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Research Article

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The Potent Effect of a Newly Synthesized N-Butylpyridoquinoxaline 1,4-dioxide (NBPQD) Derivative as Antitumor agent in solid tumor model

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ABSTRACT

The present study was carried out to evaluate the potent effect of the newly synthesizedNBPQD derivative as antitumor agent in Ehrlich ascites carcinoma (EAC) bearing mice in solid tumor model. The effect of ***NBPQD*** *as antitumor in solid tumor model was assessed by evaluating tumor volume and the contents of total protein, total lipid, DNA and RNA in liver tissues in addition to hematological profiles. Also, levels of glucose, urea and albumin in serum and the redox status were assessed. Tumor volume and DNA, RNA and malondialdehyde (MDA) levels were highly significantly increased (P< 0.001) in untreated EAC-bearing mice compared to control. However, hemoglobin, total lipid in liver tissues and glucose level in serum were highly significantly decreased in untreated EAC-bearing mice compared to control. In addition, reduced glutathione content (GSH) and activities of glutathione reductase (GSH-R), catalase and superoxide dismutase (SOD) in blood were also highly significantly decreased in untreated EAC-bearing mice compared to control. All these parameters were highly significantly (P< 0.001) restored their normal levels in* ***NBPQD*** *treated mice compared to the untreated EAC-bearing mice. These results were confirmed by the long survival time of the treated group with* ***NBPQD,*** *in addition to 80% reduction in the tumor volume. It can be concluded that,* ***NBPQD*** *exhibited a remarkable antitumor activity against EAC in Swiss albino mice in solid tumor model.*

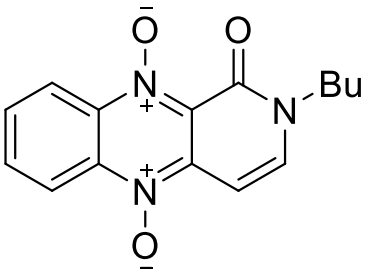
Keywords: *Ehrlich Ascites Carcinoma; SOD; MDA; NBPQD; GSH-R; GSH*

INTRODUCTION

Quinoxaline derivatives have been reported to possess a wide variety of biological activities **[1,2,3]**. Notable among these are anticancer, antioxidant, anti-inflammatory, antimicrobial and antihistamic activities. Drugs having

pyrazoline ring systemare well known to have anti-inflammatory, antioxidant, antihistamic, antimicrobial, antidepressant, hypoglycemic, hypotensive, anticarcenogenic activities etc **[4].**

The quinoxaline derivatives are a class of compounds that have biological properties and there is active interest in their medicinal chemistry **[5].** There many efforts to find quinoxaline-1,4-di-N-oxide derivatives with antimycobacterial, antiprotozoal and anti-cancer activity **[6].**



NBPQD

The oxidation of the two nitrogen atoms of this heterocyclic system, in order to obtain quinoxaline 1,4-di-N-oxide derivatives, increases the number of biological properties enormously **[5].** Specific derivatives also show selective cytotoxicity against hypoxic cells present in solid tumors **[7].**

The synthesis and biological evaluation of new agents derived from quinoxaline 1,4-di-N-oxide and related compounds have proved to be efficient cytotoxic agents for hypoxic cells in solid tumors. The poor tumor vascular structure and the inefficient blood supply along with a high interstitial pressure generate a variable increases in reactive oxygen species and promotes leukocyte proportion of viable hypoxic cells in solid tumors which is one of the causes of cell resistance to anticancer treatments. Thus, antioxidants prevent the increase in leukocyte-endothelial adhesive interactions observed in hypoxia **[8]**.

Quinoxaline-1,4-dioxide derivatives seem to have interestinganticancer activities**[9],** especially in solid tumortreatment**[10].** Moreover, quinoxaline derivatives may act by theconcept of bioreductive alkylation and could cleave theDNA under hypoxic conditions in the presence of xanthineand xanthine oxidase **[10].**

Radiolabeling of quinoxaline derivatives had been studied and its biodistribution in tumor bearing animals were investigated**[11].**

Oxidative stress is defined as a disturbance in the equilibrium between free radicals (FR), reactive oxygen species (ROS), and endogenous antioxidant defense mechanisms, or more simply, it is a disturbance in oxidant-antioxidant states, favoring the oxidant environment **[12]**.

In cancer cells, ROS increase the rate of mutagenicity, which leads to DNA damage and chromosomal instability, thereby potentiating cancer progression **[13, 14]**. Secondly, ROS may promote cell survival and proliferation, thus contributing to cancer development **[15].**In addition, the abnormal regulation of ROS has a role in pathological conditions, including inflammation, atherosclerosis, angiogenesis and aging in addition to cancer.

Cells have evolved several antioxidant defenses, including repair and detoxifying enzymes, and small scavenger molecules, such as glutathione. The intracellular ROS-scavenging system includes superoxide dismutases (SOD), glutathione peroxidase (GPx), thioredoxins (TRXs), and catalases. In mitochondria, superoxide anion (O_2^-) can be dismutated to hydrogen peroxide (H_2O_2) by two enzymes, namely copper-zinc superoxide dismutase (CuZnSOD) and manganese superoxide dismutase (MnSOD), that are present in the mitochondrial matrix and in the intermembrane space, respectively. Once generated, H_2O_2 can be quenched by GPx in mitochondria, or by catalase in the cytosol. Glutathione is the major non-enzymatic component of intracellular antioxidant defenses. It is present at millimolar

concentrations, and it works either as a nucleophile for efficient detoxification of reactive electrophiles, or as an antioxidant **[16]**.

SOD mainly functions to provide a defensive action against the potentially damaging reactivities of the superoxide radical generated by all aerobic metabolic reactions. As SOD is a free radical metabolizing enzyme, it catalyzes the dismutation of superoxide radical to H_2O_2. This protects the cell membrane from damage by ROS. But the decreased SOD levels may lead to increased lipid peroxidation resulting in the cellular rigidity and deformability**[17].**

The present work aims at studying the antitumor potent effect of **NBPQD** in mice infected with Ehrlich ascites carcinoma cells in the right thigh of the mice.

MATERIALS AND METHODS

1- Antitumor agent:

The quinoxaline derivative (NBPQD) under studying was prepared by Dr. M. A. Waly, Chemistry Department, Faculty of Science, Damietta University, Egypt. In addition, 5-Flurouracil (5-FU) was used as antitumor standard for comparison. The Labeling of NBPQD was preparedaccording to the method of Coenen et al. [18] using NaI^{125}

2-Tumor cells:

Ehrlich ascites carcinoma cells (EAC) was kindly supplied by the National Cancer Institute, Cairo university, Egypt. EAC cells were collected 7 days after intraperitoneal implantation. The harvested cells were diluted with saline to obtain a concentration of 5 x 10^6 viable EAC cells per ml. A volume of 0.2 ml saline (1 x 10^6) EAC was injected intramuscularly in right thighof the mice to produce solid tumor where the left thigh kept as control. The animals were maintained for 7-10 days till the solid tumor development.

3- Animals:

All experiments were performed with adult female Swiss albino mice purchased from Theodore Bilharz Research Institute, Giza, Egypt, with an average body weight of 25 to 30 gm. Mice were housed in steel mesh cage (10 mice/cage) and maintained for two weeks acclimatization periods on commercial standard diet and tap water. Then, these mice were randomly divided into 8 groups, 15 animals each, according to the following scheme:

Group I. Normal mice-saline treated group (GI as control) :

Eachmouse was intraperitoneally injected daily with 0.2 ml of physiological saline solution for 10 days.

Group II. Normal mice-dimethylsulphoxide (DMSO) treated group (GII):

Eachmouse was intraperitoneally injected daily with 0.2 ml of dimethyl sulphoxide solvent for 10 days.

Group III. Normal mice-NBPQD treated group (GIII):

Eachmouse was intraperitoneally injected daily with (16 mg/kg/day) of the NBPQD for 10 days.

Group IV. Normal mice-5-Flurouracil (5-FU) treated group (GIV):

Eachmouse was intraperitoneally injected daily with (20 mg/kg/day) of (5-FU) for 10 days as positive control.

Group V. Solid tumorized mice-saline treated group (GV):

Normal mice were injected intramuscular (of right thigh) with 0.2 ml saline contains 1x10^6 tumor cells/mouse. After 24 hours of tumor inoculation, the mice were treated intraperitoneally daily with 0.2ml of the physiological saline solution for 24 days .

Group VI. Solid tumorized mice-(DMSO) treated group (GVI):

Normal mice were injected intramuscular (of right thigh) with 0.2 ml contains $1x10^6$ tumor cells/mouse. After 24 hours of tumor inoculation, the mice were treated intraperitoneally daily with 0.2ml of **DMSO** solvent for 24 days.

Group VII. Solid tumorized mice-NBPQD treated group (G VII):

Normal mice were injected intramuscular (of right thigh) with 0.2 ml contains $1x10^6$ tumor cells/mouse. After 24 hours of tumor inoculation, the mice were treated intraperitoneally daily with **(16 mg/Kg/day)**of**NBPQD**for 24 days.

Group VIII. Solid tumorized mice-5-FU treated group (G VII):

Normal mice inoculated intramuscular (of right thigh) with 0.2 ml contains $1x10^6$ tumor cells/mouse. After 24 hours of tumor inoculation, the mice were treated intraperitoneally daily with**(20 mg/Kg/day)**of **5-FU** for 24 days as positive control.

On days 9 to 24, tumor volume of bearing thigh of each animal was measured with the help of vernier caliper. Tumor volume of each animal was calculated using the following formula:

Tumor volume(mm^3)= length(mm) x [width(mm)]^2 x 0.5

5- Biochemical tests:

Total proteins were determined in liver homogenate by the method of Lowery *et al*. [19]. Nucleic acids were extracted from liver homogenate [20] and DNA content was determined in the extract using the diphenylamine procedure [21] while RNA content was measured by the orcinol procedure [22]. Lipids were extracted from liver tissues by the method of Littelfield*et al.* [24] while their concentration was determined by the sulfophosphovanilin method [24]. Total cholesterol, was estimated in serum using a commercial kit (Biocon's, India) according to the instruction of the manufacturer. The triglyceride are enzymatically determined according to the method of Fossati and Principe (1982) [25]. Glucose in serum was determined enzymatically [26] and serum albumin was determined by bromocresol green method [27].

Erythrocyte reduced glutathione (GSH) was determined by the method of Beutler*et al*. [28]. Malondialdehyde (MDA) was determined in liver tissue by the method of Ohkawa*et al.* [29]. Superoxide dismutase (SOD) activity in serum was assayed spectrophotometrically [30]. Catalase activity was determined by the method of Bergmeyer (1974) [31]. Blood hemoglobin (Hb) concentration was colourimetrically determined [32] while total counts of WBCs and platelets were determined using an advanced Bright-Line Haemocytometer (Bosch and Lomb USA) [33]. Spleen, kidney, liver and total body were weighed.

6. Statistical analysis:

The statistical analysis of the results were carried out using Instate software computer program, version 2.03 (Graph pad, USA) and IBM PC compatible computer. A difference was said to be significant, and highly significant, when the corresponding value of probability (P) was ≤ 0.05 and ≤ 0.001, respectively. Correlation coefficient (r) was used for measuring the relationship between two variables. The correlation is weak at r = 0.50, moderate at r = 0.50 – 0.75 and strong at r = 0.80 – 1.00.

RESULTS

b- Biodistribution of ^{125}I- NBPQD:

In solid tumor bearing mice, the biodistribution of ^{125}I- **NBPQD** in solid tumor bearing mice was found to be greatest in blood, stomach and heart at 15 minutes post injection and lowest in left leg, spleen and thyroid **(Table 1)**. The biodistribution of ^{125}I- **NBPQD** in the right thigh (inoculated) was greater than that of left one. The uptake of ^{125}I-**NBPQD** in right thigh is significantly increased with the time and reached maximum at 24 hour of injection.

In Table 2, the weights of liver, spleen and kidney and white blood cells count illustrate nonsignificant changes between the different groups under study in solid tumorized mice. On the other hand, the levels of haemoglobin (Hb) in solid tumorized mice saline treated (GV) and solid tumorized mice DMSO treated (GVI) are significantly decreased compared to control. However, the levels of haemoglobin in solid tumorized mice NBPQD treated (GVII) retained their normal values. Also, the levels of haemoglobin in the normal mice treated with DMSO, NBPQD and 5-FU showed non significant change compared to control.

The mean levels of MDA, DNA and RNA in liver tissue homogenate of solid tumorized mice saline treated (GV) and solid tumorized mice DMSO treated are highly significantly increased than control (GI). However, MDA, DNA and RNA levels in liver tissue homogenate of solid tumorized mice NBPQD treated (GVII) and solid tumorized mice 5-FU treated (GVIII) showed non significant change compared to the control. Also, the normal mice treated with DMSO, NBPQD and 5-FU showed non significant change compared to control **(Table 3).**

Table.1. Biodistribution of ^{125}I-NBPQD in solid tumor bearing mice

Organs & Body fluids	**% ^{125}I-NBPQD/gram organ, Time post injection**			
	15 min	**1 hr**	**12 hr**	**24 hr**
Blood	22.8 ± 1.8	14 ± 1.2*	7.5 ± 0.2*	4.6 ± 1.3*
Bone	1.2 ± 0.1	1.8 ± 0.1*	2.4 ± 0.1*	1 ± 0.02*
Liver	4.4 ± 0.27	3.7 ± 0.25*	2.17 ± 0.1*	1.6 ± 0.1*
Lung	5.5 ± 0.1	6.5 ± 0.2*	4.0 ± 0.2*	3.1 ± 0.3*
Heart	12 ± 0.8	3 ± 0.2*	2 ± 0.1*	2 ± 0.01
Stomach	11.1 ± 0.6	16.1 ± 0.8*	10.8 ± 0.5*	8.0 ± 0.6*
Intestine	2.9 ± 0.2	6.0 ± 0.5*	4 ± 0.22*	3.5 ± 0.15
Kidney	2.3 ± 0.9	7 ± 0.2*	4.6 ± 0.08*	2. ± 0.16*
Spleen	2 ± 0.1	3.0 ± 0.2*	2.3 ± 0.2*	1 ± 0.05*
Thyroid	2 ± 0.02	4 ± 0.22*	6.5 ± 0.4*	6.4 ± 0.3
Left leg	0.8 ± 0.05	0.9 ± 0.03*	1.1 ± 0.07*	0.5 ± 0.3*
Right leg	2.7 ± 0.2	5.5 ± 0.5*	7.0 ± 0.04*	7.3 ± 0.4

This table was subtracted from our previous article (11)

Values represent the mean ± SEM n = 6

Significantly different from the initial value of each organ ($P<0.05$).

Table.2: Weights of liver, spleen and kidney corresponding to total body weight , blood haemoglobin (gm%) and white blood corpuscle/c.mm in mice of groups I-VIII:

Group	Percent of liver weight to total body weight (%)	Percent of spleen weight to total body weight (%)	Percent of kidney weight to total body weight (%)	Blood haemoglobin (gm%)	White blood corpuscle/c.mm
G I M ± S.D	8.0 ± 0.8	0.9 ± 0.31	2.0 ± 0.14	15.3 ± 2.1	7700 ± 2714.2
G II M ± S.D	8.0 ± 0.6^{ns}	0.88 ± 0.1^{ns}	2.2± 0.12^{ns}	14.16 ± 1.1^{ns}	7200 ± 346.4^{ns}
G III M ± S.D	8.0 ± 1.1^{ns}	0.89 ± 0.31^{ns}	2.0 ± 0.4^{ns}	15.1 ± 1.8^{ns}	7700 ± 1979.8^{ns}
GIV M ± S.D	8.0 ± 0.3^{ns}	0.9 ± 0.2^{ns}	2. 2 ± 0.34^{ns}	15.3 ± 1.3^{ns}	7400 ± 1979.8^{ns}
G V M ± S.D	7.2 ± 0.8^{ns}	0.74 ± 0.63^{ns}	2.0 ± 0.34^{ns}	11.13 ± 1.0*	7800 ± 424.3^{ns}
G VI M ± S.D	7.4 ± 1.5^{ns}	0.71 ± 0.72^{ns}	2.0 ± 0.6^{ns}	11.0 ± 3.0*	7950 ± 212.13^{ns}
G VII M ± S.D	8.0 ±1.03 $^{ns, b}$	0.84 ± 0.4^{ns,b}	2.0 ± 0.43^{ns,b}	14.24 ± 0.4^{ns,a}	7566 ± 2500^{ns,b}
G VIII M ± S.D	7.3 ±1.1 $^{ns, b}$	0.81± 0.52^{ns,b}	2.0± 0.1^{ns,b}	11.4 ± 0.52^{*,b}	7133 ±1401^{ns,b}

Group I=Normal mice saline treated . **Group II =** Normal mice DMSO treated.

Group III= Normal mice NBPQD treated . **Group IV=** Normal mice 5-FU treated.

Group V= solid tumrized mice saline treated .**Group VI=** solid tumrized mice DMSO treated.

Group VII = solid tumrized mice NBPQD treated .**Group VIII =** solid tumrized mice 5-FU treated.

^{ns}Non significant (p>0.05) when compared to group I.

^{b}Non significant (p>0.05) when group VII and group VIII compared to group V.

Table.3: Levels of malondialdhyde (MDA), DNA and RNA in liver tissue homogenate in mice of groups I-VIII

Parameter / Group	MDA (µmole/gm tissue)x10^{-6}	DNA (mg/gm tissue)	RNA (mg/gm tissue)
Group I M ± S.D	1.63 ± 0.6	1.7 ± 0.3	8.2 ± 1.13
Group II M ± S.D	1.70 ± 0.41^{ns}	1.8 ± 0.33^{ns}	8.0 ±0.4 ^{ns}
Group III M ± S.D	1.72 ± 0.7^{ns}	1.7 ± 0.32^{ns}	8.0 ± 0.7^{ns}
Group IV M ± S.D	1.75 ± 0.20^{ns}	1.8 ± 0.33^{ns}	8.0 ± 0.9^{ns}
Group V M ± S.D	4.01 ± 0.2**	2.7 ± 0.2*	15.25 ± 1.06 *
Group VI M ± S.D	3.30 ± 0.74*	2.6 ± 0.2 *	12.0 ± 1.3*
Group VII M ± S.D	1.7 ± 0.54^{ns,a}	1.8 ± 0.3^{ns,a}	8.0 ± 0.9^{ns,a}
Group VIII M ± S.D	1.9 ± 0.54^{ns,a}	2.01 ± 0.1^{ns,a}	9.4 ± 0.1^{ns,a}

^{ns}Non significant ($p>0.05$) when compared to group I.

*significant ($p<0.05$) and ** highly significant ($p<0.001$) when compared to group I.

^{a} significant ($p<0.05$) when compared group VII and group VIII with group V.

Table.4: Levels of cholesterol and triglyceride in serum and total lipid level in liver tissue homogenate in mice of groups I-VIII

parameter / Group	Cholesterol (mg%)	Triglyceride (mg%)	Total lipid (mg/gm tissue)
Group I M ± S.D	63.0 ± 10.0	165.0 ± 55.1	374.1 ± 58.0
Group II M ± S.D	61.0 ± 6.0^{ns}	163.33 ± 23.41^{ns}	374.0 ± 104.1^{ns}
Group III M ± S.D	62.13 ± 4.0^{ns}	164.21 ± 45.3^{ns}	373.33 ± 11.54^{ns}
Group IV M ± S.D	67.33 ± 12.32^{ns}	167.0±12.2^{ns}	372.31 ± 56.1^{ns}
Group V M ± S.D	83.0 ± 8.3*	275.0 ± 24.0*	221.0 ± 30.1*
Group VI M ± S.D	80.32 ± 17.0*	266.0 ± 14.0*	276.0 ± 32.0*
Group VII M ± S.D	62.22 ± 5.1^{ns,b}	165.0 ± 44.0^{ns,b}	361.4 ± 62.04$^{ns\ ,\ b}$
Group VIII M ± S.D	60.0 ± 9.43^{ns,b}	167.0 ± 9.42^{ns,b}	346.0 ± 63.0$^{ns\ ,\ b}$

^{ns} Non significant ($p>0.05$) when compared to group I.

*Significant ($p<0.05$) when compared to group I.

^{b}Non significant ($p > 0.05$) when group VII and group VIII compared to group V.

The mean values of cholesterol and triglyceride in serum of solid tumorized mice saline treated (GV) and solid tumorized mice DMSO treated (GVI) are significantly increased compared to control group (GI). However, cholesterol and triglyceride levels in serum of solid tumorized mice NBPQD treated (GVII) and solid tumorized mice 5-FU treated (GVIII) showed non significant change compared to control. Also, these parameters in normal mice treated with DMSO, NBPQD and 5-FU showed non significant change compared to control. On the other hand, the mean values of total lipid in liver tissue homogenate of solid tumorized mice saline treated (GV) and solid tumorized mice DMSO treated (GVI) are significantly decreased compared to control. However, liver total lipid in solid tumorized mice NBPQD treated (GVII) and solid tumorized mice 5-FU treated (GVIII) showed non significant change compared to control **(Table 4)**.

Table.5: Levels of glucose, albumin and urea in serum and total protein in liver tissue homogenate in mice of groups I-VIII

parameter / Group	Glucose (mg%)	Albumin (gm%)	Urea (mg%)	Total protein (mg/gm tissue)
Group I				
M ± S.D	154.0 ± 35.3	3.0 ± 0.11	28.0 ± 5.0	20.0 ± 0.12
Group II				
M ± S.D	134.24 ± 18.4*	3.0 ±1.0 ^{ns}	28.0 ± 10.3^{ns}	20.0 ± 1.0^{ns}
Group III				
M ± S.D	153.0 ± 39.0^{ns}	3.0 ± 0.2^{ns}	28.0 ± 2.0^{ns}	19.8 ± 1.44^{ns}
Group IV				
M ± S.D	154.1 ± 55.1^{ns}	3.0 ± 0.3^{ns}	28.0 ±4.0^{ns}	20.0 ± 0.3^{ns}
Group V				
M ± S.D	79.0 ± 19.0*	2.4 ± 0.1^{ns}	22.0 ± 7.0^{ns}	19.0 ± 0.5^{ns}
Group VI				
M ± S.D	53.4 ±6.0*	2.5 ± 0.3^{ns}	22.4 ± 10.2^{ns}	19.1± 0.12^{ns}
Group VII				
M ± S.D	82.1 ± 33.41^{*,b}	3.0 ± 0.23^{ns,b}	26.0 ±7.0 $^{ns, b}$	19.5 ± 0.7$^{ns ,b}$
Group VIII				
M ± S.D	81.0 ± 14.3^{*,b}	2.42 ± 0.4^{ns,b}	22.0 ± 4.2^{ns,b}	19.0 ± 1.41$^{ns , b}$

^{ns} Non significant ($p>0.05$) when compared to group I.

*Significant ($p<0.05$) when compared to group I.

^{b}Non significant ($p>0.05$) when group VII and group VIII compared to group V.

Table 5, shows a significant decrease in blood glucose level in solid tumorized mice saline treated (GV) and solid tumorized mice DMSO treated (GVI) when compared to control (GI). Also, glucose level in solid tumorized mice NBPQD treated (GVII) and solid tumorized mice 5-FU treated (GVIII) are significantly increased compared to solid tumorized mice saline treated (GV) and solid tumorized mice DMSO treated (GVI), but still less than control. However, the glucose level in normal mice treated with NBPQD, DMSO and 5-FU showed non significant change compared to the control**.** On the other hand, the albumin and urea contents in serum and total protein in liver tissues of all groups showed non significant change compared to control.

Table. 6: GSH content and GSH-R activities in RBCs and catalase and SOD in liver tissue homogenate in mice of groups I-VIII

parameter / Group	GSH (mmole /ml cells)	GSH-R (U/L)	Catalase (U/gm tissue)	SOD (% of inhibition)
Group I M ± S.D	1.51 ± 0 .3	61.0 ± 5.44	58.0±11.0	33.0 ± 11.0
Group II M ± S.D	1.50 ± 0.23^{ns}	63.0 ± 9.4^{ns}	50.44 ± 3.41^{ns}	32.0 ± 5.0^{ns}
Group III M ± S.D	1.45 ± 0.10^{ns}	60.42 ± 5.0^{ns}	50.0 ± 8.0^{ns}	29.4 ± 2.20^{ns}
Group IV M ± S.D	1.47 ± 0.3^{ns}	57.0 ± 11.1^{ns}	51.2 ± 2.14^{ns}	29.5 ± 5.0^{ns}
Group V M ± S.D	0.90 ± 0.3*	27.0 ± 10.11*	31.44 ± 13.42*	15.3 ± 4.1*
Group VI M ± S.D	0.51 ± 0.11*	30.1 ± 10.0*	34.0 ± 7.4*	15.41 ± 2.0*
Group VII M ± S.D	1.5 ± 0.7^{ns,a}	60.0 ± 18.1$^{ns\ ,\ a}$	43.21 ± 4.2^{ns,a}	30.3 ± 5.0^{ns,a}
Group VIII M ± S.D	1.0 ± 0.2$^{*\ ,\ b}$	41.1 ±9.0 $^{ns\ ,\ b}$	38.9 ± 7.7^{ns,b}	22.0 ± 7.0^{ns,b}

^{ns}Non significant ($p>0.05$) when compared to group I.

*Significant ($p<0.05$) when compared to group I.

^{a}Significant ($p<0.05$) when group VII compared to group V.

^{b}Non significant ($p>0.05$) when group VII and group VIII compared to group V.

The results in **Table 6** show a significant decrease in GSH content and GSH-R activity in erythrocytes, and catalase and SOD activities in liver tissue homogenateof solid tumorized mice saline treated and solid tumorized mice DMSO treated compared to control group (GI). However, the values of GSH content and GSH-R activity in erythrocytes, and catalase and SOD activities in liver tissue homogenateof solid tumorized mice NBPQD treated showed non significant change compared to the control.Also, these parameters in normal mice treated with DMSO, NBPQD and 5-FU showed non significant change in compared to control,

Table.7. Solid tumor volume in mice of groups (V-VIII) with and without treatment

Days	Solid tumor volume (mm^3)			
	solid tumorized mice saline treated (GV)	**solid tumorized mice DMSO treated (GVI)**	**solid tumorized mice NBPQD treated(GVII)**	**solid tumorized mice 5-FU treated (GVIII)**
3 days	0	0	0	0
6 days	60.35±5.3	55.14±9.8	25.24±4.21	31.46±5.33
9 days	111.0±9.2	100.4±19.0	57.0±11.23	94.5±20.31
12 days	239.2±10.14	197.0±22.4	138.14±15.0	172.61±68.0
15 days	519.0±157.54	396.0±68.0	288.0±57.0	324.0±135.0
18 days	1024.1±303.02	858.0±183.0	315.1±122.0	425.33±51.0
21 days	1756.22±258.0	1608.43±330.0	484.0±72.11	1083.0±373.0
24 days	2252.4±526.43	2071.21±369.14	426.0±161.0	1487.0±511.0

As shown in **Table 7,** the solid tumor volume in mice treated with saline increased with the time, after 9 days of the inoculation, the tumor volume was 111.0 ± 9.2 mm^3 and reached 2252.4 ± 526.43 mm^3 after 24 days of inoculation. Also, the solid tumor volume in mice treated with DMSO after 9 days of the inoculation was 100.4±19.0 mm^3 and reached 2071.21±369.14 mm^3 after 24 days of inoculation. However, the volume of solid tumor in mice treated with NBPQD after 9 days of the inoculation was 57.0±11.23 mm^3 and reached 426.0±161.0 mm^3 after 24 days of inoculation, comparing with the solid tumor volume in mice treated with 5-FU (standard anticancer) after 9 days of the inoculation which was 94.5±20.31mm^3 and reached1487.0±511.0 mm^3after 24 days of inoculation (Figure 2).

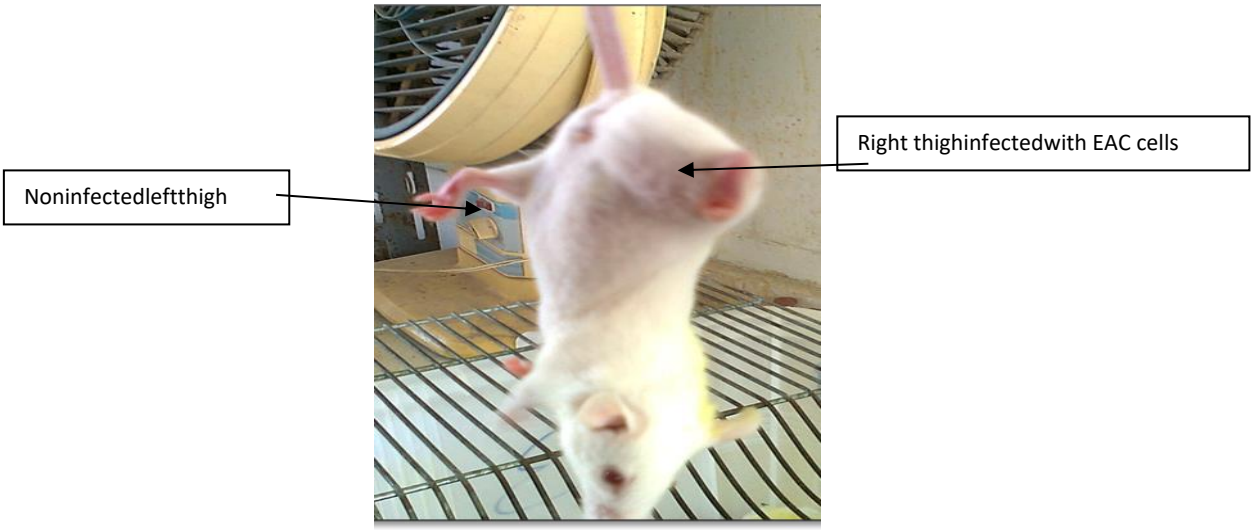


Figure 2. Solid tumor in right thigh of mice infected with EAC cells.

DISCISSION

Quinoxaline derivatives have been reported to possess a wide variety of biological activities**,** include anticancer **[34, 35, 36]**.

In solid tumor bearing mice, the uptake of ^{125}I-NBNPQD in inoculated leg increased with time and reached maximum after 24 hr, while it declined in the majority of organs, probably due to high proliferation rate in tumor site **(37)**. The

increase in ^{125}I-NBNPQD uptake in tumor to non tumor ratio was represented by ^{125}I- NBNPQD uptake in right leg/left leg. This may be due to proliferation rate, which is high in right leg than in left one **[38]**.

The uptake of ^{125}I-NBPQD in solid tumor bearing mice showed that, the concentration of ^{125}I-NBPQD increased in organs of high proliferation like stomach, while it decreased rapidly from other organs. The uptake of ^{125}I- NBPQD in right thigh was rapidly occurred as it was 2.7 % per gram at 15 min post injection, 5.5% per gram after 1 hr and 7.o % per gram after 12 hours and reached 7.8 % per gram after 24 hours. The longer persistence of its activity in tumor site may be due to the binding of ^{125}I-NBPQD with DNA of tumor tissues and takes the chance to exert their effect as antitumor drug. These results were in agreement withthat obtained for ^{25}I-UdR **[39]**.

In cancer chemotherapy the major problems are of myelosuppression and anemia. The anemia encountered in tumor bearing mice is mainly due to the reduction in RBC or hemoglobin percentage and this may occur either due to iron deficiency or due to hemolytic or myelopathic conditions **[40].** Also, there is an association between high WBC count and cancer mortality [**41]**.

In the present study, the treatment of the infected mice with **NBPQD** brought back the hemoglobin content and WBC cells count near to the normal values. This indicates that **NBPQD** posses protective action for the hematopoietic system.

Oxygen free radicals are extremely reactive and unstable and react with lipids, proteins, carbohydrates and nucleic acids in the body and generate a cascade of lipid peroxidation, a major mechanism of cell membrane distraction and cell damage **[42]**.

Increased lipid peroxidation causes degeneration of tissues, and lipid peroxides formed in the primary site are transferred through the circulation to other tissues and provoke damage by propagating the process of lipid peroxidation. MDA is the end-product of lipid peroxidation and its level is higher in carcinomatous tissue than in non-diseased organs **[40].** Malondialdehyde (MDA) is formed during oxidative degeneration, which is accepted as an indicator of lipid peroxidation **[43]**. The present study showed that the level of MDA in solid tumorized mice was highly significant increased than normal mice. This increase in MDA in tumorized mice was reduced in tumorized mice treated with NBPQD .

Since the liver is the most sensitive target in animals with extrahepatic tumors, due to tumor infiltration to the liver, in the present study, liver DNA and RNA contents were significantly increased in the infected mice and significantly decreased after treatment with **NBPQD**. These observations support that, the **NBPQD** has marked beneficial effects in this tumor model.

In contrast, liver lipid content in tumorized mice was significantly decreased and restored their normal value after treatment with **NBPQD** compared with those of saline treated. These results are in agreement with the findings of **El-Sayde, [44] and Toson*, et al.* [45].**

In the present study**,** there is a significant decreased in blood glucose level of EAC-bearing mice saline treated compared to control group**.** However, the level of blood glucose in EAC-bearing mice treated with NBPQD was increased than the treated with saline but still less than the normal level**.**

The non significant change in total proteins and albumin levels spite of the decrease in total lipid, indicates that the metastasis in liver has not been happened[**46**].

Reduced glutathione is an important nonenzymatic antioxidant. Under oxidative stress, glutathione is consumed by the glutathione-related enzymes to detoxify peroxides produced due to lipid peroxidation **[45].** The observed reduction in GSH levels in tumor-bearing mice has been previously reported **[47].** Increased rate of transformation of GSH to GSSG is a result of GSH consumption to get rid of H_2O_2, and the increased in inhibition of GSH synthesis due to lack of the amino acids used in GSH synthesis. In the present study, the depletion in GSH level in EAC-bearing mice saline treated was repaired in treatment the infected mice with NBPQD.

The decrease in the activity of SOD in tumor-bearing mice has also been reported before. This reduction in SOD activity could alter antioxidant defenses, resulting in enhanced oxidation due to the accumulation of H_2O_2**[47**]. The current study demonstrates, therefore, that EAC inoculation induces a significant decrease in hepatic GSH content associated with an inhibition of hepatic SOD activity **[48]**. The impairment of the cellular redox status may be attributed to an increase in the production of reactive oxygen species and a reduction of the anti-oxidants in liver tissues. SOD acts to trap superoxide radicals, while GSH can chemically detoxify H_2O_2 **[47].**

On the other hand the free radical scavenging system, SOD and catalase are present in all oxygen metabolizing cells and their function is to provide a defense against the potentially damaging reactivity's of superoxide and hydrogen peroxide**. The** decrease in SOD activity in EAC bearing mice which might be due to loss of MnSOD activity in EAC cells and the loss of mitochondria, leading to a decrease in total SOD activity in the liver. The inhibition of SOD and CAT activities as a result of tumor growth was also reported before [**40]**.

In the present study, inoculation of tumor bearing mice with **NBPQD** increased the SOD activity in a dose dependent manner, which may indicate the antioxidant and free radical scavenging property of **NBPQD**.

The significant inhibitions of SOD and GSH-R activities, as well as reduction of their GSH content were observed in EAC-bearing mice saline treated. These results are in agreement with previous finding of **EL-Sayde, (1998) [44].**

In addition, a significant decrease in the activity of SOD was observed in tumor-bearing mice saline treated. It has been proved that SOD inhibits OH^- production by scavenging O^{-2}, and hence, it would lead to decrease in the initiation of lipid peroxidation. Therefore, the observed increase in lipid peroxidation in tumorized-saline treated mice can be associated with the decrease in SOD level **[48]**.

Biochemical measurements of these parameters in the present study showed that no significant hepatotoxicity was observed in normal mice treated with **NBPQD**, since the values of these parameters remaine within the normal ranges in the treated group.

Quinoxaline 1,4-di-N-oxide derivatives seem to have very interesting anticancer activity**[49]**, especially in solid tumor**(50)**. Quinoxaline derivatives may act by theconcept of bioreductive alkylation and could cleave theDNA under hypoxic conditions in the presence of xanthineand xanthine oxidase **[51].**

In the synthesis and biological evaluation of new agents derived from quinoxaline 1,4-di-N-oxide and related compounds, have been proved to be efficient cytotoxic agents for hypoxic cells in solid tumors. The poor tumor vascular structure and the inefficient blood supply along with a high interstitial pressure, generate a variable increases in reactive oxygen species and promotes leukocyte proportion of viable hypoxic cells in solid tumors which is one of the causes of cell resistance to anticancer treatments. Systemic hypoxia-endothelial adherence via reactive oxidant generation has been prevented by antioxidants. **[8].**

In the present study, the tumor volume is highly significant decreased in the infected mice treated with **NBPQD** (426.0±161.0) compared to the infected mice treated with saline (2252.4±526.43). It can been seen that, there is 81.0% reduction in tumor volume in solid tumorized mice (right leg) treated with (**NBPQD**) compared to solid tumorized mice saline treated.

These results agreeing with the result that obtained by **Brown, and Koong, (1999) [9], and Ibrahim, and Wally, (2009) [10]** who reported that quinoxaline-1,4- dioxide derivatives seem to have interestinganticanceractivitiesespecially in solid tumortreatment**.**

Also, the **NBPQD** treated animals at the doses of **16 mg/kg/day** brought back the hematological parameters to more or less normal levels. Also, restored the hepatic lipid peroxidation and free radical scavenging enzyme GSH as well as antioxidant enzymes such as SOD and GSH-R in tumor bearing mice to near the normal levels.

It can be concluded that, **NBPQD** compound was biodistributed in different organs of the EAC bearing mice , (right thigh). So NBPQD observed high effect as antitumor in solid model in mice infected with EAC-cells.

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