green RT Mix (Fermentaz, USA), 2 μ l of the extracted RNA (50 ng/ μ l), 1 μ l of 25 pM/ μ l forward (F) primer, 1 μ l of 25 pM/ μ l reverse (R) primer (**Table 1**), 9.5 μ l of RNAase free water for a total of 25 μ l. Samples were spun before loading in the rotor's wells. The real time PCR program was performed as follows: initial denaturation at 95°C for 10 min.; 40 cycles of 64°C for 15 sec for *p53*, annealing at 63°C for 30 sec for *Bcl2* and extension at 72°C for 30 sec. Data acquisition performed during the extension step. This reaction was performed using Rotor-Gene 6000 system (QIAGEN, USA).

2.5.9. Molecular Data Analysis

Comparative quantitation analysis was performed using Rotor-Gene-6000 Series Software based on the following equation:

$$\text{Ratio target gene expression} = \frac{\text{Fold change in target gene expression} \left(\frac{\text{sample}}{\text{control}} \right)}{\text{Fold change in reference gene expression} \left(\frac{\text{house keeping gene}}{\text{control}} \right)}$$

Real-time PCR data of all samples were analyzed with appropriate bioinformatics and statistical program for the estimation of the relative expression of genes using real-time PCR and the result normalized to its gene (Reference gene). The data were statistically evaluated, interpreted and analyzed using Rotor-Gene-6000 version 1.7.

3. Results

As in **Table 2**, the sera bilirubin and tumour markers such as arginase and alpha-l-fucosidase levels were significant increase in the transplant EAC group ($p \leq 0.001$)

when compared with the control group. The extracts of seeds and pulp fruits of *Adansonia* showed significant decrease in the tumor markers when compared with EAC group. Moreover, the level of bilirubin was ameliorated with the administration of the extracts of seeds and fruits of *Adansonia*.

The EAC group in **Table 3**, showed very high significant decrease ($P \leq 0.001$) in the sera superoxide dismutase and glutathione peroxidase activities and reduced glutathione content when compared with control group. The oxidative stress was determined as an increment in

Table 1. Primers nucleotides sequence used in this study.

Primers name	Primer sequence from 5'-3'	Annealing temp.
<i>P53</i>	F-AGGGATACTATTTCAGCCCGAGGTG	64°C
	R-ACTGCCACTCCTTGCCCATTC	
<i>Bcl2</i>	F-ATGTGTGTGGAGAGCGTCAACC	63°C
	R-TGAGCAGAGTCTTCAGAGACAGCC	
GPDH (Housekeeping gene)	F-ATTGACCACTACCTGGGCAA	60°C - 65°C
	R-GAGATACACTTCAACACTTTGACCT	

GPDH: Glyceraldehyde-3-phosphate dehydrogenase.

Table 2. Serum bilirubin and tumor markers of different treated groups.

Groups Parameters	Control	EAC	EAC+ Seeds Extract	EAC+ Fruit Extract
Bilirubin (U/dl)	0.51 ± 0.08	1.1 ± 0.12 ^{***i} ii	0.87 ± 0.14 ^{***i} ii	0.79 ± 0.02 ^{***i} ii
Arginase (U/dl)	31.8 ± 2.2	110.6 ± 5.1 ^{***}	63.6 ± 2.5 ^{***i} ii	72.0 ± 1.6 ^{***i} ii
L-Fucosidase (U/dl)	21.8 ± 10.4	75.1 ± 6.9 ^{***}	56.9 ± 11.2 ^{***i} i	53.0 ± 3.9 ^{***i} ii

All data ± standard deviation (SD), each group equal 5 rats, $p \leq 0.001^{***}$, represented the comparison of EAC, EAC+ Seeds Extract and EAC+ Fruit Extract with the control group; $p \leq 0.001^{iii}$, represented the comparison of EAC+ Seeds Extract and EAC+ Fruit Extract with the EAC group.

Table 3. The serum antioxidants activities and oxidative stress in different treated groups.

Groups Parameters	Control	EAC	EAC+Seeds Extract	EAC+Fruit Extract
SOD (U/ml)	3.5 ± 0.1	1.3 ± 0.2 ^{***}	2.6 ± 0.4 ^{***i} ii	2.3 ± 0.4 ^{***i} ii
GSH-Px (U/ml)	162.6 ± 19.7	55.2 ± 7.8 ^{***}	74.1 ± 5.7 ^{***i}	77.6 ± 5.9 ^{***i} i
GSH (mg/mg protein)	28.7 ± 3.3	14.1 ± 0.87 ^{***}	18.4 ± 1.78 ^{***i} i	18.1 ± 1.5 ^{***i} i
MDA (n mol/ml)	82.7 ± 5.3	124.2 ± 9.1 ^{***}	101.8 ± 9.1 ^{***i} ii	98.2 ± 5.9 ^{***i} ii
H ₂ O ₂ (U/ml)	71.1 ± 10.7	146.8 ± 7.4 ^{***}	128.1 ± 3.8 ^{***i} ii	118.9 ± 3.6 ^{***i} ii

All data ± standard deviation (SD), each group equal 5 rats, $p \leq 0.001^{***}$, represented the comparison of EAC, EAC+ Seeds Extract and EAC+ Fruit Extract with the control group; $p \leq 0.01^{ii}$, $p < 0.001^{iii}$, represented the comparison of EAC+ Seeds Extract and EAC+ Fruit Extract with the EAC group.

TBARS and H₂O₂ in the EAC group. The methanolic extracts of seeds and fruits of *Adansonia* ameliorate the deflection of antioxidants status as they increase the antioxidant enzymes as well as decrease the TBARS and H₂O₂ in the EAC+ Seeds Extract and EAC+ Fruit Extract groups when compared with EAC group.

As in **Table 4** the EAC+ Seeds Extract and EAC+ Fruit Extract groups showed high significant increase in the Ascites fluid superoxide dismutase and glutathione peroxidase activities and reduced glutathione content when compared with EAC group. The extracts of seeds and fruits of *Adansonia* showed high significant decrease the TBARS and H₂O₂ in the EAC+ Seeds Extract and EAC+ Fruit Extract groups when compared with EAC group.

As in **Table 5** the EAC+ Seeds Extract and EAC+ Fruit Extract groups showed high significant increase in the EAC cells superoxide dismutase and glutathione peroxidase activities and reduced glutathione content when compared with EAC group. The extracts of seeds and fruits of *Adansonia* ameliorate the imbalance in antioxidants status as they increase the antioxidant enzymes as well as decrease the TBARS and H₂O₂ in the EAC+ Seeds Extract and EAC+ Fruit Extract groups when compared with EAC group.

The EAC group in **Table 6**, showed very high significant decrease ($P \leq 0.001$) in the liver superoxide dismutase and glutathione peroxidase activities and reduced glutathione content when compared with control group. The oxidative stress was estimated as an increment in TBARS and H₂O₂ in the EAC group. The extracts of seeds and fruits of *Adansonia* ameliorate the imbalance

Table 4. The Ascites fluid antioxidants activities and oxidative stress in different treated groups.

Groups Parameters	EAC	EAC+ Seeds Extract	EAC+ Fruit Extract
SOD (U/ml)	90.4 ± 9.7	180.3 ± 14.5 ^{***}	193.6 ± 12.1 ^{***}
GSH-Px (U/ml)	101.48 ± 6	117 ± 5.5 ^{**}	124.6 ± 10.4 ^{***}
GSH (mg/ml)	19 ± 0.98	29.1 ± 1.3 ^{***}	31.1 ± 3.3 ^{***}
MDA (nmol/ml)	145.6 ± 8.3	111.8 ± 13.8 ^{***}	126.4 ± 11.1 [*]
H ₂ O ₂ (U/ml)	17.8 ± 1.2	11.8 ± 0.91 ^{***}	12.1 ± 0.61 ^{***}

All data ± standard deviation (SD), each group equal 5 rats, $p < 0.05^*$, $p \leq 0.01^{**}$, $p \leq 0.001^{***}$, represented the comparison of EAC+ Seeds Extract and EAC+ Fruit Extract with the EAC group.

Table 5. The EAC cells antioxidants activities and oxidative stress in different treated groups.

Groups Parameters	EAC	EAC+ Seeds Extract	EAC+ Fruit Extract
SOD (U/g protein)	1.6 ± 0.13	2.1 ± 0.12 ^{**}	1.9 ± 0.46 [*]
GSH-Px (U/g protein)	92 ± 4.9	104.5 ± 8.4 ^{**}	110.9 ± 7.4 ^{***}
GSH (U/g protein)	32.9 ± 17.3	46.6 ± 27.7 ^{***}	43.9 ± 29.4 ^{***}
MDA (n mol/g protein)	148.6 ± 11.9	121.8 ± 6.8 ^{***}	128.4 ± 14.6 ^{**}
H ₂ O ₂ (U/g protein)	15.7 ± 1.8	10.4 ± 0.76 ^{***}	11.1 ± 1.5 ^{***}

All data ± standard deviation (SD), each group equal 5 rats, $p < 0.05^*$, $p \leq 0.01^{**}$, $p \leq 0.001^{***}$, represented the comparison of EAC+ Seeds Extract and EAC+ Fruit Extract with the EAC group.

antioxidants status as they increase the antioxidant enzymes as well as decrease the TBARS and H_2O_2 in the EAC+ Seeds Extract and EAC+ Fruit Extract groups when compared with EAC group.

The real time PCR results (**Figure 1**) showed that the expression of *p53* and *Bcl2* genes was decreased in EAC+ Seeds extract group when compared with the EAC one. Also the extract of fruit extract decreased the expression of both genes EAC+ Fruit Extract treated group when compared with the EAC group.

4. Discussion

The transplantation of EAC neoplastic cells into the mice causes deflection in the balance of antioxidant/oxidative stress system both in EAC and liver tissue. The inhibition of SOD and GSH-Px activities as a result of EAC growth was reported in liver [39]. Corresponding decrease in the levels of SOD and GSH was observed in the liver of EAC-bearing mice [12]. The decrease in the activities of antioxidants may be due to the increase of oxidative stress induced by the inoculation of EAC. There was increase in the levels of thiobarbituric acid reactive substance representing by malondialdehyde (MDA) and hydrogen peroxide in the EAC cells, ascetic fluid and liver homogenates in EAC group when compared with the

EAC treated groups. The extracts of seeds and fruits pulp of *Adansonia* moderate the levels of MDA and H_2O_2 in EAC cells, ascetic fluid and liver homogenates. This amelioration may be due to the antioxidant effect of *Adansonia*. Vertuani [40] showed that the fruit pulp exhibited strong anti-oxidant activity corresponding to 6 - 7 mmol/g of Trolox, in comparison to the fruit pulps of orange (0.1 mmol/g), strawberry (0.90 mmol/g), apple (0.16 mmol/g) and kiwi (0.34 mmol/g). The antioxidant activity of fresh ripe fruit of *A. digitata* was 1000 mg AEAC/100g (ascorbic acid equivalent antioxidant content) [41]. So the significant increase in the antioxidant enzymes activity such as SOD, GSH-Px in EAC and liver due to the higher antioxidant effect of *Adansonia*. Nhukarume [42] investigated the ability of solvent extracts of various fruits including baobab to inhibit the peroxidation of lipids. This may explain the significant decrease in the MDA and H_2O_2 levels after the treatment with the extract of *Adansonia*. The hepatoprotective activity of a water extract of the fruit pulp was evaluated in vivo against chemical-induced toxicity with CCL4 in rats [23]. In the present study, EAC-bearing mice showed to be under higher oxidative stress than control animals indicated by elevated lipid peroxidation and reduced endogenous antioxidants in the liver. Tumors in the human body or in

Table 6. The liver antioxidants activities and oxidative stress in different treated groups.

Groups Parameters	Control	EAC	EAC+Seeds Extract	EAC+Fruit Extract
SOD (U/g tissue)	32.4 ± 2.2	14.4 ± 7.04***	22.1 ± 2.01*** ⁱ	21.8 ± 1.9*** ⁱⁱ
GSH-Px (U/g tissue)	107.1 ± 6.5	86.5 ± 10.5***	103.3 ± 8.9 ⁱ	97.6 ± 11*
GSH(mg/g tissue)	96.31 ± 29.1	54.58 ± 14.3***	84.13 ± 25.1*** ⁱⁱ	84.34 ± 38.6*** ⁱⁱⁱ
MDA (n mol/g tissue)	92.8 ± 13.7	148.2 ± 6***	126.3 ± 2.3***	132 ± 5.2***
H_2O_2 (U/g tissue)	9.0 ± 0.91	16.8 ± 2.2***	9.9 ± 0.89 ⁱ	11.0 ± 1.9 ⁱⁱ

All data ± standard deviation (SD), each group equal 5 rats, $p < 0.05^*$, $p \leq 0.001^{***}$, represented the comparison of EAC, EAC+ Seeds Extract and EAC+ Fruit Extract with the control group; $p \leq 0.01^{ii}$, represented the comparison of EAC+ Seeds Extract and EAC+ Fruit Extract with the EAC group.

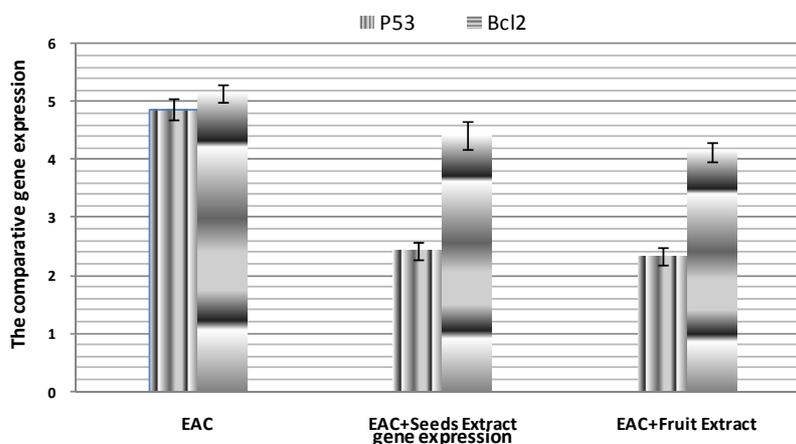


Figure 1. P53 and Bcl2 genes expression in Ehrlich Ascites Carcinoma cells of different groups of rats.

experimental animals are known to affect many functions of the vital organs, especially the liver, even when the site of the tumor does not interfere directly with organ function [43]. However, the decrease in bilirubin indicates the improvement of liver function, so the treated mice with *adansonia* improve the liver function. This is may be due totriterpenoids, β -sitosterol, β -amyripalmi-tate, and/or α -amyrin and ursolic acid present in thefruit pulp [24]. The reduction in oxidative stress leads to subsequent decrease in injury and damage of the hepatocytes membranes. It is suggested that if oxidative stress is involved in the origin of EAC-induced liver oxidative injury, then a successful extract with antioxidant potential should protect against that injury. Seeds and fruit pulp of *Adansonia* ameliorated oxidative stress in seeds and fruit pulp + EAC-bearing mice and prevented cellular injury as evidenced by a decrease in levels of TBARS in liver. This is associated with increase in GSH level as well as the activities of SOD, and GSH-Px compared with EAC-implanted mice. The control of endogenous redox state might contribute to the demonstrated increase in the life span of EAC-tumor bearing mice treated with *Adansonia*. Thus, the tumor markers such as α -l-fucosidase and arginase indicate that *Adansonia* exhibited significant anti-tumor and might show potential antioxidant activity in EAC-bearing mice. This might be via changes in the expression of endogenous antioxidants. The administration of seeds and fruit pulp of *Adansonia* significantly increased the levels of GSH, SOD and GSH-Px as well as decreased lipid peroxidation in EAC cells, ascetic fluid and liver homogenate compared with that obtained from control EAC group. This indicates that *Adansonia* extracts altered the redox state in the sera, EAC cells, ascetic fluid and liver tissue.

Apoptosis is a programmed cell death where the cell expends energy towards its own end. It is well known that functional *p53* provides a protective mechanism against tumor growth. It acts as a tumor suppressor through its capacity to induce cell cycle arrest and apoptosis in response to a variety of chemotherapeutic drugs [44]. Cell growth and its proliferation are two tightly linked processes. Both are characterized by increased protein synthesis which is achieved by changes in the activity of ribosomal biogenesis and *p53* expression [16]. It is reported that deregulated signaling in cancer cells drives excessive ribosome biogenesis within the nucleolus, which elicits uncontrolled cell growth and proliferation. It is controlled by a complex interconnection of proteins such as *p53* and is often triggered by oxidative stress and the release of cytochrome *c* from the mitochondria [19]. *Bcl-2* indicates a family of genes and proteins found in rats that regulates the outer membrane permeability of mitochondria, and most importantly the fate of the cell through programmed cell death, apoptosis.

The increased level of ROS is involved in generating the signal that causes change the permeability of the mitochondrial membrane, and, thus, the release of cytochrome *c* into the cytosol. Once this occurs, the initiation of the cascade of caspases occurs [45]. Activation of caspases will eventually lead to the death of the cell and other surrounding cells. H_2O_2 -induced apoptosis is accompanied by changes in apoptosis-related factors such as the *Bcl-2* family of regulatory proteins [19]. However, ROS such as H_2O_2 act not only as cellular messengers capable of causing oxidative damage to macromolecules, but also as signaling molecules that activate protein kinase cascades [46]. The high expression of *p53* and *Bcl-2* genes due to their molecular changes accompanied with tumor development in mice. The impairment of nucleolar function might stabilize *p53* by preventing its degradation and, therefore, for the arrest of cell-cycle progression [10]. Therefore, the present finding indicates that seeds and fruit pulp of *Adansonia* might be able to attenuate apoptotic mechanisms through the changes of *p53* and *Bcl-2* gene expression leading to the control of growth and proliferation of EAC cells.

5. Conclusion

The study demonstrated that extracts of seeds and fruit pulp of *Adansonia* could have anti-tumor action through modulation of redox state in sera, EAC cells, ascetic fluid and liver tissue. These extracts were able to restore and modulate the tumor markers levels such as α -l-fucosidase and arginase activity in the sera of EAC bearing mice. Also *Adansonia* attenuates the *p53* and *Bcl-2* gene expression as pro-apoptotic and anti-apoptotic genes leading to management of tumor growth. The study initiates human to use *Adansonia* as a good edible stuff that has strong antioxidant action and anti-tumor agent.

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