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Research Article

PHYSIOLOGICAL AND BIOCHEMICAL RESPONSES OF RAT STRESS MODELS TO NEUROTRANSMITTERS PRETREATMENT

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ABSTRACT
Pathophysiological changes associated with stress can be evaluated as indicator for therapeutic intervention. Due to their inhibitory versus excitatory effect, glycine and taurine versus L-arginine have been tested when administered prior to stress induction. Four groups of Wistar albino rats were stressed under the effect of aspirin, anoxic tolerance, writhing, tick infestation and surgical intervention. Liver and kidney functions, leukogram, hemogram, corticosterone, Gsh-PX, SOD, CAT, TNF-α, IL-2, IL-4 and IL-5 were estimated. ALT was significantly increased in writhing stressed rats. AST activities were elevated after surgically-induced stress and aspirin induced stress. Such hepatotoxic effect has been ameliorated on pretreatment with L-Arginine and taurine. TLC was markedly increased in rats stressed by tick infestation and glycine was effective in reducing such increase. Restored higher levels than control of Rbcs, PCV and blood indices were observed on pretreatments with glycine and taurine in tick infestation and surgically induced stress. The significant increases of Gsh-PX and SOD activities in stressed rats were restored near the values of control pretreatment with L-arginine and glycine. The same effect of L-Arginine and taurine was observed in alleviating CAT activities in aspirin induced stress model. The highest levels of TNF-α was under writhing stress has been effectively retained to normal with L-Arginine pretreatment. L-arginine and glycine pretreatment have decreased IL-4 in anoxic tolerance and writhing stressed animals. L-Arginine has the same effect in writhing stressed rats. The significant reduction of IL-2 and IL-5 under anoxia tolerance has been alleviated with L-arginine pretreatment. The results provide significant evidence of the medicinal use of neurotransmitters in stress disorders.

Keywords: *Stress, Neurotransmitters, Corticosterone, Rats, Gsh-PX, SOD, CAT, TNF-α, IL*

INTRODUCTION
Events induced by stress are complicated phenomena. Stress undoubtedly has become an integral part of human and animal lives. Stressful conditions have a derogative effect on normal physiological functions leading to a variety of disease states. Laboratory animals models were previously used to the study the neuro-immune-hormonal interactions and stress, and consequently bring lights our understanding of what stress is about.
In this regard, most studies have established animal models with behavioral psychopathology result, few have mentioned therapeutic intervention.
Neurotransmitters are substances synthesized, stored and released by presynaptic neurons that are involved in neuron-effector interaction with postsynaptic effect on target cells and must mimic the action of endogenous substance released from fired neuron (Richmond, 2007; Thomas and Starke, 2008).
Glycine is a nonessential amino acid and considered as inhibitory neurotransmitter in the central nervous system. It can stimulate glycine-gated chloride channels, leading to increased chloride influx that hyperpolarizes neuronal membranes and depresses excitatory signal transduction (Rajendra *et al.,* 1997). Glycine ameliorates kidney and liver injuries during endotoxin shock (Ikejima *et al.,* 1996), an effect that was due to the blocking of intracellular calcium signaling and the production of TNF-α in hepatic Kupffer cells via a glycine-gated chloride channel (Wheeler *et al.,* 1999). Glycine was mentioned previously with antioxidant effects (Mauriz *et al.,* 2001). Deters *et al.,* (1998) reported that glycine prevented damage induced in isolated perfused rat livers by some hepatotoxic agents. This protection was evaluated by the

reduction of lipid peroxidation (LPO) and the increase of GSH levels (Alcaraz-Contreras *et al.,* 2011; Ruiz-Ramirez *et al.,* 2014; Wang *et al.*, 2014).

Mikalauskas *et al.,* (2011) reported that glycine protect against chemotherapy-induced hepatotoxicity. In addition, glycine has been mentioned to protect against hypertension in a variety of experimental animal models (El Hafidi *et al.,* 2006).

Taurine is amino acids and considered as inhibitory neurotransmitter that crosses the blood-brain barrier (Salimäki *et al.,* 2003). Furthermore, taurine is important in preventing the harmful effects of excess glutamate and maintaining fluid balance (Hultman *et al.*, 2007). Taurine is prominent in many excitable tissues including the retina, brain, skeletal and cardiac muscles (Chen *et al.*, 2009). Physiological actions of taurine are widely involved in membrane stabilization, neuromodulation, regulation of calcium homeostasis, and antioxidation (Bouckenooghe *et al.*, 2006). In addition, taurine has protective effects in diabetic retinopathy (Yu *et al.*, 2008) and photochemical stress (Yu *et al.*, 2007). Taurine has also been shown to render neurons (Sun and Xu, 2008) and cardiomyocytes (Takatani *et al.*, 2004 a,b) resistant to an array of detrimental stimuli such as hypoxia and ischemia. Through scavenging ROS and attenuating lipid peroxidation, taurine stabilizes biological membrane, protect liver and heart in type I diabetic rats (Higuchi *et al.*, 2012; Wang *et al.*, 2013; Zhang *et al.*, 2014).

Results from the experiments of (Kong *et al.*, 2006) suggested that taurine produced an anxiolytic-like effect and neuro-protective function in animal models and might act as a modulator agent in the central nervous system. Wu and Prentice (2010) have shown that taurine can effectively prevent glutamate-induced neuronal injury in vitro. In addition, they have also demonstrated that taurine could protect against H2O2- induced cell injury in H2O2-induced stress (Pan *et al.*, 2010). It was generally believed that taurine's neuro protective functions were due to its role in reducing intracellular free Ca2+ concentration, and its anti-oxidative stress capacity (Schaffer *et al.*, 2009).

Koppelmann *et al.,* (2012) reported that Arginine is a non-essential amino acid processed metabolically by the urea cycle. It involved in multiple metabolic and biologic processes including release of several hormones, immune response, the regulation of inflammation, collagen synthesis during wound healing, and tumor biology. L-Arginine is the precursor for the formation of nitric oxide (NO), an important signaling molecule involved in neurotransmission, and host defense (Anggard, 1994). In addition, arginine is an essential metabolic substrate for immune cells and required for normal lymphocyte function (Popovic *et al.*, 2008). Arginine and NO were critical to the normal physiology of the digestive system. It has been suggested that L-Arginine has potent anti-stress activity during hypoxia exposure (Gupta *et al.*, 2004).

Therefore, the aim of this study was to investigate the influence of pretreatment of five types of rat stress models with excitatory neurotransmitters (L-Arginine) or inhibitory neurotransmitters (glycine & taurine) on enzymatic activities of liver, antioxidant enzymes, hemogram, leucogram, corticosterone, and pro inflammatory cytokines TNF-alpha and IL-2 and anti inflammatory cytokines IL-4 and IL-5.

MATERIALS AND METHODS

Animals: Seventy healthy male Wistar albino rats (150 - 200 g) were utilized throughout this study in the Department of Veterinary Medicine, College of Agriculture and Veterinary Medicine, Qassim University, Saudi Arabia. The animals were housed under standard conditions in appropriate cages (37 x 31 x 16 cm, 3-5 rats). All rats used in the study were received a commercial diet obtained from General Company of Feed Silo and Powder Mint two weeks before starting the experiment. The diet formulated to furnish all the nutrient requirements recommended by (NRC, 1985) for rats. The commercial diet and water were offered *ad libitum* before and throughout the experiment. The experiment was conducted during the light period (08.00 - 16.00 hours). The Animal Ethics Committee of the Institute duly approved the NIH guide for the care and use of laboratory animals.

Neurotransmitters pretreatment: Rats were received 2% taurine (New Zealand Pharmaceuticals, Ltd.) as drinking solution (Nandhini *et al.*, 2005). For L-Arginine, rats were given i.p. injections of 500 mg/100 g body weight L-arginine monohydrochloride (Sigma-Aldrich, Ltd Co., U.S.A) in saline (Mizunuma *et*

al., 1984). Control group was given the same volume of saline. For glycine, rats were fed a diet containing 5% glycine (Harlan Teklad, Madison, Wis.) and 20% casein (glycine groups) or 20% casein (control group) as described by Li *et al.,* (2001). All pretreatments were applied for three days.

Application of stress: Aspirin (Acetylsalicylic acid -Bayer Aspirin) was administered (200 mg / kg, b.w.) for three successive days (Juvekar and Nachankar, 2005). Anoxic stress was applied on rats by keeping them in a confined airtight 250 ml glass jar (Ray *et al.,* 1992). The time taken for the rats to exhibit the first colonic convulsion was taken as the end point. Writhing was induced 30 minutes after i.p injection of 0.1 ml of 0.4% (0.4 ml / 20 mg, i.p.) glacial acetic acid (Bhattacharya *et al.,* 1999). The numbers of writhing responses produced were observed for 20 minutes. Tick infestation stress was applied on rats by experimentally infested by nymphs of *Hyalomma dromederii*. Tick nymphs were manually removed from a heavily infested camel (El Ghali and Hassan, 2010). Nymphs were examined under a stereomicroscope to ensure they have intact mouthparts. Batches of 10 nymphs were applied to the abdomen in a well-closed belly bag (tubular cloth extend along the body from just behind the forelimb to the middle of the abdomen) designed for rats. Rats were kept separately, fed pelleted feed, allowed free access to water and observed daily for 5 days. After 5 days, blood samples were collected. Animals from each group were anesthetized and blood samples were taken. The effect of surgical stress was made by a transverse scrotal incision; testicles exposed and returned back. The scrotal incision was then closed using simple stitch (Jana and Samanta, 2006).

Five control groups of unstressed rats and five groups of stressed rats for each type of stress models. Three groups pretreated with L-Arginine for aspirin, anoxic tolerance and writhing stress models. Two groups pretreated with taurine for aspirin and surgical stress. Two groups pretreated with glycine for anoxic tolerance and tick-infestation-induced stress. Groups were illustrated in table 1.

Table 1: Stress models and pretreatment groups

Types of stress	Control	Stress	L-Arginine	Taurine	Glycine
Aspirin	√	√	√	√	
Anoxic tolerance	√	√	√		√
Writhing	√	√	√		
Tick	√	√			√
Surgery	√	√		√	

√ = *Group*

Sampling: Animals from each group were anesthetized with ether at the fourth day and blood samples were taken from the inner canthus of the eye using capillary tubes. Two samples of blood were collected from each rat. One sample was collected in EDTA-tubes for hematological studies and the other sample was collected in plain tubes and allowed to clot to obtain serum.

Measurements: The EDTA-blood was used for the determination of hemoglobin (Hb) by cyanomet hemoglobin method (Kuwahara, 1974), total red cells by haemocytometer (Schalm *et al.,* 1975), Packed cell volume (PCV %) by microhaematocrit method (Schalm *et al.,* 1975). Color Index (CI), Mean Corpuscular Volume (MCV), Mean Corpuscular Hemoglobin (MCH) and Mean Corpuscular Hemoglobin Concentration (MCHC) were calculated according to Schalm *et al.,* (1975). The white cells were counted by haermocytometer according to Coles (1980). Differential leucocytic count by using cross sectional method according to Schalm *et al.,* (1975). Serum was used for the determination of total protein and albumin using SPECTRUM kits. Determination of serum globulin was obtained by subtracting albumin from total protein of the same samples. Determination of serum creatinine was performed using Linear Chemicals. S.L. Kits. Corticosterone was determined using Alpaco Diagnostics Competitive Immunoassay ELISA kits (Catalog No.: 43-CORMS-E01). The activity of AST and ALT and ALP enzymes was determined using Linear Chemicals. S.L. Kits. Glutathione peroxidase, superoxide dismutase and catalase assay using BIODIAGNOSTIC Kits (CAT. No 2524, CAT. No. 25 21 and CAT. No. 25 17 respectively).

Determination of cytokines: TNF-α was determined by the Assay Max Rat TNF-α ELISA kit (Catalog No.07671211). IL-4 was assayed by kit that pre-coated with an antibody specific to IL-4. (CUSABIO BIOTECH CO., LTD. Lot: 004152648). Interleukin-2 and Interleukin-5 were done using Kits from CUSABIO BIOTECH CO., LTD. Lot: 004152647 and 004152649 as the same procedure for IL-4. The standard curves of cytokines were constructed before measurements. All data presented in the current study were subjected to statistical analysis according to Snedecor and Cochran (1994). All values were presented as the mean $\pm$ SEM.

RESULTS AND DISCUSSION

Results

Slight increased levels of total protein (Table 2) were obtained under most stresses. Tick infestation caused slight decrease in protein level which improved on pretreatment with glycine. Slight fluctuation between stressed groups on serum levels of albumin was observed. Anoxic tolerance stressed animals showed significant increase in globulin levels. Under writhing and tick infestation, changes in A/G ratio were occurred. Pretreatments seemed to be not effective towards normal. The liver enzyme, ALP activities were remarkably increased under tick infestation stresses. Glycine pretreatments have retained normal activities of ALP in animals under tick infestation. The ALT activities were almost constant being not affected by stresses except writhing. Pretreatments showed satisfactory controlling effect on ALT activities. Increased activities of AST were recorded particularly those stressed by aspirin interference. An approximate improvement of AST activities were induced on L-Arginin pretreatments against stresses. Creatinine in sera of the stressed animals has increased under tick infestation. However, other stressed animals have shown slight increase in creatinine levels. Pretreatments seemed to have no effect on retaining creatinine levels in sera of the stressed animals.

Remarkably reduced levels of Hb (Table 3) were obtained in samples from stressed animals subjected to all types of stress. The total erythrocytic count showed remarkable decrease in the blood of animals stressed under aspirin and writhing. Pretreatments with taurine, L-arginine and glycine appeared to have controlled the effect of aspirin and anoxic tolerance**.** Table (3) showed slight or almost no changes in the PCV levels. Pretreatment seemed to have no controlling effects on the PCV in the different groups. The highest CI was observed in samples from surgically stressed animals. None of the pretreated animals induced satisfactory CI towards normal. Stresses were proved to have no effect on MCH. None of the pretreatment appeared to result controlled levels of MCH in the different groups. Animals of different stressed groups showed variable increased MCV. Stress groups showed slight variation either by increase or decrease in the MCHC in the stressed animals. None of the pretreatments seemed to have a controlled effect on the MCHC.

The TLC in the stressed animals (Table 4) showed increased levels although tick appeared to have significant effect. None of the pretreatments seemed to have an effective retaining effect to the normal levels except glycine. In concern of lymphocytes, results depicted that all the stressed animals were having decreased levels of lymphocytes particularly those under anoxic tolerance and aspirin. Although L-arginine and taurine gave almost the same results, they did not improve the lymphocytic changes due to aspirin. L- arginine was superior to glycine as it lead to remarkable increase of lymphocytes in the anoxic tolerance stressed animals. From table, it was noticed that except aspirin and tick infestation among the different stress factors have reduced the level of monocytes. None of the different pretreatments led to recognized improved changes in the monocytes percentage under stress. Significant changes in the percentage of neutrophils were observed in most stressed animals. Results showed that there here was an increased N /L ratio in animals under all stressors particularly in animals stressed by aspirin. Anti stressors seemed to have no effect.

Measurements of corticosterone (Table 5) indicated that increased levels being recorded at different groups. L-Arginine pretreated animals showed improved levels in animals subjected to aspirin and writhing effects. An overall decreased activity of GSh-PX, SOD and CAT was recorded in all animals. Taurine effect was superior to that of L-arginine in the aspirin pretreated animals. L-arginine treatment

almost did not affect the stress induced by writhing but did so with anoxic tolerance stress. CAT activities in animals stressed by tick infestation have been greatly elevated to the normal level when pretreated with glycine. Under surgical stress, taurine seemed to be effective as indicated by CAT activities towards control.

Variable increases in the levels of TNF-α were observed. Writhing and surgical stress have the most stressing effect as indicated by marked increase in the TNF-α level. TNF-α levels showed a significant decrease on pretreatment with taurine, L-arginine and glycine in surgical, writhing and anoxic tolerance stressed rats respectively. From the table (5) while some stressors have no effect on the IL-2, other stressors resulted in an decrease in the IL-2 levels particularly tick infestation and anoxic tolerance. The effect of pretreatment on IL-2 levels showed no significant difference between stress levels and taurine, L-arginine and glycine pretreated rats.

While levels of IL-4 (Table 5) showed marked decrease in the anoxic tolerance and writhing stressed animals, other stress seemed to have no effect. However, pretreatment proved that L-arginine and glycine have increasing effect. IL-5 levels were significantly lowered only in anoxic tolerance stress and tick infestation-induced stress models. Meanwhile, it is increased with tick infestation. Stress due writhing did not affect the IL-5 levels. L-Arginine proved to be effective in alleviating the stress effects of aspirin and anoxic tolerance as indicated by decreased or increased IL-5 levels towards control respectively.

Discussion

There were great variation in the effect of the different stressors; while some have no effects, others seem to be significantly effective. Liver enzymes such as serum AST, ALT, and ALP are the most sensitive markers of liver damage because they are cytoplasmic in location and are released into the circulation after hepatocellular damage (Pari and Amali, 2005). ALT was significantly increased in writhing stressed rats. AST activities were elevated after surgically-induced stress and aspirin – induced stress. Such hepatotoxic effect has been ameliorated on pretreatment with L-Arginine and taurine as being mentioned by Zhang *et al.*, (2014).

Serum levels of ALP and total protein are closely dependent on the integrity of the hepatic cell (Rafi *et al.*, 2013). Both the increased ALP and globulin levels under tick infestation and anoxia have been improved with glycine pretreatment as it stimulates protein synthesis (Wang *et al.*, 2014). The parenchymal cells are responsible for synthesis of fibrinogen, albumin and other coagulation factors and most of α and β-globulins. The stressed animals that showed no any changes in the liver enzymes might be due to the time of stress was not long or strong enough, the extent of the of liver cell damage was not particularly serious as previously recorded by Gao *et al.*, (2013). They observed no significant differences in ALT and AST levels between the normal group and the stressed groups.

Slight increased levels of total protein were obtained under most stresses. Tick infestation caused slight decrease in protein level which improved on pretreatment with glycine. Slight fluctuation between stressed groups on serum levels of albumin was observed. Anoxic tolerance stressed animals showed significant increase in globulin levels. Under writhing and tick infestation, changes in A/G ratio were occurred. Pretreatments seemed to be not effective to direct values towards normal.

Total leucocytic count was markedly increased in rats stressed by tick infestation where tick saliva modulates T-lymphocytes, macrophage responsiveness and induces Th2 type responses (Kazimirova and Stibraniova, 2013). Glycine was effective in reducing such increase. L-arginine as immune-nutrient reduces inflammation due to excessive inadequate exercise as in writhing (Cruzat *et al.*, 2014).

Pretreatment of aspirin-induced stress and writhing stress with L-arginine was accompanied by a significant increase in Hb concentration than stressed groups. Restored higher levels than control of total erythrocyte count, PCV and blood indices CI, MCH, MCV and MCHC were observed on pretreatments with glycine and taurine in tick infestation and surgically induced stress. This is due to the ability of taurine and glycine as anti-oxidants to repair tissues and built up the proteins (Shivananjappa, 2012).

Table 2: Effect of pretreatment with neurotransmitters on Liver and kidney function test

Parameters	Types of stress	Control	Stress	L-Arginine	Taurine	Glycine
Total protein (gm/100ml)	Aspirin-	5.35±0.335	5.45±0.324	5.95±0.274	5.83±0.129	-
	Anoxic tolerance	5.47±0.196	5.85±0.253	5.54±0.308	-	5.57±0.118
	Writhing	5.35±0.206	5.72±0.155	5.85±0.238	-	
	Tick	5.85±0.196	5.47±0.221	-	-	5.72±0.452
	Surgery	5.37±0.390	5.42±0.278	-	5.53±0.271	-
Albumin (gm/100ml)	Aspirin	3.20±0.235	3.25±0.125	3.23±0.149	3.30±0.122	-
	Anoxic tolerance	3.25±0.091	3.13±0.065	3.13±0.278	-	3.15±0.051
	Writhing	3.07±0.095	3.23±0.225	3.45±0.147	-	
	Tick	3.23±0.125	3.48±0.111	-	-	3.57±0.268
	Surgery	3.15±0.202	3.00±0.058	-	3.10±0.216	-
Globulin (gm/100ml)	Aspirin-	2.18±0.165	2.18±0.221	2.68±0.193	2.54±0.158	-
	Anoxic tolerance	2.24±0.041	2.73±0.149*	2.38±0.085	-	2.43±0.149
	Writhing	2.28±0.165	2.56±0.108	2.44±0.122	-	
	Tick	2.33±0.253	2.25±0.171	-	-	2.23±0.197
	Surgery	2.23±0.315	2.47±0.256	-	2.40±0.248	
A/ G Ratio	Aspirin-	1.34±0.129	1.22±0.092	1.52±0.12	1.49±0.124	
	Anoxic tolerance	1.32±0.097	1.32±0.113	1.16±0.072	-	1.46±0.089
	Writhing	1.198±0.103	1.42±0.062	1.31±0.148	-	
	Tick	1.578±0.066	1.26±0.153	-	-	1.56±0.201
	Surgery	1.38±0.28	1.21±0.16	-	1.50±0.024	
Alkaline phosphatase (ALP) (µmole/L)	Aspirin-	60.96±3.89	61.51±3.33	55.28±1.48	55.06±3.57	
	Anoxic tolerance	63.46±6.24	69.18±1.55	59.35±2.32	-	59.42±5.32
	Writhing	60.96±3.89	62.76±2.75	54.98±1.06	-	
	Tick	63.05±2.61	78.54±2.26**	-	-	61.37±3.31
	Surgery	63.46±2.87	59.44±0.68	-	58.66±3.87	-
AIanine aminotransferase (ALT) (µl/110ml)	Aspirin-	25.7±3.23	26.03±3.55	24.22±3.12	26.19±2.45	-
	Anoxic tolerance	26.92±3.97	26.19±2.45	25.67±3.59	-	25.53±2.94
	Writhing	21.67±1.36	36.45±3.95*	25.52±2.21	-	
Aspartate	Tick	23.67±4.04	24.54±3.51	-	-	23.61±1.93
	Surgery	26.92±3.97	22.76±1.81	-	24.84±1.83	-
aminotransferase (AST) (µl/110ml)	Aspirin-	112.65±2.82	126.85±2.17*	117.87±1.92[A]	118.75±3.59	-
	Anoxic tolerance	120.9±7.68	125.56±2.83	125.59±3.41	-	118.16±1.99

	Writhing	122.65±7.68	124.04±4.09	118.51±3.35	-	
	Tick	125.36±4.71	120.69±3.68	-	-	123.34±2.77
	Surgery	114.40±8.13	130.02±1.7*	-	130.16±2.36	-
Creatinine (mg %)	Aspirin-	7.88±0.69	8.28±0.29	8.37±0.36	8.56±0.15	-
	Anoxic tolerance	8.13±0.59	8.33±0.31	8.27±0.38	-	8.19±0.30
	Writhing	8.63±0.44	8.59±0.30	8.26±0.32	-	-
	Tick	7.72±0.45	8.94±0.19*	-	-	8.86±0.36
	Surgery	7.38±0.64	8.73±0.21	-	8.53±0.29	

(*) (**)Values of the stressed and treated groups were different significantly from the value of control group in the same row at P- < 0.05 and P < 0.01 respectively
(A) Values of the treated groups were different significantly from the value of stressed group in the same row at P<0.05

Table 3: Effect of pretreatment with neurotransmitters on hemogram

Parameters	**Types of stress**	**Control**	**Stress**	**L-Arginine**	**Taurine**	**Glycine**
Haemoglobin (HB) (Gm%):	Aspirin-	17.01±0.671	13.57±0.675**	17.53±1.17^{A}	13.93±1.58	-
	Anoxic tolerance	18.22±0.769	15.56±0.460*	15.33±1.05	-	15.10±1.15
	Writhing	15.41±0.583	13.08±0.583*	15.08±0.583^{A}	-	-
	Tick	14.63±0.684	13.50±0.483	-	-	14.30±0.540
	Surgery	13±0.577	13.95±0.521	-	13.27±1.13	-
Total Erythrocyte Count (10^6/µl)	Aspirin	5.06±0.12	3.86±0.25**	4.54±0.19	4.84±0.22^{A}	-
	Anoxic tolerance	5.18±0.17	4.84±0.22	5.09±0.26	-	4.86±0.25
	Writhing	5.16±0.14	4.42±0.23*	4.38±0.20	-	
	Tick	5.95±0.33	5.02±0.30	-	-	5.86±0.25
	Surgery	6.11±0.20	5.16±0.21	-	5.86±0.25	-
Packed Cell Volume (PCV) (%)	Aspirin-	38.833±0.601	38.833±0.700	38.833±0.989	39.173±1.016	-
	Anoxic tolerance	39.090±0.894	37.333±0.715	37.667±0.843	-	39.333±0.882
	Writhing	38.833±0.980	38.522±0.764	39.503±1.34	-	-

	Tick	38.833±0.910	39.172±1.140	-	-	38.830±1.082
	Surgery	39.651±0.816	37.333±0.715	-	39.333±0.882	-
Color Index (CI)	Aspirin-	1.165±0.051	1.154±0.117	0.935±0.050	1.283±0.163	
	Anoxic tolerance	1.093±0.068	1.023±0.063	0.944±0.051	-	0.836±0.059
	Writhing	0.945±0.035	0.934±0.054	0.877±0.028	-	-
	Tick	0.778±0.0513	0.714±0.058	-	-	1.182±0.089^{B}
	Surgery	0.501±0.010	0.614±0.038*	-	1.173±0.115^{B}	-
Mean Corpuscular Haemoglobin (MCH) (Pg%)	Aspirin-	33.76±1.62	36.94±3.73	29.92±1.61	44.59±4.35^{A}	-
	Anoxic tolerance	34.99±2.18	32.58±2.03	30.22±1.62	-	46.30±6.25
	Writhing	29.93±1.13	29.88±1.73	28.09±0.854	-	
	Tick	24.90±1.64	22.84±1.84	-	-	37.83±2.86^{B}
	Surgery	23.14±1.79	27.31±2.96	-	38.07±3.00^{A}	-
Mean Corpuscular Volume (MCV) (Fl)	Aspirin-	77.09±2.91	79.90±3.74	85.05±3.57	81.85±3.36	
	Anoxic tolerance	75.55±2.73	77.75±2.71	74.75±3.18	-	75.38±2.82
	Writhing	75.65±3.30	88.03±4.21*	74.19±4.63^{A}	-	
	Tick	66.64±5.24	65.56±2.58	-	-	105.5±7.65^{B}
	Surgery	48.17±1.37	52.40±2.05	-	66.24±4.31^{A}	-
Mean Corpuscular Hemoglobin Concentration (Mchc) (%)	Aspirin-	43.94±2.16	45.86±3.27	35.84±2.00^{A}	39.84±4.44	-
	Anoxic tolerance	46.38±2.66	41.80±1.61	40.78±2.86	-	38.39±4.44
	Writhing	39.68±1.97	35.01±1.23	40.24±2.18	-	
	Tick	37.74±2.42	36.22±1.44	-	-	35.89±1.24
	Surgery	33.46±1.87	37.43±1.56	-	38.39±4.4	-

(*) (**)Values of the stressed and treated groups were differs significantly from the value of control group in the same row at P < 0.05 and P < 0.01 respectively.
(A) (B) Values of the treated groups were differs significantly from the value of stressed group in the same row at P<0.05 and P< 0.01 respectively.

Table 4: Effect of pretreatment with neurotransmitters on leukogram

Parameters	Types of stress	Control	Stress	L-Arginine	Taurine	Glycine
Total leucocytes count (TLC) (10^3/mm³)	Aspirin-	6.967±0.640	7.800±0.757	9.700±1.137	6.500±0.443	-
	Anoxic tolerance	7.400±0.542	8.967±0.460	8.867±0.943	-	6.167±0.348 A
	Writhing	7.300±0.565	9.667±0.796	8.767±0.491	-	
	Tick	8.600±0.621	10.800±0.666*	-	-	7.600±0.689 A
	Surgery	9.550±1.142	9.667±0.711	-	7.000±0.801	-
Lymphocytes (%)	Aspirin	69±3.43	57.17±2.59*	60±4.44	60.33±5.10	-
	Anoxic tolerance	75±3.20	59±3.24**	69.17±2.47 B	-	62±5.35
	Writhing	79.50±2.08	73.17±2.73	72±3.08		
	Tick	69.50±3.67	65.67±2.40	-		59.67±4.82
	Surgery	74.17±2.77	70.33±2.20	-	59.67±3.16 C	-
Acidophils (%)	Aspirin-	4.17±1.14	5.57±0.74	5.83±0.703	5.54±0.85	-
	Anoxic tolerance	3.83±0.72	4.5±0.76	4.50±0.64	-	5.52±0.67
	Writhing	4.53±0.764	4.5±0.70	3. 33±0.73	-	-
	Tick	3.83±0.60	4.53±0.72	-	-	4.08±0.73
	Surgery	4.54±0.64	4.59±0.73	-	4.66±0.55	-
Monocytes (%)	Aspirin-	4.54±0.76	3.33±0.94	7.17±1.01	5.16±0.72	-
	Anoxic tolerance	4.31±0.77	5.81±0.57	3.57±0.76	-	4. 67±0.80
	Writhing	4.66±0.66	5. 67±0.47	3.66±0.66	-	-
	Tick	4.83±0.70	4. 35±0.64	-	-	4.31±0.15
	Surgery	4.57±0.64	5.16±0.01	-	5.16±0.72	-
Neutrophils (%)	Aspirin-	22.33±2.67	23.67±2.22	27±3.44	17.33±1.40 A	-
	Anoxic tolerance	18±3.44	17.17±3.31	22.83±2.51	-	18.67±1.17
	Writhing	18.50±2.14	27.33±3.39*	20.50±2.97	-	-
	Tick	17.83±3.35	25.33±1.07*	-		23.09±2.89
	Surgery	16.83±2.86	26±2.38*	-	25.33±2.91	-
N /L Ratio	Aspirin-	0.327±0.063	0.480±0.037*	0.360±0.047	0.337±0.057	-
	Anoxic tolerance	0.320±0.043	0.337±0.048	0.248±0.062	-	0.252±0.058
	Writhing	0.350±0.059	0.297±0.053	0.248±0.055	-	
	Tick	0.378±0.044	0.400±0.056	-	-	0.330±0.063
	Surgery	0.365±0.059	0.422±0.040	-	0.237±0.050	-

(*) Values of the stressed and treated groups were differs significantly from the value of control group in the same row at P < 0.05
(A) (B) (C) Values of the treated groups were differs significantly from the value of stressed group in the same row at P- < 0.05 , P < 0.01 and P< 0.001 respectively

Table 5: Effect of pretreatment with neurotransmitters on Cortiosterone GSH-PX, SOD, CAT, TNF-A, IL-2, IL4 and IL-5

Parameters	Types of stress	Control	Stress	L-Arginine	Taurine	Glycine
Cortiosterone (Ng/Ml)	Aspirin-	156.20±2.69	506±29.1***	232.9±13.1[B]	315±80.7	-
	Anoxic tolerance	244.2±12.8	360.8±16.5	342±30.1	-	316.2±70.6
	Writhing	175±25.9	480.6±24.7***	266.6±38.6[B]	-	-
	Tick	165.3±10.3	490.7±15.7***		-	343.6±93.6
	Surgery	175.1±25.2	527.3±49.3**		313.9±79.1	-
	Aspirin	7.13±0.70	4.84±0.41*	5.02±1.47	5.41±0.80	-
Gsh-PX (U/Ml)	Anoxic tolerance	7.10±0.41	5.31±0.55*	6.18±0.92	-	5.93±0.56
	Writhing	7.14±0.55	5.10±0.33**	5.76±1.65	-	-
	Tick	7.83±0.28	5.23±0.45*	-	-	5.80±0.53
	Surgery	7.45±0.49	5.17±0.56*	-	5.48±0.29	-
SOD (U/Ml)	Aspirin-	109.24±2.53	91.54±1.71***	95.45±6.46	95.85±4.87	-
	Anoxic tolerance	105.84±2.95	88.54±2.36**	85.99±3.38[C]	-	84.31±3.69[C]
	Writhing	104.70±2.39	76.47±2.20***	94.29±1.50	-	-
	Tick	92.18±3.35	84.58±1.89	-	-	86.99±3.98
	Surgery	104.9±2.84	87.12±3.51*	-	83.53±3.51[B]	-
CAT (MU/L)	Aspirin-	152.20±5.80	126.56±3.19*	132.38±3.50	142.27±1.36[A]	
	Anoxic tolerance	147.03±9.13	131.45±4.50	141.82±4.28	-	125.06±3.14
	Writhing	151.80±2.57	124.35±2.63***	125.28±3.28	-	147.79±3.43[A]
	Tick	147.15±4.36	125.22±5.47*	-	-	-
	Surgery	142.20±6.00	116.43±2.45*	-	145.07±2.15[C]	-
TNF-A (Ng/Ml)	Aspirin-	12.85±1.14	13.87±1.03	12.90±0.81	13.93±1.53	-
	Anoxic tolerance	15.67±1.54	14.77±1.01	12.07±1.49	-	11.53±0.819[A]
	Writhing	11.42±1.05	16.92±1.23**	11.17±1.01[B]	-	
	Tick	13.80±1.02	11.667±0.833	-	-	13.63±0.532
	Surgery	12.5±0.619	15.45±0.82*	-	11.7±0.93[A]	
IL-2 (Ng/Ml)	Aspirin-	12.25±2.63	15.53±2.11	12.94±1.43	14.65±2.41	

	Anoxic tolerance	16.57±2.02	11.31 ±1.83*	16.32±0.93 A	-	13.82±1.32
	Writhing	13.94±0.74	12.04±1.84	12.21±1.27	-	
	Tick	15.94±1.02	12.84±1.09*	-	-	13.43±1.52
	Surgery	13.54±2.81	13.13±2.11	-	13.21±0.99	-
IL4 (Pg/Ml)	Aspirin-	17.02±0.67	17.53±1.17	14.50±0.67	15.95±1.83	-
	Anoxic tolerance	18.00±0.77	15.57±0.46*	15.33±1.05	-	18.58±0.99 A
	Writhing	15.42±0.58	13.08±0.58*	15.88±0.78 A	-	-
	Tick	14.63±0.68	13.50±0.48	-	-	16.45±0.99 A
	Surgery	13.00±0.56	13.95±0.52	-	15.78±1.54	-
IL-5 (Pg/Ml)	Aspirin-	13.54±1.11	15.32±2.03	14.32±1.65	13.65±1.44	-
	Anoxic tolerance	14.53±2.94	11.53±2.94*	15.32±1.54	-	14.32±0.45
	Writhing	15.21±3.54	15.43±4.73	13.43±1.02	-	-
	Tick	12.53±1.94	16.54±1.01*	-	-	14.65±1.73
	Surgery	12.12±1.54	11.53±1.11*	-	13.66±1.65	-

(*)(**) (***)Values of the stressed and treated groups were differs significantly from the value of control group in the same row at P < 0.05 , P < 0.01 and P< 0.001 respectively .
(A) (B) (C) Values of the treated groups were differs significantly from the value of stressed group in the same row at P- < 0.05, P < 0.01 and P< 0.001 respectively.

Immuno-hormonal and enzymatic stress indicators also were variable. Effectiveness of L-Arginine in reducing the significantly increased levels of corticosterone in Aspirin, anoxic tolerance and writhing stressed groups is coincident with the previous observations (Gupta *et al.,* 2004; Cruzat *et al.,* 2014).
The significant decrease of Gsh-PX activities in stressed rats were restored near the values of control pretreatment with L-arginine and glycine particularly in Anoxia stressed animals where glycine increases the glutathione synthetase and consequently restored the glutathione levels (Ruiz-Ramirez *et al.*, 2014).
L-arginine and taurine pretreatment have retained SOD activities toward normal values in aspirin induced stress and writhing stress groups but not in anoxic tolerance and surgical stressed groups. Such effects of L-Arginine and taurine have been documented (Gupta *et al.*, 2004; Wang *et al.*, 2013). The same effect of L-Arginine and taurine was observed in alleviating CAT activities in aspirin induced stress model.
The highest levels of TNF-α was under writhing stress, which has been effectively retained to normal with L-Arginine pretreatment. Such observation has been reported (Cruzat *et al.,* 2014) where L-Arginine has reduced immunosuppression and excessive inflammation in excessive exercise such as writhing.
IL-4 levels were significantly increased by glycine pre treatment in anoxic tolerance stressed rats. L-Arginine has the same effect in writhing stressed rats. That could be due to stimulated protein synthesis by glycine (Wang *et al.,* 2014) and the immune immune-nutrient effect of L-Arginine (Cruzat *et al.,* 2014). Concerning IL-2 and IL-5 the significant reduction occurred under anoxia tolerance has been alleviated with L-arginine pretreatment.
The obtained results provide significant evidence of the medicinal use of neurotransmitters in stress disorders. The cooperation between excitatory and inhibitory neurotransmitters needs to be considered as a therapeutic target when developing new interventions for stress disorders.

REFERENCES

Alcaraz-Contreras Y, Garza-Ocañas L, Carcaño-Díaz K and Ramírez-Gómez XS (2011). Effect of Glycine on Lead Mobilization, Lead-Induced Oxidative Stress, and Hepatic Toxicity in Rats. *Journal of Toxicology,* Article ID 430539 7.

Anggard E (1994). Nitric oxide: mediator, murderer, and medicine. *Lancet* 14;**343**(8907) 1199-1206.

Bhattacharya D and Sur TK (1999). The effect of Panax ginseng and diazepam on norepinephrine levels of whole brain and hypothalamus during stress. *Indian Journal of Pharmacology* **31** 124–127.

Bouckenooghe T, Remacle C and Reusens B (2006). Is taurine a functional nutrient? *Current Opinion in Clinical Nutrition and Metabolic Care* **9** 728–733.

Chen K, Zhang Q, Wang J, Liu F, Mi M, Xu H, Chen F and Zeng K (2009). Taurine protects transformed rat retinal ganglion cells from hypoxia-induced apoptosis by preventing mitochondrial dysfunction. *Journal of Brain Research* **1279** 131-138.

Coles EH (1980). *Veterinary Clinical Pathology*, 3rd edition (W.B. Sanders Co. Philadelphia) 10 –20.

Cruzat VF, Kraus M and Newsholme P (2014). Amino acid supplementation and impact on immune function in the context of exercise. *Journal of the International Society of Sport Nutrition* **11** 61 -73.

Deters M, Siegers CP and Strubelt O (1998). Influence of glycine on the damage induced in isolated perfused rat liver by five hepatotoxic agents. *Toxicology* **128**(1) 63–72.

El Ghali A and Hassan SM (2010). Life cycle of the camel tick *Hyalomma dromedarii* (Acari: Ixodidae) under field conditions in Northern Sudan. *Veterinary Parasitology* **174**(3-4) 305-312.

El Hafidi M, P´erez I, Carrillo S, Cardoso G, Zamora J, Chavira R and Ba˜nos G (2006). Effect of sex hormones on non-esterified fatty acids, intra-abdominal fat accumulation, and hypertension induced by sucrose diet in male rats. *Clinical and Experimental Hypertension* **28** 669–681.

Gao X, Zeng Y, Liu S and Wang S (2013). Acute stress show great influences on liver function and the expression of hepatic genes associated with lipid metabolism in rats. *Lipids in Health and Disease* **12** 118.

Gupta V, Gupta A, Saggu S, Divekar HM, Grover SK and Kumar R (2005). Anti-stress and Adaptogenic Activity of L-Arginine Supplementation. *Evidence-based Complementary and Alternative Medicine (ECAM)* **2**(1) 93 – 97.

Higuchi M, Celino FT, Shimizu-Yamaguchi S, Miura S and Miura T (2012). Taurine plays an important role in the protection of spermatogonia from oxidative stress. *Amino Acids* **43** 2359 – 2369.

Hultman K, Alexanderson C, Manneras L, Sandberg M, Holmang A and Jansson T (2007). Maternal taurine supplementation in the late pregnant rat stimulates postnatal growth and induces obesity and insulin resistance in adult offspring. *Journal of Physiology* **579**(3) 823–833.

Ikejima K, Iimuro Y, Forman DT and Thurman RG (1996). A diet containing glycine improves survival in endotoxin shock in the rat. *American Journal of Physiology* **271** G97–G103.

Jana K and Samanta PK (2006). Evaluation of single intratesticular injection of calcium chloride for nonsurgical sterilization in adult albino rats. *Contraception* **73** 289– 300.

Juvekar AR and Nachankar RS (2005). Restraint stress induced changes and their modification by Spirulina platensis in albino rats: An experimental study. *Acta Horticulturae* (ISHS) **680** 49-55.

Kazimirova M and Stibraniova I (2013). Tick salivary compounds: their role in modulation of host defenses and pathogen transmission. *Frontiers in Cellular and Infection Microbilogy* **3**(Article43) 1-19.

Kong WX, Chen SW, Li YL, Zhang YJ, Wang R, Min L and Mi X (2006). Effects of taurine on rat behaviors in three anxiety models. *Pharmacology Biochemistry and Behavior* **83**(2) 271-6.

Koppelmann T, Pollak Y, Mogilner J, Bejar J, Coran AG and Sukhotnik I (2012). Dietary L-arginine supplementation reduces Methotrexate-induced intestinal mucosal injury in rat. *BMC Gastroenterology* **12** 41, Available: doi:10.1186/1471-230X-12-41.

Kuwahara SS (1974). Effect of anticoagulants upon the cyanomethemoglobin method for hemoglobin assay. *Clinical Biochemistry* **9** 53-54.

Li X, Bradford BU, Wheeler MD, Stimpson SA, Pink HM, Brodie TA, Schwab JH and Thurman RG (2001). Dietary Glycine Prevents Peptidoglycan Polysaccharide-Induced Reactive Arthritis in the Rat: Role for Glycine-Gated Chloride Channel. *Infection and Immunity* **69**(9).
Mauriz JL, Matilla B, Culebras JM, Gonzalez P and Gonzalez-Gallego J (2001). Dietary glycine inhibits activation of nuclear factor kappa B and prevents liver injury in hemorrhagic shock in the rat. *Free Radical Biology & Medicine* **31**(10) 1236–1244.
Mikalauskas S, Mikalauskiene L, Bruns H, Nickkholgh A, Hoffmann K, Longerich T, Strupas K, B¨uchler MW and Schemmer P (2011). Dietary glycine protects from chemotherapy-induced hepatotoxicity. *Amino Acids* **40** 1139–1150.
Mizunuma T, Kawamura S and Kishino Y (1984). Effects of Injecting Excess Arginine on Rat Pancreas. *Journal of Nutrition* **114** 467-471.
Nandhini ATA, Thirunavukkarasu V, Ravichandran MK and Anuradha CV (2005). Effect of taurine on biomarkers of oxidative stress in tissues of fructose-fedinsulin-resistant rats. *Singapore Medical Journal* **46**(2) 82.
National Research Council (1985). *Guide for the Use and Care of Laboratory Animals*. Publication no 85-23(rev.), (Washington: NIH).
Pan C, Giraldo GS and Wu JY (2010). Taurine protection of PC12 cells against endoplasmic reticulum stress induced by oxidative stress. *Journal of Biomedical Science* **17**(Suppl 1) S17.
Pari L and Amali DR (2005). Protective role of tetrahydrocurcumin (THC) and active principle of turmeric on chloroquine induced hepatotoxicity in rats. *Journal of Pharmacy & Pharmaceutical Sciences* **8**(1) 115-123.
Popovic M, Valpoti H and Kardum Paro Marijana *et al.*, (2008). Qualitative and quantitative parameters of swine cell immunity. *Acta Veterinaria (Beograd)* **58**(2-3) 149-158.
Rafi KP, Aleemuddin MA, Sravani K and Sivarama Krishna K (2013). Hepatoprotective and antioxidant activity of Coldeniaprocumbens Linn on paracetamol induced liver damage in albino wistar rats. *European Journal of Zoological Research* **2**(4) 25-31.
Rajendra S, Lynch JW and Schofield PR (1997). The glycine receptor. *Pharmacology & Therapeutics* **73** 121–146.
Ray A, Puri S, Chakravarty AK and Sen P (1992). Central histaminergic involvement during stress in rats. *Indian Journal of Experimental Biology* **30** 724–8.
Richmond J (2007). *Synaptic function Worm Book.* The C. elegans Research Community, December 7.
Ruiz-Rammirez A, Ortiz-Balderas E, Cardozo-Saldana G, Diaz-Diaz E and El-Hafidi M (2014). Glycine restores glutathione and protects against oxidative stress in vascular tissue from sucrose-fed rats. *Clinical Science* **128** 19 – 29.
Salimäki J, Scriba G, Piepponen TP, Rautolahti N and Ahtee L (2003). The effects of systemically administered taurine and N-pivaloyltaurine on striatal extracellular dopamine and taurine in freely moving rats. *Naunyn-Schmiedeberg's Archives of Pharmacology* **368**(2) 134 - 141.
Schaffer SW, Azuma J and Mozaffari M (2009). Role of antioxidant activity of Taurine in diabetes. *Canadian Journal of Physiology and Pharmacology* **87** 91-99.
Schalm OW, Jain NC and Caroll EJ (1975). *Textbook of Veterinary Haematology* (Published by Lea and Febiger) Philadelphia 129 – 250.
Shivanajappa MM (2012). Taurine attenuates maternal and embryonic oxidative stress in streptozotocin-diabetic rat model. *Reproductive Biomedical Online* **24** 558 – 566.
Snedecor GW and Cochran WG (1994). *Statistical Methods*, 8th edition [book review].
Sun M and Xu C (2008). Neuroprotective mechanism of taurine due to up-regulating calpastatin and down-regulating calpain and caspase-3 during focal cerebral ischemia. *Cellular and Molecular Neurobiology* **28** 593–611.
Takatani T, Takahashi K, Uozumi Y, Matsuda T, Ito T, Schaffer SW, Fujio Y and Azuma J (2004b). Taurine prevents the ischemia-induced apoptosis in cultured neonatal rat cardiomyocytes through Akt/caspase-9 pathway. *Biochemical and Biophysical Research Communications* **316** 484–489.

Takatani T, Takahashi K, Uozumi Y, Shikata E, Yamamoto Y, Ito T, Matsuda T, Schaffer SW, Fujio Y and Azuma J (2004a). Taurine inhibits apoptosis by preventing formation of the Apaf-1/caspase-9 apoptosome. *American Journal of Physiology-Cell Physiology* **287** 949–953.
Thomas C and Starke K (2008). Pharmacology of Neurotransmitter Release. *Handbook of Experimental Pharmacology* 184.
Wang G, Li W, Lu X, Zhao X and Xu L (2013). Taurine attenuated oxidative stress and alleviates cardiac failure in type I rats. *Croatian Medical Journal* **45** 171-179.
Wang W, Wu Z, Lin G, Hu S, Wang B, Dai Z and Wu G (2014). Glycine stimulates protein synthesis and inhibits oxidative stress in pig small intestinal epithelial cells. *Journal of Nutrition* **144**(10) 1540 – 1548.
Wheeler MD, Ikejima K, Enomoto N and Stachlewitz RF *et al.*, (1999). Glycine: a new anti-inflammatory immunonutrient. *Cellular and Molecular Life Sciences* **56** 843–856.
Wu JY and Prentice H (2010). Role of taurine in the central nervous system. *Journal of Biomedical Science* **17**(1) S1.
Yu X, Chen K, Wei N, Zhang Q, Liu J and Mi M (2007). Dietary taurine reduces retinal damage produced by photochemical stress via antioxidant and anti-apoptotic mechanisms in Sprague–Dawley rats. *British Journal of Nutrition* **30** 1–9.
Yu X, Xu Z, Mi M, Xu H, Zhu J, Wei N, Chen K, Zhang Q, Zeng K, Wang J, Chen F and Tang Y (2008). Dietary taurine supplementation ameliorates diabetic retinopathy via anti-excitotoxicity of glutamate in streptozotocin-induced Sprague–Dawley rats. *Neurochemical Research* **33** 500–507.
Zhang Z, Liu D, Yi B, Liao Z, Tang L, Yin D and He M (2014). Taurine supplementation reduces oxidative stress and protects the liver in an iron-overload murine model. *Molecular Medicine Reports* **10** 2255 – 2262.