

ORIGINAL ARTICLE

Gallic Acid Protects Against Immobilization Stress-Induced Changes In Wistar Rats

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Background: Stress triggers a wide range of body changes. Herbal medicines are rich in non specific antistress agents.

Purpose: The present study was carried out to evaluate the antistress effect of gallic acid (GA), a naturally occurring plant phenol, on immobilization induced-stress in male albino Wistar rats.

Methods: The immobilization stress was induced in rats by putting the rats in 20 cm × 7 cm plastic tubes for 2 h/day for 21 days. Rats were post orally treated with GA at a dose of 10 mg/kg body weight via intragastric intubations.

Results: Treatment with GA significantly increased the food intake, body weight, organ weight (spleen, testis and brain) and the significant reduction was found in weight of liver, kidney, heart and adrenal glands, which was in stressed rats. GA also significantly reduced the elevated levels of plasma glucose, plasma and tissue cholesterol (CHL), triglycerides (TG), Low Density Lipid (LDL), Very Low Density Lipid (VLDL) and also significantly increased the level of High Density Lipid (HDL). A significant decrease in hematological parameters like RBC count, total and differential WBC count was also found which were increased in immobilization stress.

Conclusion: GA prevented the stress-induced physiological, biochemical and hematological changes, indicating the preventive effect against stress.

Key words: Blood cell count; gallic acid; lipid profile; stress; organ weight

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Conclusion: GA prevented the stress-induced physiological, biochemical and hematological changes, indicating the preventive effect against stress.

Key words: Blood cell count; gallic acid; lipid profile; stress; organ weight

Stress is a condition of highly individualized response of an organism to external and internal challenges which one can control with difficulties or cannot control. It is one of the important factor acting upon a large human population in the entire country. It induces the strain upon both emotional and physical endurance which has been considered

the basic factor in the etiology of a number of diseases like cardiovascular diseases, cancer, diabetes mellitus, etc (Vogel, 1993; Brown, 1993). In response to stressors, a series of behavioral, neurochemical, and immunological changes occur that ought to serve in an adaptive capacity (Brown, 1993; Anisman, 1999). However, if those systems

become overly taxed, the organism may become vulnerable to pathology. The stress response is a natural reaction by the body, against potentially harmful stimuli to enhance the chance for survival. Stress has become an increasingly popular and widely applied term in our every day languages. People have witnessed the physical damage of stress that can work on the body. A recent survey showed 70-90% of us feel stressed at work and outside. Today's fast paced life lifestyle is putting a toll on population (Moberg, 2000). According to the World Health report, approximately 450 million people suffer from a mental or behavioural disorder (WHO, Geneva, 2001). This amounts to 12.3% of the global burden of disease, and predicted to rise up to 15% by 2020 (Reynolds, 2003). The term stress is defined by Hans Selye (1930) as the sum of all the nonspecific changes caused by function or damage and a state of threatened homeostasis. Stress basically is a reaction of mind and body against change in the homeostasis. The productive stress is called Eustress while harmful stress is called Distress. If the stress is extreme, the homeostatic mechanisms of the organism become deficit and the survival of the organism is threatened. Under these conditions, stress triggers a wide range of body changes called General Adaptation Syndrome (GAS). The stimuli, which produce GAS, are called the Stressors and range from physical to psychological factors including cold, heat, infection, toxins, major personal disappointment etc (Selye, 1973; Panossian, et al., 1999). Immobilization/restraint stress is an easy and convenient method to induce both psychological and physical stress (Nayanatara, et al., 2012). Herbal medicines are rich in nonspecific antistress agents which are of increasing clinical significance, among them adaptogens are the plant

derived biological active substances which increases the power of resistance against physical, chemical or biological noxious agents (Ahmed, 1998). Plant polyphenols are well known to show biological activity, such as antimutagenicity, anticarcinogenicity and antioxidative activity. GA (3,4,5-trihydroxyl-benzoic acid), as a polyhydroxylphenolic compound is widely distributed in various plants, fruits and foods. Various biological activities of GA have been reported, including strong antioxidant (Abdelwahed et al., 2007), antibacterial (Kang et al., 2008), antiviral (Kratz et al., 2008), antiinflammatory (Kim et al., 2006), anticancer (Faried et al., 2007), antiapoptotic activities (Sameermahmood et al., 2010) and antimutagenic activities (Peyrat-Maillard et al., 2000). Many of these therapeutic effects have been confirmed by contemporary scientific research and their antistress effects have not been well researched. Therefore, the present study was planned to investigate the antistress activities of GA on immobilization induced-stress in rats.

MATERIALS AND METHODS

Animals

All the experiments were done with male albino Wistar rats weighing 150–180 g, obtained from Central Animal House, Rajah Muthiah Institute of Health Sciences, Annamalai University, Tamil Nadu, India. The experiment was carried out according to the guidelines of the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA), New Delhi, India. The experimental protocol was approved by the Animal Ethical Committee of Annamalai University (Approval No.886; 29.05.2012). Rats were housed in polypropylene cages (47 cm×34 cm×20 cm) lined with husk (replaced every 24 h) in a 12 h light–dark cycle at around 22 °C and relative humidity (55%).

Rats were fed on standard pellet diet (Amrut Laboratory Animal Feed, Pranav Agro Industries Ltd., Bangalore, India) and water ad libitum.

Drug and chemicals

GA was provided as a gift sample by Dr. N. Nalini, the Professor, Department of Biochemistry and Biotechnology, Annamalai University, Tamilnadu, India. All other chemicals and solvents used were of analytical grade and purchased from Sigma-Aldrich Co. St. Louis, Missouri, USA or Himedia Laboratories Pvt. Ltd., Mumbai, India.

Experimental design

The immobilization stress was induced in rats by putting the them in 20 cm × 7 cm plastic tubes for 2 h/day for 21 days (Marcilhac et al., 1998; Yokus et al., 2005). There are several 3 mm holes at the far end of the tubes for breathing, that allows ample air but animals will be unable to move. A pilot study was conducted with three different doses of GA (5, 10 and 20 mg/kg) to determine the dose dependent effect of GA on immobilization induced stress in rats. It was observed that after 21 days of experiment, GA treatment at the doses of 5, 10 and 20 mg/kg significantly ($p < 0.5$) lowered the elevated levels of corticosterone, alanine aminotransferase (ALT) and aspartate aminotransferase (AST) in immobilization induced-stress rats. 10 mg/kg of GA showed higher significant effect than the lower dose 5 mg/kg and highest dose 20 mg/kg. Hence, we have chosen the midst dose (10 mg/kg) for our study. All the rats were randomly divided in to four groups. Six animals were used for each group. Group I: control rats; Group II: control rats were treated with GA (10 mg/kg); Group III: stressed rats; Group IV: stressed rats were treated with GA (10 mg/kg). GA was dissolved in saline and administered to rats orally using an intragastric

tube, daily after 2 h stress for 21 days. Following stress session, rats were returned to home cages and were able to access food and water freely for remainder of the day. Food intake of the animals was studied every day and the weight of the animals was measured on the 0th and 21st day of the experimental period by a digital scale (Zardooz et al., 2006). After the experimental period, all the rats were anesthetized and then sacrificed by cervical decapitation between 10:00 and 11:00 a.m in the laboratory. After decapitation, blood and tissues were collected for the estimation of biochemical parameters such as plasma glucose (Trinder, 1969), plasma and tissue cholesterol (Allain et al., 1974), triglycerides (McGrowan et al., 1983), HDL-cholesterol (Izzo et al., 1981), and hematological parameters like RBC count, white blood cell (WBC) count and the absolute numbers of lymphocytes, monocytes, neutrophils and eosinophils (Kozinets and Makarov, 1997). The LDL and VLDL levels were calculated using the formulae (Friedewald et al., 1972). The weight of organs (liver, kidney, heart, brain, spleen, adrenal gland and testes) after washing with ethanol was recorded per 100 g body weight. Data were analyzed by one way analysis of variance (ANOVA) followed by Duncan's multiple range test (DMRT) using a commercially available statistics software package (SPSS for Windows, V. 16.0, Chicago, USA). Results were presented as means \pm SD. p values < 0.05 were regarded as statistically significant.

RESULTS

Food intake

Immobilization stress decreased the food intake in rats, and GA at a dose of 10 mg/kg showed significant increase in the food intake when compared to stressed rats (figure 1).

Body weight

Immobilization stress reduced the body weight in rats, which were significantly increased by GA at a dose of 10 mg/kg when compared to stressed rats (Table 1).

Organ weight

The weights of the organs like heart, liver, kidney and adrenal gland were increased, while the weight of brain, spleen and testis was reduced in stressed rats. Treatment with GA at a dose of 10 mg/kg significantly reduced the weight of the heart, liver, kidney, and adrenal gland, and significantly increased the weight of the brain, spleen and testis when compared to stressed rats (Table 2).

Plasma glucose

Exposure to immobilization stress resulted in an increased plasma glucose level in stressed rats (figure 2), which was significantly decreased by GA at a dose of 10 mg/kg when compared to stressed rats.

Plasma and tissue cholesterol

Immobilization stress elevated plasma and tissue cholesterol levels in stressed rats. GA at a dose of 10 mg/kg significantly decreased the

elevated levels of cholesterol when compared to stressed rats (Table 3).

Plasma and tissue triglycerides

Table 4 shows the effect of immobilization stress on plasma and tissue triglycerides. Stressed rats when treated with GA at a dose of 10 mg/kg significantly decreased the elevated levels of triglycerides when compared to stressed rats.

Lipoprotein

Immobilization stress decreased HDL cholesterol, and increased LDL and VLDL cholesterol levels in stressed rats. GA at a dose of 10 mg/kg significantly increased the HDL cholesterol levels and significantly decreased the LDL and VLDL cholesterol levels when compared to stressed rats (Figure 3).

RBC, total and differential WBC count

Exposure to immobilization stress elevated the RBC, WBC, lymphocytes, monocytes, eosinophils and neutrophils count. Treatment with GA at a dose of 10 mg/kg showed significant decline in RBC, WBC, lymphocytes, monocytes, eosinophils and neutrophils count when compared to stressed rats (Table 5).

Table 1: Effect of gallic acid on body weight in control and immobilization induced-stress rats

Groups	Initial weight (g)	Final weight (g)	Weight gain (% g)
Control	164.54 ± 5.83 ^a	178.05 ± 9.71 ^a	8.21 ± 0.45 ^a
Control + GA	164.47 ± 7.27 ^a	177.35 ± 5.61 ^a	7.83 ± 0.47 ^a
Stress	164.14 ± 7.98 ^b	146.37 ± 5.60 ^b	-11.36 ± 0.25 ^b
Stress + GA	158.15 ± 6.27 ^c	167.60 ± 6.27 ^c	5.97 ± 0.35 ^c

Values are means ± SD (n=6). GA= gallic acid (10 mg/kg body weight). Values not sharing a common alphabet as superscripts are significantly different from each other at the level of $p < 0.05$ (ANOVA followed by DMRT)

Table 2: Effect of gallic acid on organ weight in control and immobilization induced-stress rats

Groups	Organ weight (g/100 g body weight)						Testes
	Brain	Heart	Liver	Kidney	Spleen	Adrenal gland	
Control	2.23 ± 0.13 ^a	0.365 ± 0.03 ^a	3.27 ± 0.21 ^a	0.547 ± 0.04 ^a	0.56 ± 0.03 ^a	0.21 ± 0.01 ^a	1.65 ± 0.05 ^a
Control + GA	2.23 ± 0.11 ^a	0.367 ± 0.02 ^a	3.30 ± 0.12 ^a	0.531 ± 0.02 ^a	0.53 ± 0.04 ^a	0.23 ± 0.01 ^a	1.61 ± 0.06 ^a
Stress	1.36 ± 0.07 ^b	0.542 ± 0.04 ^b	5.31 ± 0.11 ^b	0.724 ± 0.06 ^b	0.28 ± 0.01 ^b	0.49 ± 0.03 ^b	1.27 ± 0.03 ^b
Stress + GA	1.82 ± 0.09 ^c	0.467 ± 0.03 ^c	4.28 ± 0.21 ^c	0.609 ± 0.03 ^c	0.42 ± 0.03 ^c	0.37 ± 0.02 ^c	1.46 ± 0.04 ^c

Values are means ± SD (n=6). GA= gallic acid (10 mg/kg body weight). Values not sharing a common alphabet as superscripts are significantly different from each other at the level of $p < 0.05$ (ANOVA followed by DMRT)

Table 3: Effect of gallic acid on plasma and tissue cholesterol in control and immobilization induced-stress rats

Groups	Cholesterol (mg/g tissue)				
	Plasma (mg/dl)	Brain	Heart	Liver	Kidney
Control	58.24 ± 1.29 ^a	3.12 ± 0.24 ^a	2.63 ± 0.12 ^a	4.32 ± 0.34 ^a	2.80 ± 0.18 ^a
Control + GA	55.65 ± 2.39 ^a	2.81 ± 0.17 ^a	2.47 ± 0.20 ^a	3.89 ± 0.27 ^a	2.64 ± 0.22 ^a
Stress	76.37 ± 5.51 ^b	4.72 ± 0.31 ^b	3.67 ± 0.17 ^b	7.43 ± 0.56 ^b	3.91 ± 0.23 ^b
Stress + GA	63.47 ± 3.10 ^c	3.53 ± 0.21 ^c	2.86 ± 0.10 ^c	4.97 ± 0.29 ^c	3.05 ± 0.12 ^c

Values are means ± SD (n=6). GA= gallic acid (10 mg/kg body weight). Values not sharing a common alphabet as superscripts are significantly different from each other at the level of $p < 0.05$ (ANOVA followed by DMRT)

Table 4: Effect of gallic acid on plasma and tissue triglycerides in control and immobilization induced-stress in rats

Groups	Triglycerides (mg/g tissue)				
	Plasma (mg/dl)	Brain	Heart	Liver	Kidney
Control	69.18 ± 4.17 ^a	3.09 ± 0.13 ^a	2.51 ± 0.16 ^a	3.61 ± 0.21 ^a	2.12 ± 0.12 ^a
Control + GA	66.13 ± 3.15 ^a	2.96 ± 0.17 ^a	2.36 ± 0.16 ^a	3.53 ± 0.15 ^a	2.02 ± 0.13 ^a
Stress	106.57 ± 7.56 ^b	4.17 ± 0.21 ^b	3.42 ± 0.12 ^b	4.32 ± 0.26 ^b	3.29 ± 0.15 ^b
Stress + GA	83.63 ± 4.836	3.65 ± 0.14 ^c	2.80 ± 0.13 ^c	3.89 ± 0.19 ^c	2.56 ± 0.17 ^c

Values are means ± SD (n=6). GA= gallic acid (10 mg/kg body weight). Values not sharing a common alphabet as superscripts are significantly different from each other at the level of $p < 0.05$ (ANOVA followed by DMRT)

Table 5: Effect of gallic acid on hematological parameters in control and immobilization induced-stress in rats

Groups	WBC/mm ³	Lymphocytes	Monocytes	Neutrophills	Eosinophills	RBC (10 ⁶ /mm ³)
Control	6875 ± 216.23 ^a	45.03 ± 1.59 ^a	1.05 ± 0.20 ^a	23.27 ± 1.78 ^a	1.21 ± 0.07 ^a	4.22 ± 0.23 ^a
Control + GA	6765 ± 135.40 ^a	46.27 ± 2.34 ^a	1.13 ± 0.50 ^a	24.17 ± 1.88 ^a	1.27 ± 0.04 ^a	4.32 ± 0.07 ^a
Stress	11648 ± 249.58 ^b	76.12 ± 2.80 ^b	3.57 ± 0.24 ^b	38.42 ± 2.58 ^b	4.76 ± 0.32 ^b	7.17 ± 0.21 ^b
Stress + GA	7217 ± 255.64 ^c	60.02 ± 3.08 ^c	2.44 ± 0.17 ^c	27.18 ± 2.01 ^c	3.47 ± 0.29 ^c	5.17 ± 0.03 ^c

Values are means ± SD (n=6). GA= gallic acid (10 mg/kg body weight). Values not sharing a common alphabet as superscripts are significantly different from each other at the level of $p < 0.05$ (ANOVA followed by DMRT)

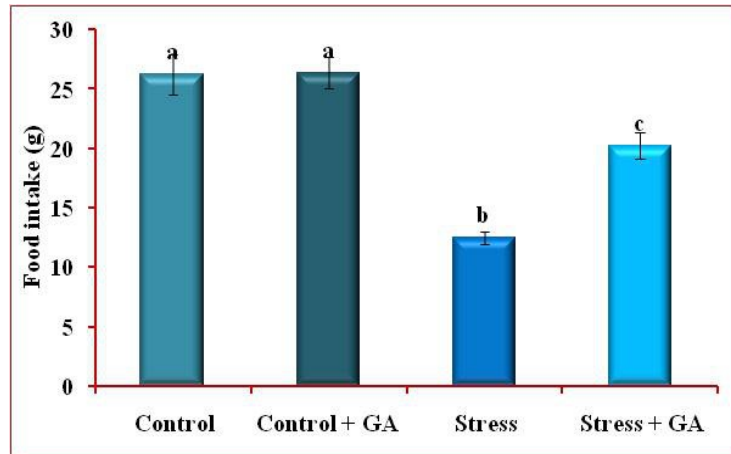


Figure 1: Effect of gallic acid on food intake in control and immobilization induced-stress rats.

Values are means \pm SD (n=6). GA= gallic acid (10 mg/kg body weight). Values not sharing a common alphabet as superscripts are significantly different from each other at the level of $p < 0.05$ (ANOVA followed by DMRT)

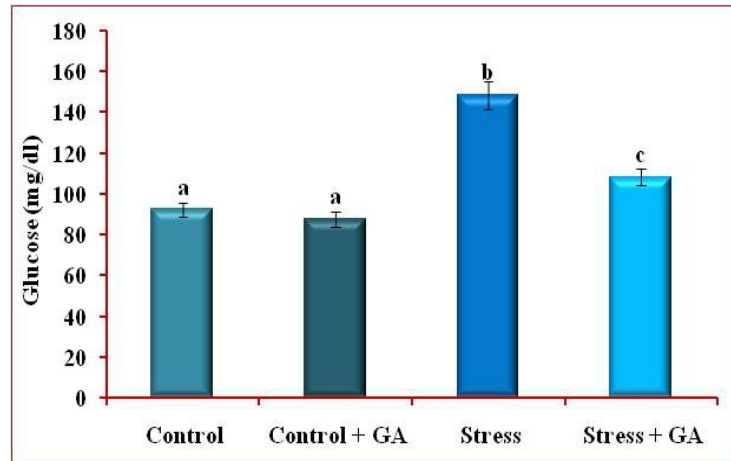


Figure 2: Effect of gallic acid on plasma glucose in control and immobilization induced-stress rats.

Values are means \pm SD (n=6). GA= gallic acid (10 mg/kg body weight). Values not sharing a common alphabet as superscripts are significantly different from each other at the level of $p < 0.05$ (ANOVA followed by DMRT)

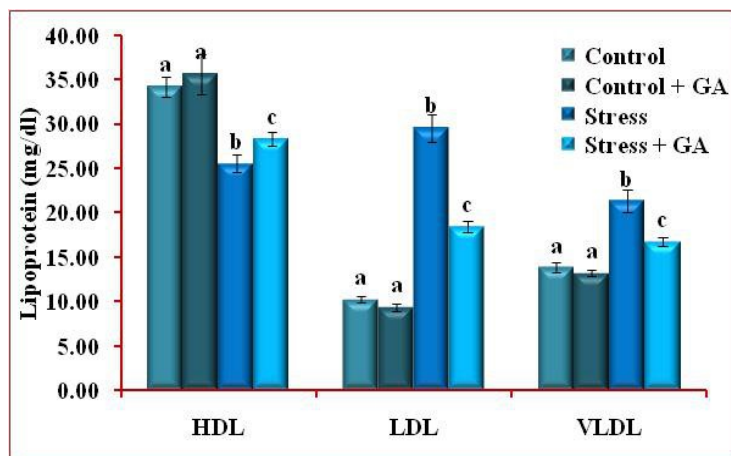


Figure 3: Effect of gallic acid on plasma lipoprotein in control and immobilization induced-stress rats.

Values are means \pm SD (n=6). GA= gallic acid (10 mg/kg body weight). Values not sharing a common alphabet as superscripts are significantly different from each other at the level of $p < 0.05$ (ANOVA followed by DMRT)

DISCUSSION

It has been proved through countless study that our mental attitude has powerful influence on our physical health. As immobilization stress is believed to be the most severe type of stress in rodent models and has a comparative effect in humans, this type of stress was used in the present study. In the present study, the exposure to immobilization stress for 2 hours for 21 days resulted in a significant reduction of body weight. The decreased body weight could be due to the decreased food intake in the rats under the influence of stress. In addition to that the decrease in the body weight might also have been presumably associated with stress induced increase in metabolic demands, reduced digestion, and increased adrenal steroid secretion (Nayanatara et al., 2012). The lower weight gain observed in stressed animals has already been reported (Pecoraro *et al.*, 2004) and may be related to the action of glucocorticoids, mobilizing energy stores and increasing hepatic gluconeogenesis (Dallman *et al.*, 2005; Torres & Nowson, 2007). Treatment with GA significantly increased the body weight in the stress loaded animals which correlates with the studies of Nitish *et al.*, (2011), who indicated the adaptogenic potential of Punarnavine during stressful events.

Increased levels of glucose are seen in the immobilization stressed rats due to the decreased secretion of insulin level (Zardooz *et al.*, 2006). In response to stress, ACTH is released which acts on adrenal cortex where by cortisol and corticosterone will be secreted. Increased plasma cortisol influences the mobilization of stored fat and carbohydrate reserves, which in turn increases blood glucose level. The increased cortisol levels and increased blood glucose level are reversed by anti-stress agents (Meera and Mustafa, 2009; Tache

and Selye, 1976; Meera and Mustafa, 2007). Treatment with GA significantly decreased plasma glucose when compared to stressed rats.

The mechanism by which stress raises cholesterol and triglyceride levels in stress induced animals is due to the enhanced activity of hypothalamo-hypophyseal axis resulting in increased liberation of catecholamines and corticosteroids (Lakshmi and Sudhakar, 2009). It is well-known that catecholamines activate lipolysis in adipose tissue and increase the free fatty acid flow to the liver where increased triglyceride synthesis and secretion occurs. The observed increased level of triglycerides may also due to the stress induced catecholamine surge (Nayanatara, et al., 2012). Numerous experimental animal studies have provided empirical support for a definite relationship between stress and lipid concentrations. Cholesterol level was significantly higher in stress group which is similar to those reported by others (Jain et al., 2000). The LDL is well recognized as a risk factor and HDL as a protective factor against arteriosclerosis (Haberland, 1988). Correlation of lipid profile and lipid peroxidation after the exposure to immobilization stress indicates that increased lipid peroxidation is associated with increased total cholesterol, LDL and VLDL (Nayanatara et al., 2012). In our study, treatment with GA significantly reduced the elevated levels of cholesterol, triglyceride, LDL and VLDL and significantly increased HDL levels might be due to inhibition of stimulation of sympathetic nervous system.

Increased weight of liver during stress could be due to the increased secretion of the stress hormones, which are known to increase the metabolic activities and mRNA levels in the hepatic cells. Changes in the homeostatic mechanism such

as increased cardiac output and blood pressure during stress might have contributed to the increased kidney weight after stress (Chang et al., 1995; Nagaraja and Jeganathan, 1999). Cardiac hypertrophy following immobilization stress observed in the present study was similar to previous reports (Urban et al., 2004). The increased heart weight following immobilization stress may be a consequence of an excessive stimulation of the hypothalamo-pituitary-adrenal axis (Nagaraja and Jeganathan, 1999). Strong stimulation of the adrenal glands during prolonged stress situations is known to cause adrenal hyperplasia and hypertrophy (Marti et al., 1993; Tuli et al., 1995). The hyperactivity of adrenals in stressed animals is due to the stress induced adrenomedullary response leading to increased production of corticotrophic hormone that leads to increase in weight of adrenals (Lakshmi and Sudhakar, 2009; Jiban et al, 2011). Treatment with GA has significantly reduced the weight of liver, kidney, heart and adrenal glands might be due to the reversal of stress induced adrenomedullary response and hence decrease production of corticotrophic hormone.

Spleen contracts during stress and releases more amount of blood (RBC) into circulation, hence its weight decreases (Sharma et al., 2007). The significant increase in the spleen weight might be due to the inhibition of recruitment of lymphocytes to blood from spleen. The weight of testis decreases because there is suppression of spermatogenesis and decrease testosterone levels during stress (Jyoti, 2003). Stress causes alteration in hematological parameters like increase in total and differential WBC counts, neutrophils (Takano, 1983; Lakshmi and Sudhakar, 2009). GA significantly reduced the RBC count, total and differential WBC

counts compared to stressed rats.

GA prevented the stress-induced alterations in body weight, organ weight, lipid profile and hematological parameters, indicating the protective effect against stress.

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