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Original Article

The Hepatoprotection Provided by Taurine and Glycine against Antineoplastic Drugs Induced Liver Injury in an *Ex Vivo* Model of Normothermic Recirculating Isolated Perfused Rat Liver

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Abstract

Taurine (2-aminoethane sulfonic acid) is a non-protein amino acid found in high concentration in different tissues. Glycine (Amino acetic acid) is the simplest amino acid incorporated in the structure of proteins. Several investigations indicate the hepatoprotective properties of these amino acids. On the other hand, antineoplastic agents-induced serum transaminase elevation and liver injury is a clinical complication. The current investigation was designed to screen the possible hepatoprotective properties of taurine and glycine against antineoplastic drugs-induced hepatic injury in an ex vivo model of isolated perfused rat liver. Rat liver was perfused with different concentration (10 µM, 100 µM and 1000 µM) of antineoplastic drugs (Mitoxantrone, Cyclophosphamide, Cisplatin, 5 Fluorouracil, Doxorubicin and Dacarbazine) via portal vein. Taurine and glycine were administered to drug-treated livers and liver perfusate samples were collected for biochemical measurements (ALT, LDH, AST, and K^+). Markers of oxidative stress (reactive oxygen species formation, lipid peroxidation, total antioxidant capacity and glutathione) were also assessed in liver tissue. Antineoplastic drugs caused significant pathological changes in perfusate biochemistry. Furthermore, markers of oxidative stress were significantly elevated in drug treated livers. It was found that taurine (5 and 10 mM) and glycine (5 and 10 mM) administration significantly mitigated the biomarkers of liver injury and attenuated drug induced oxidative stress. Our data indicate that taurine and glycine supplementation might help as potential therapeutic options to encounter anticancer drugs-induced liver injury.
Keywords: Amino acid, Chemotherapy, Cancer, Drug-Induced Liver Injury (DILI), Hepatoprotection, Hepatotoxicity.

1. Introduction

Drug-induced liver injury (DILI) is a clinical complication associated with many pharmaceuticals (1). Chemotherapeutic agents administered to treat different malignancies in humans are among the most cytotoxic drugs (2-5). These drugs are also used as animal models of liver injury (6-8). Elevated serum transaminases is a common event ensued anticancer drugs administration (4, 9-11). On the other hand, several cases of anticancer drugs-induced liver injury have been reported (12-15). Chemotherapy-induced hepatotoxicity might lead to hepatic failure and patients' death (14, 16, 17). Hence, finding hepatoprotective molecules with a safe profile of administration has clinical benefits.

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Taurine is one of the most abundant amino acid in the human body (18). Many physiological properties are attributed to this amino acid (19). Cell volume regulation, membrane stabilization, and antioxidant properties are important roles attributed to taurine in different biological systems (19-21). Taurine also showed several pharmacological properties including anti-emesis, gastroprotective, antiepileptic, and anti-inflammatory (22-26). On the other hand, taurine showed several beneficial properties in the liver and hepatocytes (27, 28). It has been reported that taurine administration could ameliorate xenobiotics-induced liver injury (29-42). Moreover, daily taurine intake in humans is high in some world regions and risk assessment investigation revealed that taurine is a very safe amino acid even at very high doses (43-45).

Glycine is the simplest amino acid incorporated in the structure of body proteins. Previous investigations revealed several beneficial effects of glycine in liver and hepatocytes (46-48), as well as other organs (49-52). It has been shown that this amino acid effectively mitigated hypoxia-induced liver injury and counteracted xenobiotics-induced hepatic damage (53, 54).

Isolated organs are intriguing animal models for screening adverse drug reactions (ADRs) and the potential therapeutic strategies against this complication (55). The isolated perfused liver is a useful and efficient model for investigating xenobiotics-induced liver injury (55-57). The isolated liver is useful for examining xenobiotics-induced liver injury without the complication of many interacting factors which are difficult to control in other experimental models (56, 57).

The current investigation aimed to evaluate and screen the potential protective properties of taurine and glycine against anticancer drugs-induced liver injury in an *ex vivo* model of isolated perfused rat liver.

Table 1. Concentration-response of the investigated antineoplastic agents in isolated perfused rat liver system.

	Markers assessed in liver perfusate			
Treatment	**LDH (U/l)**	**AST (U/l)**	**ALT (U/l)**	**K^+ (mmol/dl)**
Control (Only buffer)	17± 3	13±1	9±3	5.71±0.58
Mitoxantrone 10 µM	26±5	29±11	15±3	4.78±0.22
Mitoxantrone 100 µM	30±7	44±14*	43±3*	6.44±0.13
Mitoxantrone 1000 µM	190±15*	107±25*	60±8*	8.92±0.29*
Cyclophosphamide 10 µM	25±2	35±3*	14±4	5.38±0.12
Cyclophosphamide 100 µM	71±5*	39±5*	18±5	5.86±0.24
Cyclophosphamide 1000 µM	804±92*	102±5*	159±17*	7.88±0.42*
Cisplatin 10 µM	37±3*	53±12*	23±3*	7.22±0.34*
Cisplatin 100 µM	132±14*	149±22*	143±12*	8.65±0.49*
Cisplatin 1000 µM	153±21*	199±14*	269±30*	9.02±0.57*
Dacarbazine 10 µM	24±10	39±9*	11±1	4.65±0.13
Dacarbazine 100 µM	49±7*	29±11	12±1	5.43±0.093
Dacarbazine 1000 µM	670±100*	245±72*	132±30*	8.79±0.14*
5-FU 10 µM	24±6	13±4	15±2	5.02±0.074
5-FU 100 µM	29±4	27±12	19±4	5.21±0.31
5-FU 1000 µM	519±41*	243±74*	34±4*	7.69±0.22*
Doxorubicin 10 µM	26±10	25±2	15±3	6.34±0.62
Doxorubicin 100 µM	151±11*	98±14*	31±7*	7.44±0.049*
Doxorubicin 1000 µM	380±13*	144±40*	48±5*	8.36±0.17*

Data are represented as Mean±SD (n=6) as assessed 120 minutes after liver perfusion. *Indicates significantly higher as compared to control (only buffer group) (P<0.05).

2. Material and Methods

2.1. Chemicals

5,5′ dithionitrobenzoic acid (DTNB), 2-aminoethane sulfonic acid (Taurine), 2, 4, 6-tripyridyl-s-triazine (TPTZ), and 2′,7′dichlorofluorescein diacetate (DCF-DA), were purchased from Sigma-Aldrich (St. Louis, USA). Thiobarbituric acid (TBA), Trichloroacetic acid (TCA), Ferric chloride hexahydrate, Glycine, n-butanol and hydroxy methyl amino methane (Tris), were purchased from Merck (Dardamstd, Germany). The kits for liver biochemistry analysis (ALT, AST and LDH) were obtained from Pars Azmun® Company (Tehran, Iran). All salts for preparing buffer solutions were of the highest grade commercially available and prepared from Merck (Dardamstd, Germany).

2.2. Experimental setup

2.2.1. Isolated perfused rat liver preparation

Male Sprague-Dawley rats (200-300 g, n=104) were purchased from the Laboratory Animals Breeding Center of Shiraz University of Medical Sciences and allowed free access to food and tap water. The animals were handled and used according to the animal handling protocol approved by the ethics committee of Faculty of Pharmacy, Shiraz University of Medical Sciences, Shiraz, Iran. Animals were anesthetized with thiopental (70 mg/kg, i.p.). Rats liver were cannulated and perfused via portal vein (57,58), with hemoglobin- and albumin-free Krebs Henseleit buffer (pH=7.4, 37 °C) gassed with carbogen (95% O_2, 5% CO_2). The perfusate was pumped through the liver with a peristaltic pump at a constant flow rate of 3 mL/min/g liver weight, in a re-circulating mode. The perfusate buffer volume was 200 mL in all experiments.

2.2.2. Study procedure

Isolated rat liver was exposed to different concentrations of investigated antineoplastic

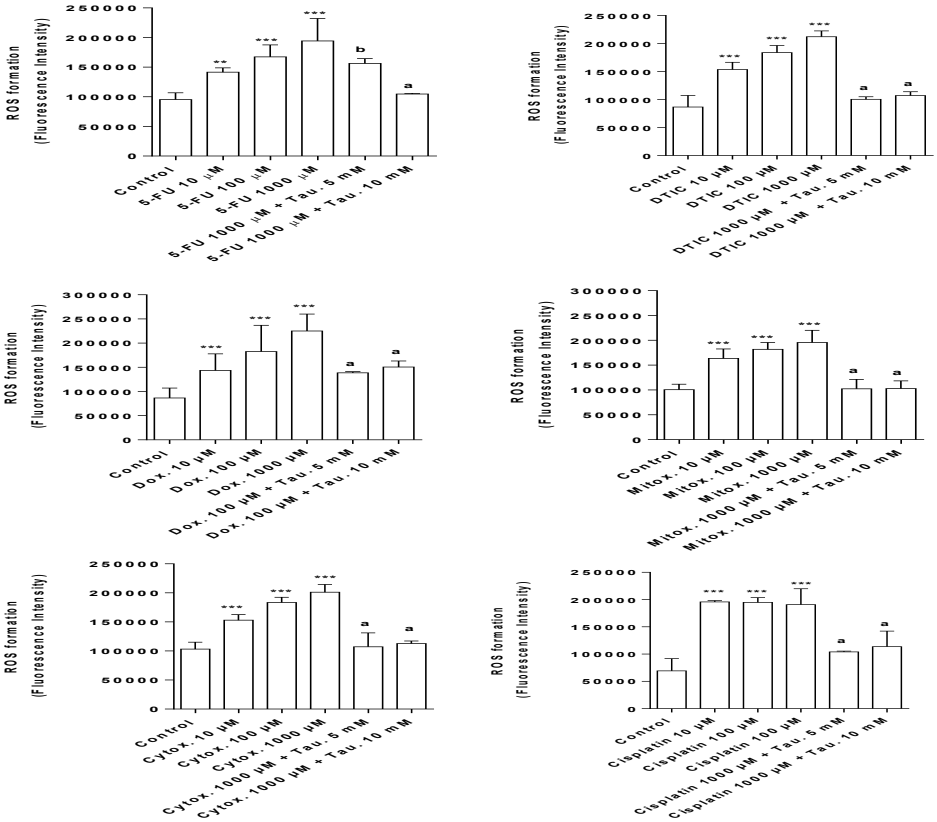


Figure 1. Reactive oxygen species (ROS) formation in the isolated perfused rat liver and effect of taurine treatment. Mitox. Mitoxantrone. Cytox: Cytoxan, Cyclophosphamide. DTIC: Dacarbazine. 5-FU: 5-Fluorouracil. Dox. Doxorubicin. Tau: Taurine. Data are given as Mean±SD (n=6). Asterisks indicate significantly different as compared to control group (**P<0.01, ***P<0.001). a Indicates significantly different as compared to antineoplastic drug-treated group (P<0.001). b Indicates significantly different as compared to antineoplastic drug-treated group (P<0.01).

drugs, taurine, and glycine for 180 minutes of organ perfusion. Hepatic injury was determined at scheduled time intervals (every 30 minutes) to evaluate the effects of the various concentrations of drugs on the liver. The injurious concentration of a drug was reported as a concentration value, which lead to a significant rise in all assessed biomarkers of liver injury after 120 minutes of organ perfusion. Samples were taken from liver perfusate at different times and assessed for biomarkers of liver injury. At the end of each experiment (180 minutes), liver samples were used to assess tissue lipid peroxidation, glutathione content, ROS formation, and total antioxidant capacity. Taurine (5 and 10 mM) and glycine (5 and 10 mM) caused no significant changes in the liver injury biomark-

Table 2. Perfusate LDH level after drug administration to isolated rat liver and the role of taurine administration.

	Perfusate LDH Level (U/l)					
	Time (minute)					
	30	**60**	**90**	**120**	**150**	**180**
Control (Only buffer)	1±0.3	5±2	10±2	15±3	21±3	29±2
Mitox. 1000 µM	34±2*	78±8*	107±19*	190±15*	260±27*	326±54*
+Taurine 5 mM	2±0.5 a	6±1a	8±2a	21±8a	33±7a	41±6a
+Taurine 10 mM	2±0.6 a	8±3a	11±2a	17±6a	27±4a	47±3a
+Glycine 5 mM	3±0.4a	11±3a	14±5a	21±2a	26±7a	31±4a
+Glycine 10 mM	2±0.6a	10±2a	15±6a	22±7a	35±4a	46±5a
Cytox. 1000 µM	91±9*	211±36*	311±15*	804±92*	1198±98*	1125±174*
+Taurine 5 mM	27±6a	65±8a	163±27a	253±34a	489±86a	762±64a
+Taurine 10 mM	15±4v	74±12 a	132±21a	304±22a	387±63a	646±75a
+Glycine 5 mM	25±7a	89±31 a	183±26a	452±74a	577±44a	802±93a
+Glycine 10 mM	30±5a	104±21 a	215±32a	571±45a	628±57a	832±61a
Cisplatin 1000 µM	42±3*	57±6*	90±11*	153±21*	215±32*	297±25*
+Taurine 5 mM	11±2a	23±5 a	46±11a	77±9a	107±11a	122±11a
+Taurine 10 mM	13±2a	26±8 a	39±10a	51±7a	84±12a	109±21a
+Glycine 5 mM	15±4a	31±12 a	45±12a	66±9a	92±14a	132±13a
+Glycine 10 mM	11±3a	22±7 a	63±14a	78±6a	114±17a	148±14a
DTIC 1000 µM	41±4*	300±57*	495±74*	670±99*	1800±304*	2017±372*
+Taurine 5 mM	8±2a	21±4 a	43±5a	58±7a	69±11a	149±17a
+Taurine 10 mM	14±2a	30±9 a	39±11a	77±13a	93±15a	171±21a
+Glycine 5 mM	14±3a	17±3 a	29±10a	33±14a	35±9a	98±16a
+Glycine 10 mM	12±3a	21±4 a	23±7a	47±11a	77±21a	102±29a
5-FU 1000 µM	143±24*	228±14*	415±27*	519±41*	679±53*	789±101*
+Taurine 5 mM	41±3a	55±4 a	76±11a	131±17a	244±22a	322±34a
+Taurine 10 mM	43±11a	48±14 a	61±12a	99±12a	127±16a	168±13a
+Glycine 5 mM	13±3a	22±9 a	27±3a	48±11a	93±23a	182±22a
+Glycine 10 mM	17±4a	23±4 a	29±11a	62±22a	88±21a	134±34a
Dox. 100 µM	48±13*	172±7*	235±11*	380±13*	441±67*	576±69*
+Taurine 5 mM	12±3a	23±3 a	29±12a	77±4a	98±7a	141±18a
+Taurine 10 mM	11±2a	15±4 a	21±4a	34±11a	45±3a	107±13a
+Glycine 5 mM	7±3a	9±2 a	13±4a	24±4a	38±11a	51±9a
+Glycine 10 mM	11±2a	16±2 a	21±6a	36±2a	44±13a	62±19a

Data are given as Mean±SD (n=6). Mitox. Mitoxantrone. Cytox: Cytoxan, Cyclophosphamide. DTIC: Dacarbazine. 5-FU: 5-Fluorouracil. Dox. Doxorubicin. *Indicates significantly higher as compared to control (only buffer group) (P<0.05). aIndicates significantly lower as compared with mitoxantrone-treated liver (P<0.05).

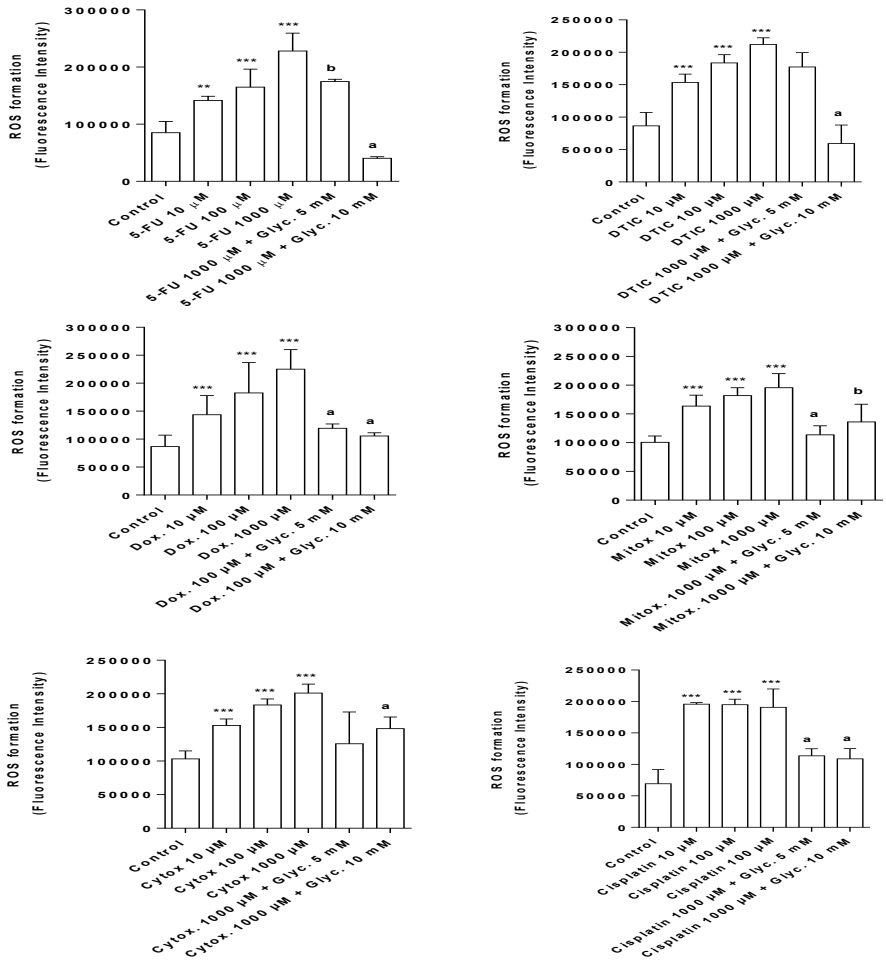


Figure 2. Effect of glycine administration on reactive oxygen species (ROS) formation in isolated perfused rat liver. Mitox. Mitoxantrone. Cytox: Cytoxan, Cyclophosphamide. DTIC: Dacarbazine. 5-FU: 5-Fluoro Uracil. Dox. Doxorubicin. Glyc: Glycine. Data are given as Mean±SD (n=6). Asterisks indicate significantly different as compared to control group (**P<0.01, *** P<0.001). a Indicates significantly different as compared to antineoplastic drug-treated group (P<0.001). b Indicates significantly different as compared to antineoplastic drug-treated group (P<0.01).

ers when they were administered alone at the mentioned concentrations in the current investigation.

2.3. Reactive oxygen species (ROS) formation in liver tissue

ROS level in liver tissue was estimated by a method described by Gupta *et al* (59), with some modifications. Briefly, at the end of each experiment (after 180 minutes of organ perfusion), liver samples (200 mg) were homogenized in ice-cold Tris-HCl buffer (40 mM, pH=7.4) (1:10 w/v). Then, 100 µL of tissue homogenate was mixed with 1 mL of Tris-HCl buffer (40 mM, pH=7.4) and 5 µL of 2′, 7′ dichlorofluorescein diacetate (Final concentration of 10 µM). The mixture was incubated for 30 minutes in 37 °C. Finally, the fluorescence intensity of the samples were assessed using a FLUOstar Omega® multifunctional microplate reader ($\lambda_{excitation}$=485 nm and $\lambda_{emission}$=525 nm) (60, 61).

2.4. Liver glutathione content

The glutathione contents of the liver were assessed using the Ellman reagent (DTNB) (62). Briefly, tissue samples (200 mg) were homogenized in 8 ml of ice-cooled EDTA solution (0.02 M). Then, 5 mL of liver homogenate was mixed with 4 mL of distilled water and 1 mL of 50% trichloroacetic acid (TCA). The mixture was vortexed and centrifuged (765 g, 15 minutes, 4 °C) (63, 64). Then, 2 mL of supernatant was added to 4 mL of Tris buffer (pH= 8.9) and 100 µl of DTNB solution (0.01 M in methanol) (62). The absorbance

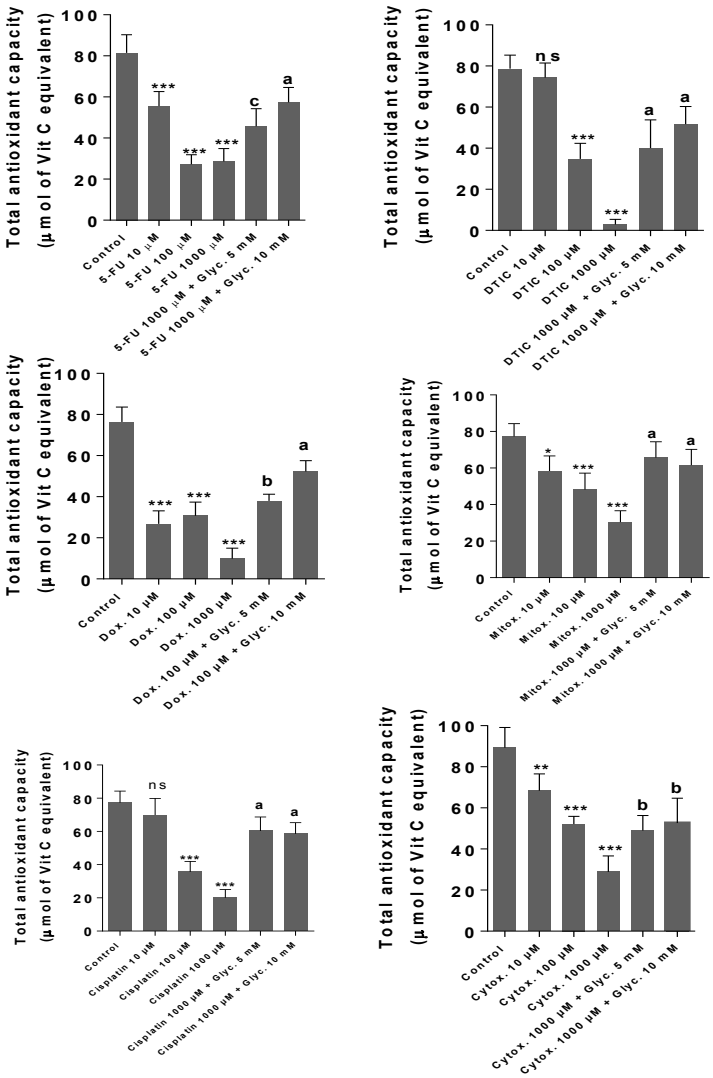


Figure 3. Effect of taurine on the total antioxidant capacity of isolated perfused liver. Cytox: Cytoxan, Cyclophosphamide. DTIC: Dacarbazine. 5-FU: 5-Fluorouracil. Dox. Doxorubicin. Tau: Taurine. Data are shown as Mean±SD (n=6). Asterisks indicate significantly different as compared to control group (***P*<0.01, ****P*<0.001). a Indicates significantly different as compared to antineoplastic drug-treated group (*P*<0.001). b Indicates significantly different as compared to antineoplastic drug-treated group (*P*<0.01). c Indicates significantly different as compared to antineoplastic drug-treated group (*P*<0.05). ns: not significant as compared to control (*P*>0.05).

of developed yellow color was read at 412 nm using an Ultrospec 2000®UV spectrophotometer.

2.5. Lipid peroxidation

The level of lipid peroxidation in the isolated perfused liver was assessed by thiobarbituric acid reactive substances (TBARS) test (66). The reaction mixture consisted of 0.5 mL of 10% liver homogenate, 3 ml phosphoric acid 1% (w/v) and 1 mL of 1% (w/v) thiobarbituric acid (TBA) (63, 64, 67). The mixture was vortexed and then heated in boiling water (100 ºC) for 45 minutes. Afterward, 4 mL of n-butanol was added to reaction mixture and vigorously mixed. After centrifugation (765 g, 5 min), the absorbance of developed color in n butanol phase was read at 532 nm using an Ultrospec 2000®UV spectrophotometer (66).

2.6. Total antioxidant capacity of liver

The ferric reducing antioxidant power (FRAP) of liver tissue was assessed in each experimental group. The working FRAP reagent was

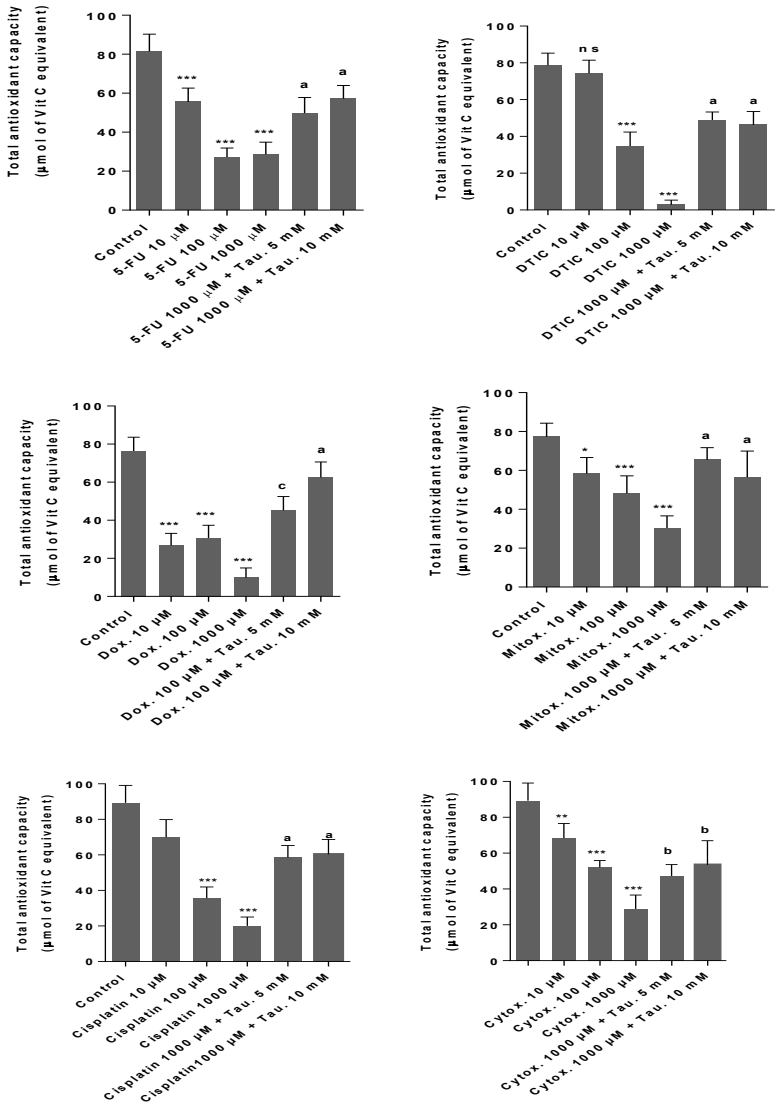


Figure 4. Effect of glycine administration on the total antioxidant capacity of isolated perfused rat liver. Cytox: Cytoxan, Cyclophosphamide. DTIC: Dacarbazine. 5-FU: 5-Fluorouracil. Dox. Doxorubicin. Tau: Taurine. Data are represented as Mean±SD (n=6). Asterisks indicate significantly different as compared to control group (**P<0.01, *** P<0.001). a Indicates significantly different as compared to antineoplastic drug-treated group (P<0.001). b Indicates significantly different as compared to antineoplastic drug-treated group (P<0.01). c Indicates significantly different as compared to antineoplastic drug-treated group P<0.05). ns: not statistically significant as compared to control (Only buffer group)(P>0.05).

prepared by mixing 10 volumes of 300 mmol/L acetate buffer, pH 3.6, with 1 volume of 10 mmol/L TPTZ (2, 4, 6-tripyridyl-s-triazine, in 40 mmol/L hydrochloric acid) and with 1 volume of 20 mmol/L ferric chloride. All solutions were used on the day of the experiment. Liver tissue was homogenized in cooled Tris buffer (0.25M, containing 0.2M sucrose and 5mM DTT, pH 7.4). Then, 50 µL of tissue homogenate and 150 µL of deionized water was added to 1.5 mL of the FRAP reagent. The reaction mixture was incubated at 37 ºC for 5 minutes. Finally, samples were centrifuged (1000 g, 1 min) and the absorbance of developed color in the supernatant was measured at 595 nm by an Ultrospec2000® spectrophotometer (68).

2.7. Perfusate biochemistry

A Mindray BS-200® auto analyzer and standard kits were employed to assess liver perfusate level of alanine aminotransferase (ALT), aspar-

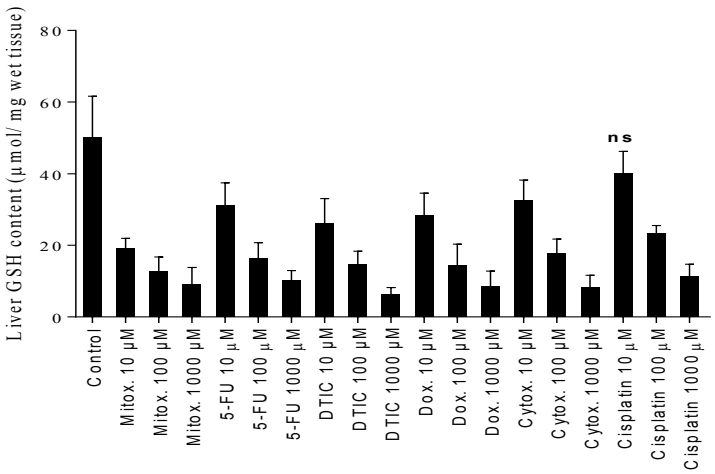


Figure 5. Effect of antineoplastic agents on hepatic glutathione content.
Data are given as Mean±SD (n=6). Mitox. Mitoxantrone. Cytox: Cytoxan, Cyclophosphamide. DTIC: Dacarbazine. 5-FU: 5-fluoro uracil. Dox. Doxorubicin. All antineoplastic agents significantly depleted hepatic glutathione content at mentioned doses (P<0.001). ns: not significant as compared to control (P>0.05).

tate aminotransferase (AST), and lactate dehydrogenase (LDH) (69). Perfusate potassium ion (K^+) level was measured using a flame photometer.

2.8. Statistical analysis

Data are given as the Mean±SD. Commercially available software GraphPad Prism (GraphPad Prism 6 for Windows, version 6.01) was used for statistical evaluation. Data comparison was performed by the one-way analysis of variance (ANOVA) with Tukey's multiple comparison test as a post hoc. Differences were considered statistically significant when P<0.05.

3. Results

Isolated rat liver was perfused with different concentrations of anticancer drugs (Table 1). It was found that cisplatin; cyclophosphamide, mitoxantrone, dacarbazine and 5-FU caused a significant elevation in all assessed biomarkers of liver injury in the concentration of 1000 µM, after 120 minutes of liver perfusion (Table 1). Doxorubicin caused significant changes in biomarkers of liver injury at the concentration of 100 µM (Table 1).

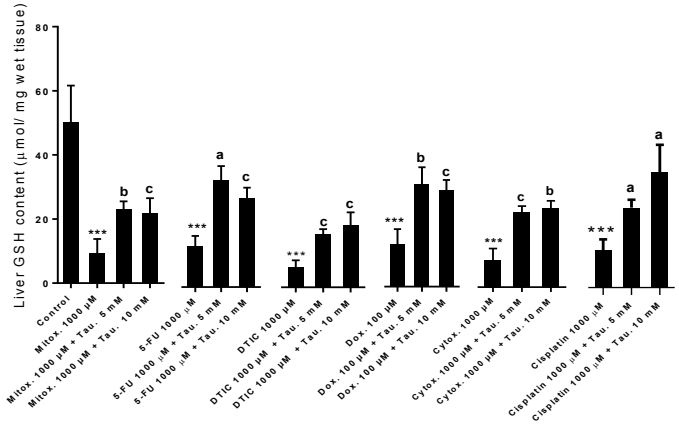


Figure 6. Effect of taurine administration on the liver glutathione content of antineoplastic treated groups. Data are given as Mean±SD (n=6). Mitox. Mitoxantrone. Cytox: Cytoxan, Cyclophosphamide. DTIC: Dacarbazine. 5-FU: 5-fluoro uracil. Dox. Doxorubicin. ***Indicates significantly different as compared to control (only buffer group) (P<0.001). a Indicates significantly lower as compared with drug-treated liver (P<0.001). b Indicates significantly different from drug-treated group (P<0.01). c Indicates significantly different from drug-treated group (P<0.05). ns: no significant difference as compared to drug-treated group (P>0.05).

Table 3. Liver perfusate ALT level and the role of glycine and taurine administration.

Liver Perfusate ALT Level (U/l)						
Time (minute):	**30**	**60**	**90**	**120**	**150**	**180**
Control (Only buffer)	3±1	5±1	7±1	10±3	18±2	24±3
Mitox. 1000 µM	17±2*	15±4*	42±7*	60±8*	76±9*	91±13*
+Taurine 5 mM	4±1[a]	7±1	17±4[a]	19±6[a]	28±4[a]	32±2[a]
+Taurine 10 mM	3±1[a]	6±2	15±3[a]	22±1[a]	34±6[a]	39±7[a]
+Glycine 5 mM	3±1[a]	8±2	19±3[a]	34±4[a]	46±4[a]	55±6[a]
+Glycine 10 mM	4±1[a]	7±2	11±4[a]	19±4[a]	25±6[a]	41±6[a]
Cytox. 1000 µM	18±3*	25±3*	52±5*	159±17*	375±26*	439±22*
+Taurine 5 mM	4±1[a]	7±2[a]	19±3[a]	77±11[a]	155±23[a]	209±24[a]
+Taurine 10 mM	5±1[a]	8±3[a]	11±4[a]	89±12[a]	211±27[a]	247±22[a]
+Glycine 5 mM	3±1[a]	11±2[a]	27±2[a]	76±6[a]	253±31[a]	294±17[a]
+Glycine 10 mM	3±1[a]	17±4[a]	22±7[a]	63±11[a]	206±22[a]	247±39[a]
Cisplatin 1000 µM	23±2*	78±17*	177±22*	269±30*	308±22*	377±29*
+Taurine 5 mM	4±1[a]	12±3[a]	19±4[a]	56±12[a]	78±9[a]	109±17[a]
+Taurine 10 mM	3±1[a]	16±5[a]	25±3[a]	44±9[a]	57±11[a]	77±15[a]
+Glycine 5 mM	3±1[a]	11±4[a]	37±11[a]	76±14[a]	124±22[a]	177±19[a]
+Glycine 10 mM	5±1[a]	19±6[a]	49±11[a]	72±15[a]	147±11[a]	169±29[a]
DTIC 1000 µM	35±4*	85±14*	106±32*	132±29*	177±12*	217±5*
+Taurine 5 mM	22±2[a]	39±3[a]	67±11[a]	79±17[a]	83±4[a]	101±16[a]
+Taurine 10 mM	14±3[a]	55±6[a]	67±7[a]	79±11[a]	88±9[a]	111±4[a]
+Glycine 5 mM	21±2[a]	33±4[a]	38±4[a]	41±7[a]	76±10[a]	97±11[a]
+Glycine 10 mM	17±3[a]	22±2[a]	34±3[a]	39±3[a]	45±5[a]	51±12[a]
5-FU 1000 µM	10±1*	29±4*	39±4*	34±4*	44±7*	52±7*
+Taurine 5 mM	7±2[a]	11±1[a]	13±1[a]	17±3[a]	23±2[a]	34±4[a]
+Taurine 10 mM	3±1[a]	10±2[a]	11±2[a]	16±2[a]	21±4[a]	26±2[a]
+Glycine 5 mM	5±1	7±1[a]	13±3[a]	14±5[a]	21±3[a]	39±3[a]
+Glycine 10 mM	7±2	11±3[a]	16±5[a]	19±2[a]	24±3[a]	33±3[a]
Dox. 100 µM	7±3*	15±5*	23±7*	31±8*	37±9*	42±14*
+Taurine 5 mM	2±0.6	5±2[a]	7±3[a]	12±3[a]	14±3[a]	20±3[a]
+Taurine 10 mM	1±0.3[a]	3±1[a]	4±1[a]	11±3[a]	13±2[a]	22±2[a]
+Glycine 5 mM	3±1	5±1[a]	7±1[a]	7±1[a]	14±3[a]	17±3[a]
+Glycine 10 mM	6±2	4±1[a]	8±2[a]	10±1[a]	11±1[a]	21±2[a]

Data are given as Mean±SD for six independent experiments. Mitox. Mitoxantrone. Cytox: Cytoxan, Cyclophosphamide. DTIC: Dacarbazine. 5-FU: 5-Fluorouracil. Dox. Doxorubicin. *Indicates significantly higher as compared to control (only buffer group) ($P<0.05$). [a]Indicates significantly lower as compared with antineoplastic drugs-treated liver ($P<0.05$).

The injurious concentrations of anticancer drugs in the current *ex vivo* system were selected for further experiments.

All investigated drugs caused significant LDH leakage from the liver at different time intervals (Table 2). It was found that taurine (5 mM and 10 mM) and glycine (5 mM and 10 mM) significantly ameliorated drugs-induced organ LDH release (Table 2). Perfusate level of ALT was also significantly higher in anticancer drugs-treated liver at different time points (Table 3). Taurine (5 and 10 mM) and glycine (5 and 10 mM) administration significantly mitigated antineoplastic drugs-induced ALT release in isolated rat liver (Table 3). An elevated perfusate AST level was detected when rat liver was treated with antineo-

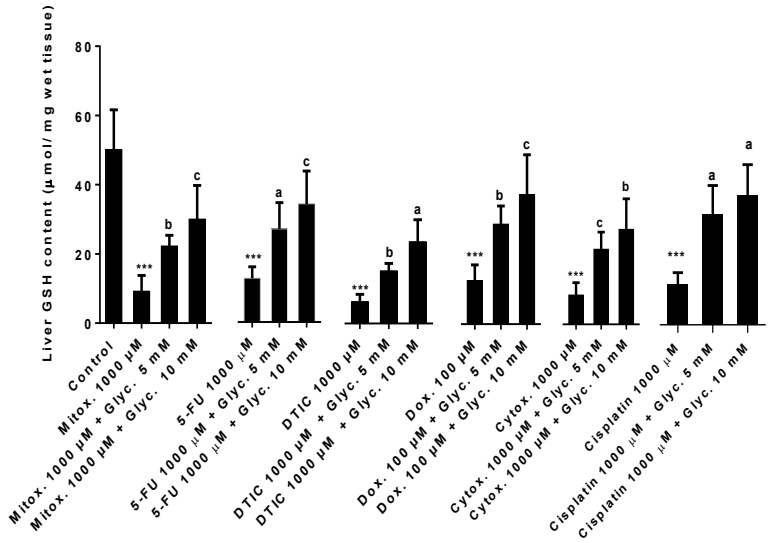


Figure 7. Effect of glycine administration on liver glutathione content of antineoplastic-treated groups. Data are given as Mean±SD (n=6). Mitox. Mitoxantrone. Cytox: Cytoxan, Cyclophosphamide. DTIC: Dacarbazine. 5-FU: 5-fluoro uracil. Dox. Doxorubicin. ***Indicates significantly different as compared to control (only buffer group) (P<0.001). a Indicates significantly lower as compared with drug-treated liver (P<0.001). b Indicates significantly different from drug-treated group (P<0.01). c Indicates significantly different from drug-treated group (P<0.01). ns: no significant difference as compared to drug-treated group (P>0.05).

plastic drugs (Table 4). It was found that taurine and/or glycine effectively ameliorated anticancer-induced AST release in different groups (Table 4).

A significant amount of K^+ ion was released to the liver perfusate when the liver was treated with different concentrations of antineoplastic drugs (Table 5). Taurine (5 and 10 mM) and glycine (5 and 10 mM) significantly prevented antineoplastics-induced K^+ release to liver perfusate (Table 5).

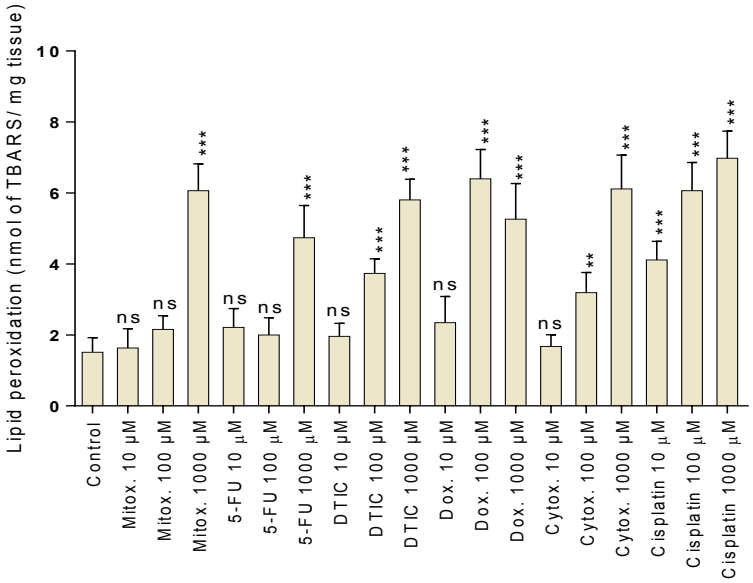


Figure 8. Effect of antineoplastic agents on liver lipid peroxidation. Data are given as Mean±SD (n=6). Mitox. Mitoxantrone. Cytox: Cytoxan, Cyclophosphamide. DTIC: Dacarbazine. 5-FU: 5-fluoro uracil. Dox. Doxorubicin. ***Indicates significantly different as compared to control (only buffer group) (P<0.001). **Indicates significantly different as compared to control (only buffer group) (P<0.01). ns: no significant difference as compared to control (only buffer group) (P>0.05).

Table 4. Perfusate AST level in isolated rat liver model treated with antineoplastic drugs, taurine and glycine.

Liver Perfusate AST level (U/l)						
Time (minute):	**30**	**60**	**90**	**120**	**150**	**180**
Control (Only buffer)	5±1	9±3	9±1	13±2	27±3	37±5
Mitox. 1000 µM	14±4	50±12*	74±18*	107±25*	145±36*	183±45*
+Taurine 5 mM	2±0.6[a]	11±4[a]	17±8[a]	26±3[a]	34±9[a]	51±6[a]
+Taurine 10 mM	4±1	9±2[a]	13±2[a]	26±7[a]	39±4[a]	45±4[a]
+Glycine 5 mM	2±0.3[a]	6±1[a]	21±7[a]	35±4[a]	51±3[a]	64±7[a]
+Glycine 10 mM	3±1[a]	4±1[a]	17±3[a]	23±6	37±4[a]	45±3[a]
Cytox. 1000 µM	17±1*	27±6*	48±8*	102±5*	206±17*	270±14*
+Taurine 5 mM	6±2[a]	11±4[a]	21±6[a]	65±6[a]	121±17[a]	145±11[a]
+Taurine 10 mM	3±1[a]	9±4[a]	22±5[a]	37±11[a]	79±14[a]	123±9[a]
+Glycine 5 mM	7±2[a]	13±4[a]	38±13[a]	82±11	130±24[a]	162±21[a]
+Glycine 10 mM	4±1[a]	11±3[a]	33±9[a]	75±6[a]	133±31[a]	179±21[a]
Cisplatin 1000 µM	31±3*	64±7*	146±19*	199±14*	239±21*	257±11*
+Taurine 5 mM	9±2[a]	15±4[a]	21±6[a]	77±11[a]	94±6[a]	141±22[a]
+Taurine 10 mM	6±2[a]	11±2[a]	32±6[a]	65±4[a]	77±19[a]	104±11[a]
+Glycine 5 mM	7±2[a]	11±5[a]	21±4[a]	65±12[a]	83±12[a]	111±22[a]
+Glycine 10 mM	3±1[a]	12±4[a]	31±9[a]	46±11[a]	78±19[a]	141±34[a]
DTIC 1000 µM	95±3*	105±13*	150±42*	245±72*	311±89*	478±137*
+Taurine 5 mM	11±1[a]	20±1[a]	29±3[a]	37±4[a]	44±12[a]	56±15[a]
+Taurine 10 mM	12±2[a]	15±2[a]	32±3[a]	45±12[a]	71±9[a]	108±21[a]
+Glycine 5 mM	14±3[a]	17±3[a]	29±10[a]	33±14[a]	35±9[a]	98±16[a]
+Glycine 10 mM	12±3[a]	21±4[a]	23±7[a]	47±11[a]	77±21[a]	102±29[a]
5-FU 1000 µM	66±24*	126±47*	220±71*	243±74*	313±98*	427±117*
+Taurine 5 mM	21±11[a]	32±9[a]	39±9[a]	47±12[a]	78±22[a]	131±34[a]
+Taurine 10 mM	34±9[a]	55±17[a]	67±12[a]	109±34[a]	121±21[a]	174±29[a]
+Glycine 5 mM	13±3[a]	22±9[a]	27±3[a]	48±11[a]	93±23[a]	182±22[a]
+Glycine 10 mM	17±4[a]	23±4[a]	29±11[a]	62±22[a]	88±21[a]	134±34[a]
Dox. 100 µM	31±4*	63±9*	87±12*	98±14*	113±13*	130±16*
+Taurine 5 mM	11±4[a]	17±3[a]	23±3[a]	44±11[a]	51±4[a]	72±16[a]
+Taurine 5 mM	16±3[a]	19±2[a]	27±2[a]	39±10[a]	44±9[a]	91±21
+Glycine 5 mM	7±3[a]	9±2[a]	13±4[a]	24±4[a]	38±11[a]	51±9[a]
+Glycine 10 mM	11±2[a]	16±2[a]	21±6[a]	36±2[a]	44±13[a]	62±19[a]

Data are given as Mean±SD (n=6). Mitox. Mitoxantrone. Cytox: Cytoxan, Cyclophosphamide. DTIC: Dacarbazine. 5-FU: 5-Fluorouracil. Dox. Doxorubicin. *Indicates significantly higher as compared to control (only buffer group) (P<0.05). [a]Indicates significantly lower as compared with only anticancer agent treated isolated liver (P<0.05).

Reactive oxygen species (ROS) formation assessment in the liver tissue revealed a high level of ROS in drug-treated livers (Figure 1). Tissue ROS formation was significantly lowered when isolated livers were treated with taurine and/or glycine (Figure 1 and 2). The antioxidant capacity of liver tissue was significantly lower in drug-treated groups (Figure 3 and 4). On the other hand, the total antioxidant capacity of the liver tissue was significantly improved when rats liver were treated with taurine (5 mM and 10 mM) and/or glycine (5 mM and 10 mM) (Figure 3 and 4).

Liver glutathione content was lower in anticancer drugs-treated groups (Figure 5). Taurine and glycine administration significantly prevented anticancer drugs-induced liver gluta-

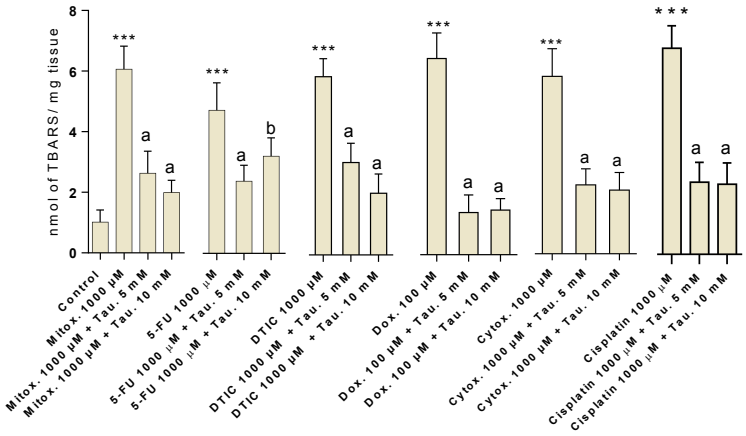


Figure 9. The role of taurine administration on antineoplastic agents-induced lipid peroxidation in the isolated perfused rat liver. Data are shown as Mean±SD (n=6). Mitox. Mitoxantrone. Cytox: Cytoxan, Cyclophosphamide. DTIC: Dacarbazine. 5-FU: 5-Fluorouracil. Dox. Doxorubicin. *Indicates significantly higher as compared to control (only buffer group) (P<0.05). a Indicates significantly lower as compared with anticancer drugs-treated liver (P<0.05).

thione depletion (Figure 6 and 7). A significant amount of thiobarbituric acid reactive substances (TBARS), as an index of tissue lipid peroxidation, were formed in antineoplastic drugs-treated perfused liver (Figure 8). Administration of taurine (5 and 10 mM) and glycine (5and 10 mM) to anticancer drugs-treated groups ameliorated lipid peroxidation of liver tissue (Figure 9 and 10).

4. Discussion

Chemotherapy-associated hepatotoxicity is a clinical complication. There is no safe and promising protective agent against chemotherapy drugs-induced liver injury. The current investigation aimed to screen the potential protective properties of the amino acids taurine and glycine against different commonly administered cancer chemotherapy drugs.

Taurine is present in the human body at high concentrations (69). The beneficial effects of taurine in liver and its protective properties have been shown in several previous investigations (34, 37, 39, 70-74). Several pharmacological effects including membrane stabilization and antioxidant effects are attributed to taurine (22, 75). The excessive reactive oxygen species and oxidative stress is believed to be involved in chemotherapy-induced hepatotoxicity (11, 14). Dacarbazine, cyclophosphamide, cisplatin, and mitoxantrone are

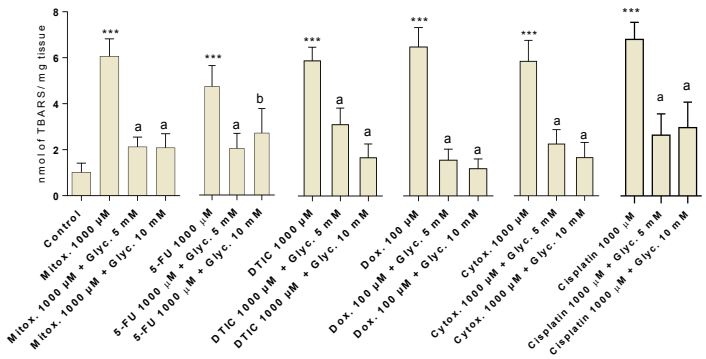


Figure 10. The effect of glycine administration on antineoplastic agents-induced lipid peroxidation in the isolated perfused rat liver. Data are given as Mean±SD (n=6). Mitox. Mitoxantrone. Cytox: Cytoxan, Cyclophosphamide. DTIC: Dacarbazine. 5-FU: 5-fluoro uracil. Dox. Doxorubicin. *Indicates significantly higher as compared to control (only buffer group) (P<0.05). a Indicates significantly lower as compared with drug-treated liver (P<0.05).

Table 5. Perfusate potassium (K^+) level.

	Liver Perfusate K^+ Level (mmol/dl)					
Time (minute):	**30**	**60**	**90**	**120**	**150**	**180**
Control (Only buffer)	4.35±0.25	5.08±0.38	5.64±0.11	5.71±0.58	5.94±0.10	6.08±0.21
Mitox. 1000 µM	5.31±0.16*	6.89±0.24*	7.94±0.13*	8.92±0.29*	9.35±0.16*	11.22±0.25*
+Taurine 5 mM	4.97±0.22	5.69±0.19[a]	6.33±0.07[a]	6.59±0.14[a]	7.08±0.11[a]	7.45±0.22[a]
+Taurine 10 mM	4.08±0.15[a]	4.33±0.11[a]	5.56±0.27[a]	6.04±0.19[a]	6.34±0.07[a]	6.89±0.12[a]
+Glycine 5 mM	4.56±0.17[a]	5.21±0.09[a]	5.76±0.10[a]	6.43±0.22[a]	7.89±0.34[a]	8.08±0.16[a]
+Glycine 10 mM	4.89±0.23	5.02±0.11[a]	6.34±0.08[a]	6.88±0.19[a]	7.39±0.33[a]	8.66±0.40[a]
Cytox. 1000 µM	5.96±0.28	6.44±0.63 *	7.29±11 *	7.88±0.42*	8.51±0.47*	9.40±0.23*
+Taurine 5 mM	4.28±0.26[a]	4.96±0.19[a]	5.78±0.45[a]	6.04±0.07[a]	6.23±0.31[a]	6.92±0.46[a]
+Taurine 10 mM	4.22±0.41[a]	4.67±0.22[a]	4.96±0.45[a]	5.11±0.37[a]	5.28±0.30[a]	6.02±0.12[a]
+Glycine 5 mM	5.28±0.19	5.57±0.28[a]	5.89±0.42[a]	6.05±0.20[a]	6.29±0.15[a]	6.74±0.27[a]
+Glycine 10 mM	4.76±0.21[a]	5.44±0.09[a]	5.65±0.12[a]	6.21±0.31[a]	7.02±0.44[a]	7.33±0.13[a]
Cisplatin 1000 µM	6.23±0.51*	8.07±0.35*	8.44±0.15*	9.02±0.57*	9.38±0.28*	10.04±0.19*
+Taurine 5 mM	4.89±0.32	5.33±0.64[a]	5.67±0.50[a]	6.28±0.22[a]	6.76±0.15[a]	7.44±0.35[a]
+Taurine 10 mM	4.52±0.09[a]	4.88±0.18[a]	5.63±0.06[a]	5.92±30[a]	6.27±0.35[a]	6.64±0.22[a]
+Glycine 5 mM	5.01±0.27[a]	5.32±0.16[a]	6.21±0.27[a]	6.89±0.33[a]	7.05 ± 0.49[a]	7.48±0.12[a]
+Glycine 10 mM	4.23±0.30[a]	4.47±0.22[a]	5.88±0.47[a]	6.34±0.28[a]	6.58±0.22[a]	7.09±0.17[a]
DTIC 1000 µM	6.20±0.22*	6.89±0.48*	7.47±0.33*	8.79±0.14*	9.24±0.11*	10.71±0.59*
+Taurine 5 mM	5.21±0.04[a]	5.77±0.10[a]	6.02±0.16[a]	6.59±0.30[a]	7.27±0.19[a]	8.06±0.50[a]
+Taurine 10 mM	5.02±0.12[a]	5.69±0.06[a]	6.34±0.10[a]	6.88±0.27[a]	7.45±0.20[a]	8.11±0.20[a]
+Glycine 5 mM	4.38±0.17[a]	5.22±0.11[a]	5.79±0.22[a]	6.04±0.49[a]	7.11±0.07[a]	7.56±0.15[a]
+Glycine 10 mM	4.77±0.34[a]	5.79±0.22[a]	5.90±0.17[a]	6.16±0.11[a]	6.33±0.21[a]	7.09±0.17[a]
5-FU 1000 µM	5.88±0.29*	6.92±0.55*	7.35±0.19*	7.69±0.22*	8.12±0.32*	8.96±0.40*
+Taurine 5 mM	4.92±0.15[a]	5.33±0.14[a]	5.40±0.36[a]	5.78±0.48[a]	6.15±0.04[a]	6.23±0.17[a]
+Taurine 10 mM	5.31±0.10[a]	5.76±0.22[a]	6.22±0.13[a]	6.70±0.10[a]	6.95±0.26[a]	7.14±0.20[a]
+Glycine 5 mM	5.24±0.31	5.41±0.07[a]	6.58±0.10[a]	7.34±0.11	7.58±0.05[a]	8.11±0.03[a]
+Glycine 10 mM	4.80±0.47	5.08±0.10[a]	5.44±0.51[a]	6.03±0.07[a]	7.68±0.29[a]	7.94±0.43[a]
Dox. 100 µM	4.71±0.25*	6.01±0.14*	6.39±0.61*	7.44±0.04*	8.39±0.08*	8.74±0.11*
+Taurine 5 mM	4.39±0.51	4.91±0.16[a]	5.18±0.12[a]	5.39±0.17[a]	6.00±0.67[a]	6.58±0.48[a]
+Taurine 10 mM	4.79±0.24	5.13±0.18[a]	5.01±0.04[a]	5.44±0.12[a]	5.90±0.30[a]	6.33±0.21[a]
+Glycine 5 mM	4.44±0.12	4.89±0.07[a]	5.20±0.11[a]	5.61±0.44[a]	6.12±0.02[a]	6.49±0.15[a]
+Glycine 10 mM	4.97±0.20	5.69±0.06[a]	6.77±0.05	7.34±0.23	7.21±0.10[a]	8.23±0.22

Data are given as Mean±SD for six independent experiments. Mitox. Mitoxantrone. Cytox: Cytoxan, Cyclophosphamide. DTIC: Dacarbazine. 5-FU: 5-Fluorouracil. Dox. Doxorubicin.

*Indicates significantly different as compared to control (only buffer) group ($P<0.05$).

[a]Indicates significantly lower as compared with antineoplastic drug-treated liver ($P<0.05$).

converted to reactive intermediates in liver which are capable to induce oxidative stress and cellular injury (76-80). As taurine serves as an antioxidant agent (81-84), a large part of its protective properties against chemotherapy-induced liver injury might be mediated through its effect of antioxidant enzymes and preventing biomembranes destruction (42, 85).

Potassium is the most abundant intracellular ion. As taurine and glycine prevented antineoplastic drugs induced increase in perfusate level of K^+, this might indicate their role in preserving cell membrane integrity.

It has been shown that glycine protected the liver from injury in various models (86-88). It has been reported that glycine ameliorated iri-

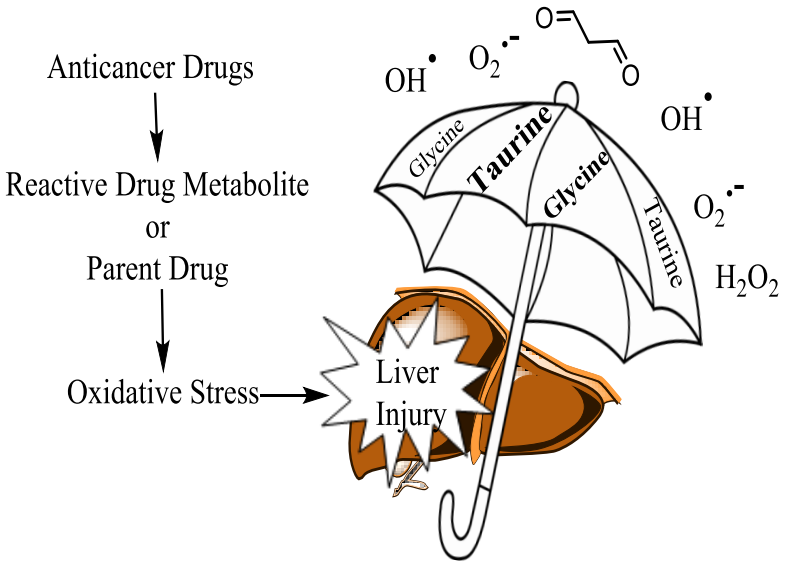
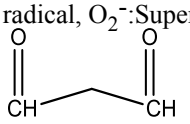


Figure 11. Taurine and glycine encounter xenobiotics-induced oxidative stress and protect the liver. OH :Hydroxyl radical, O_2^-:Superoxide anion, H_2O_2:Hydrogen peroxide.



: Malondialdehyde.

notecan, 5-FU and oxaliplatin adverse effects in an animal model (89). This indicates the potential protective effects of this amino acid against cancer chemotherapy adverse effects (89). We found that glycine not only ameliorated 5-FU adverse effects toward liver but also significantly mitigated other antineoplastic drugs-induced liver injury.

It has been shown that taurine administration not only caused no significant changes in the antineoplastic pharmacological effects, but also hasten the anticancer effects of some chemotherapy drugs (90). On the other hand, it has been reported that taurine level is significantly decreased in several tissues after cancer chemotherapy (91). As taurine serves as a protective agent in many tissues (18, 20), its depletion by chemotherapy (91), might sensitize different organs to injury. Some investigators indicated the beneficial effects of taurine against nausea and emesis as a common complication associated with cancer chemotherapy (23). These investigations indicate that taurine and glycine supplementation might help as potential safe adjuvant therapy to encounter anticancer drugs-induced tissue injury in addition of chemotherapy adverse effects in patients.

Some investigations indicated the beneficial role of other amino acids such as glutamine against chemotherapy toxicity (92). Although not evaluated in the current investigation, a cocktail of amino acids such as glycine, taurine and glutamine might be an effective option to protect the liver as well as other tissues during chemotherapy. Although some evidence suggest that taurine might potentiate anticancer effects of chemotherapy agents (90), further investigation, especially on tumor-bearing animal models, are required to exclude the fact that taurine and/or glycine may not affect the therapeutic efficacy of the antineoplastic drugs. In the absence of such investigations, despite the tremendous protective effects of these amino acids against chemotherapy-induced liver injury in the current investigation, the potential therapeutic efficacy of taurine and glycine as an adjuvant in cancer chemotherapy cannot be drawn.

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Conflict of Interest

None declared.

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