

Full Length Research Paper

Effects of *Emilia praetermissa* leaf extract on the haematological and biochemical parameters of stress induced ulcerated Wistar rats

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Accepted 2 November, 2012

Effects of *Emilia praetermissa* leaf extract on the haematological and biochemical parameters of cold-water stress induced ulceration in albino rats were the focus of this study. Blood samples collected from stress induced ulcerated Wistar rats by cold water immersion were used to evaluate the haematological and biochemical parameters. This study was conducted using twenty rats randomly divided into four groups, Group A which was control group and three test Groups B, C and D. All three test groups were subjected to cold water induced stress ulceration. Groups B and C were treated with normal saline (10 ml/kg body weight) and *E. praetermissa* (500 mg/kg body weight) respectively, for seven days after stress induced ulceration while the Group D was pretreated with *E. praetermissa* (500 mg/kg) for fourteen days before cold water stress induced ulceration. The results showed that Stress reduced all blood parameters tested except total white blood cell and platelet counts when compared with control while *E. praetermissa* increased all the blood parameters tested above that of control despite the induced stress. However, the extract significantly ($p < 0.05$) reduced biochemical parameters such as alanine aminotransaminase (ALT) and aspartate aminotransaminase (AST). The results therefore suggest that *E. praetermissa* has haemopoietic potential, it could stimulate blood cell formation and also powerful enough to suppress effects of stress on haematological parameters in stress-ulcerated Wistar rats.

Key words: *Emilia praetermissa*, cold water immersion, ulcer, haematological and biochemical parameters.

INTRODUCTION

Medicinal plants have been used as traditional treatment for numerous human diseases for thousands of years and in many parts of the world (Palombo, 2011). This interest in medicinal herbs has increased scientific scrutiny of the therapeutic potential and safety (O'Hara et al., 1998). *Emilia praetermissa* which belongs to the family of Asteraceae is a useful plant of west tropical Africa used generally as food and medicine for general healing

(Burkill, 1985). *E. praetermissa* has been established as an anti-ulcerogenic plant producing complete mucosal cytoprotection at a dose of 500 mg/kg (Tan et al., 1997).

Stress is a normal physical response to events that causes threat or upset an individual's balance in some way. It refers to any condition that arouses anxiety or fear. Anxiety can be defined as an emotion characterized by feeling of anticipated danger, tension and distress and by tendencies to avoid or escape (Qureshi et al., 2002). Stress can be helpful in small doses but long term exposure to stress can lead to serious health problems ranging from major damage to health to decrease level of productivity and quality of life. Physiological studies have

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shown that stress from any source can influence the endocrine, haemopoietic and immune systems. Cytokines and cortisol seem to play an important role in the communication between these systems (Maes et al., 1998; Benoit et al., 2001). Stress related mucosal disease is an acute erosive gastritis representing conditions ranging from stress related injury to stress ulcer (Spirt, 2004; Stollman and Metz, 2005). Stress related injury is superficial mucosa damage that present primarily as erosions whereas stress ulcers are deep focal mucosa damage penetrating the submucosa with high risk for gastrointestinal bleeding (Spirt, 2004).

The underlying cause of stress related mucosal disease is hypoperfusion of the mucosa in the upper gastrointestinal tract (Spirt and Stanley, 2006). Gastrointestinal microcirculation and the mucus layer normally maintain the integrity of the gastric mucosa by providing nourishment eliminating hydrogen ion, oxygen radicals and other toxic substances, increasing bicarbonate secretion to neutralize hydrogen ions (Spirt, 2004). Stress related mucosa damage occurs when the mucosal barrier is compromised and can no longer block the detrimental effects of hydrogen ions and oxygen radicals (Spirt and Stanley, 2006). Stress reduces blood parameters such as hematocrit, haemoglobin, erythrocyte count but increased leucocyte count (Gbore et al., 2006). Circulating levels of alanine transaminase (ALT) and aspartate transaminase (AST) increase under psychological and toxic stress; reflecting liver injury. This suggests that chronic stress relates to hepatic damage (Roland et al., 2009).

Cold-water restraint has been a useful procedure for the study of the underlying mechanisms involved in stress induced ulceration in which conscious animals have been noticed to develop more severe gastric damage compared to anesthetized animals, therefore the animal's conscious activity during cold-water immersion has been known to increase the severity of gastric mucosal damage (Fernandez, 2004). Since information on the effects of this plant on blood cells and biochemical parameters during stress has not been established, therefore the aim of this study was to investigate the effects of *E. praetermissa* leaf extract on the haematological and biochemical parameters of cold-water stress-induced ulceration in albino Wistar rats.

MATERIALS AND METHODS

Extract preparation

Leaves of *E. praetermissa* were collected from the Botanical garden of University of Port Harcourt, River State, Nigeria. The botanical identification and authentication were done by the Chief Herbarium Officer, University of Port Harcourt, River State, Nigeria. The leaves were dried, milled to fine powder in manual engine grinder (Modelcorene, A.5 lander YCIA S.A) and extracted using methanol. The extracted residue was dried in air oven of 50°C to obtain deep

green homogenous substance which readily dissolves in distilled water.

Animal selection/handling

Twenty healthy albino Wistar rats were used for the study. They were kept in a spacious and well ventilated cage with suitable temperature, relative humidity, food and drinking water for 14 days to acclimatize before proceeding on the experiment. The animals with body weight ranging from 110 g to 220 g were randomly grouped into 4 groups. Group A served as control which received normal rat chow and water throughout the experiment. Group B represent animals treated with normal saline (10 ml/kg) for 7 days after ulcer induction. Group C animals were treated with extract of *E. praetermissa* (500 mg/kg) for 7 days after ulcer induction. The Group D animals were pre-treated with extract of *E. praetermissa* (500 mg/kg) for two weeks before ulcer induction.

Experimental procedure

Stress induced gastric ulcer was achieved using the cold water immersion method. After 48 h of food deprivation but free access to water, the animals were immersed in cold water of about 21°C and left to swim in the water tank of 17.5 cm long and having internal diameter of 5.2 cm (Landeira-Fernandez, 2004) for 3 h after which they were allowed to dry under 60 watts bulb lamp for another 3 h. Normal saline ((10ml/kg body weight)) was administered orally to animals in Group B while Group C animals were given the extract of *E. praetermissa* for 7 days respectively. The Group D animals were pretreated with the extract of *E. praetermissa* for 14 days before ulcer induction. All administration was through the oral route. 500 mg/kg of the crude extract of *E. praetermissa* was given to the experimental rats according to their weight. The rats were injected with thiopental which rendered them inactive for about 5 min putting them to sleep. The blood samples for analysis were collected through cardiac puncture and the samples were collected into two different containers. The first container was a plain bottle containing no anticoagulant with the sample used for biochemical determination while the second container containing EDTA anticoagulant was used for haematological parameters.

Blood analysis

The blood samples were analyzed to determine the haematological parameters such as: Packed cell volume (PCV), red blood cell (RBC) count, white blood cell (WBC) count, platelet count and haemoglobin concentration (Hb conc.) using an automated haematology ANALYZER KX-21N, made by sysmex Japan. The sysmex KX-21N is an automatic multi-pair blood cell counter for *in vitro* diagnostic use in clinical laboratory. It performs speedy and accurate analysis of blood parameters and detects the abnormal samples. The automated haematology analyzer reading correlated well with readings by the standard manual methods (Samuel et al., 2010). Mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH) and mean corpuscular haemoglobin concentration (MCHC) were calculated from values of RBC, PCV and HbC as follows:

$$\text{MCV (fL)} = \text{PCV (\%)} \times 10 / \text{RBC count};$$

$$\text{MCH (pg)} = \text{HbC (g/dl)} \times 10 / \text{RBC count}$$

And,

$$\text{MCHC (g/dl)} = \text{HbC (g/dl)} \times 100 / \text{PCV (\%)}.$$

Table 1. Haematological parameters in control and stress-induced ulceration treated with normal saline and *Emilia praetermissa* groups of rats (n=5).

Parameters	Group A	Group B	Group C	Group D
HbC (g/dl)	7.38 ± 0.13	4.90 ± 1.33	9.62 ± 0.34 ^a	9.06 ± 0.78 ^a
RBC (× 10 ⁶ mm ³)	4.55 ± 0.09	4.06 ± 0.59	5.44 ± 0.43	5.90 ± 0.50
MCV (fl)	55.05 ± 0.78	55.8 ± 0.71	60.4 ± 0.60*	56.1 ± 1.90
MCH (pg)	16.18 ± 0.24	11.36 ± 1.41*	18.26 ± 2.89 ^a	15.66 ± 2.89
HCT (%)	25.6 ± 0.50	22.8 ± 3.65	32.8 ± 2.49 ^a	33.0 ± 2.89 ^a
MCHC (g/dl)	29.4 ± 0.27	20.3 ± 2.33*	30.2 ± 1.76 ^a	28.1 ± 2.72 ^a
PLT (× 10 ³ mm ³)	104.2 ± 3.79	192.4 ± 79.82*	194.2 ± 58.68*	229.2 ± 138.3 ^a
WBC (× 10 ³ -mm ³)	3.3 ± 0.25	5.14 ± 0.52	6.88 ± 1.48*	5.46 ± 1.01
LYMP (%)	84.1 ± 0.67	72.0 ± 4.54*	70.3 ± 2.07*	59.3 ± 1.18 ^a

*P<0.05 vs. control, Values are mean ± SEM; ^aP<0.05 Group B vs. Groups C and D. Values are mean ± SEM.

Biochemical analysis of the serum enzymes for ALT and AST was by the method of Reitman and Frankel (1957). Bilirubin concentration determination was by 2.5-dichlorophenyldiazonium method described by Schlebusch et al. (1988).

Statistical analysis

All data were presented as mean ± SEM. The one way ANOVA was used to analyze the data, followed by a post-hoc test (LSD). The results were considered significant at P<0.05.

RESULTS

Effect of stress-induced gastric ulceration on haematological parameters in albino rats

Table 1 shows the haematological parameters of the various groups after the duration of the experiment. The mean values for haemoglobin concentration (HbC), red blood cell count (RBC), hematocrit (HCT), mean corpuscular haemoglobin and mean corpuscular volume (MCV) were lower in Group B when compared with Group A and these differences were not statistically significant (P>0.05) while values for mean corpuscular haemoglobin (MCH) and mean corpuscular haemoglobin concentration (MCHC) and lymphocyte count were significantly lower in Group B when compared with Group A (p<0.05). White blood cell count and platelet counts were higher in Group B than Group A and the difference were statistically significant (p<0.05).

Effect of *E. praetermissa* on haematological parameters in stress induced gastric ulceration in albino rats

All values except the value for lymphocyte count were higher in Groups C and D when compared with Groups A and B respectively. The values were higher in Group D than in Group C. Significant differences were noticed in

haemoglobin concentration, mean corpuscular volume, haematocrit, platelet and white blood cell count in Groups C and D when compared with Group A (Control). Haemoglobin concentration, haematocrit, mean corpuscular haemoglobin and mean corpuscular haemoglobin concentration in rats treated with *E. praetermissa* after ulcer induction (Group C) were significantly higher (p<0.05) than those in which ulcer was induced by stress only (Group B). The rats pretreated with *E. praetermissa* (Group D) had higher values for haemoglobin concentration, hematocrit and platelet count than those in Group B. The increases were statistically significant (p<0.05). Lymphocyte count in Group D was significantly reduced when compared with Group B.

Effect of stress-induced gastric ulceration on biochemical parameters in albino rats

Table 2 shows the concentration of alanine amino transferase (ALT), aspartate amino transferase (AST) and bilirubin in the blood of control and treated rats. There were slight increases in the concentration of alanine amino transferase (ALT), aspartate amino transferase (AST) and slight decrease in bilirubin concentration in Group B when compared with Group A. The differences observed were not statistically significant.

Effect of *E. praetermissa* on biochemical parameters in stress induced gastric ulceration in albino rats

There were significant decreases (p<0.05) in alanine amino transferase (ALT) and aspartate amino transferase (AST) in Groups C and D when compared with Group A (Control) and Group B (stress-ulcerated rats). Bilirubin concentration in Groups C and D appeared to be unchanged when compared with the control.

Table 2. Biochemical parameters in control and stress-induced ulceration treated with normal saline and *Emilia praetermissa* groups of rats (n=5).

Parameters	Group A	Group B	Group C	Group D
Aspartate amino transferase (μL)	196.2 \pm 9.24	198.8 \pm 2.03	126.8 \pm 13.1 ^a	171.6 \pm 26.71*
Alanine amino transferase (μL)	64 \pm 4.9	67 \pm 2.0	25.4 \pm 3.26 ^a	53.6 \pm 10.86 ^a
Bilirubin ($\mu\text{mol/L}$)	7.2 \pm 0.23	5.96 \pm 0.62	8.74 \pm 0.77	8.76 \pm 2.19

* P<0.05 vs. control, Values are mean \pm SEM; ^a P<0.05 Group B vs. Groups C and D. Values are mean \pm SEM.

DISCUSSION

The changes in haematological and biochemical parameters of stress-ulcerated animals treated with *E. praetermissa* extract were the focus of this study. Physiological studies have shown that stress from any source can influence on the endocrine, hemopoietic and immune systems. Cytokines and cortisol seem to play an important role in the communication between these systems (Maes et al., 1998 and Benoit et al., 2001). Previous studies have shown that stress increases erythrocytes, neutrophils and platelets counts, whereas lymphocytes, eosinophils and monocytes decrease in number (Dhabhar, 1995). The magnitude of stress-induced changes is significantly reduced in adrenalectomised animals. It is suggested that endocrine factors released during stress modulate leucocyte trafficking and result in the redistribution of leucocytes between the blood and other immune compartments (Dhabhar, 1995). The activation of sympathetic nervous system may also have a role to play. Lymphocytes and monocytes express receptors for several stress hormones, including norepinephrine and epinephrine. Thus stressful events could alter immune function. This alteration in immune function due to decrease in lymphocytes and basophils was found in the subjects of this study, confirming the stress related changes reported in the literature.

The results of this study showed that there were no significant changes in RBC and HCT in response to stress. This is similar to no significant change in red blood cell or haematocrit observed by Qureshi et al. (2002). The reason for this might be due to physiological factor pertaining to female animals such as low level of testosterone, No significant changes in MCV and Hb observed in this study contrast significant increase Hb and MCV reported by Maes et al. (1998). The differences might be due to the fact that we used majorly female animals which normally have low red blood cell count because of the reason stated above. The significant increase in Platelet count and decrease in lymphocyte count were consistent with the earlier findings reported by Qureshi et al. (2002) and Dhabhar et al. (1995). However, nearly all the values of haematological parameters (HbC, RBC, HCT, MCV, MCH, MCHC and Platelet) determined except for lymphocyte increased in the groups treated with *E. praetermissa* before and after

stress induction. Owing to the fact that it is a vegetable eaten as food (Burkill, 1985), the increase might be due to the presence of blood forming vitamins (Bamidele et al, 2010).

The slight increases in ALT and AST in stress-ulcerated rats were closely related to the findings of earlier researchers (Roland et al., 2009). The leaf extract of *E. praetermissa* decreased the activities of liver enzymes ALT and AST in stress-induced rats. Liver enzymes (ALT and AST) are released into the blood whenever liver cells are damaged and enzyme activity in the plasma is increased (Edwards et al., 1995). The fact that the enzyme activities were reduced showed that the extract protects against hepatic damage. The hepatoprotective functions of the plant might be due the presence of flavonoids, which are antioxidants that mop up super oxide anions. No significant change in bilirubin concentration observed in this present work can be attributed to any change in the RBC or HbC in the stress ulcerated animals (destruction of red blood cells produce bilirubin).

Conclusion

The results suggest that *E. praetermissa* improves haemopoiesis even in the presence of stress induced ulceration in albino Wistar rats. It also protects against hepatic damage. Therefore *E. Praetermissa* consumption as vegetable as well as anti-ulcerogenic agent may be encouraged since it brings about reduction on the effect of stress on haematological parameters.

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