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Effect of *Labisia pumila* (Kacip Fatimah) on stress induced changes in leukocyte count and organ weight of albino rats

Dissertation submitted in partial fulfillment for the
Degree of Bachelor of Science (Health) in Biomedicine

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2004

CERTIFICATE

This is to certify that the dissertation entitled

"EFFECT OF *LABISIA PUMILA* (KACIP FATIMAH) ON STRESS INDUCED CHANGES IN LEUKOCYTE COUNT AND ORGAN WEIGHT OF ALBINO RATS" is the bona fide record of research work done by **Ms. FADILLAH BINTI ALIM** during the period from **August 2003** to **March 2004** under our supervision.

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Acknowledgements

First of all, I would like to express sincere gratitude to my supervisor Associate Professor Dr. K. Sembulingam, for his patience and guidance throughout this study. Under his supervision, I have managed to expand my knowledge in the art of conducting research experiments, especially in the field of noise.

A special thanks to Associate Professor Dr. Prema Sembulingam, for helping me in this study.

I would like to extend my gratitude to Dr. Siti Amrah bt. Sulaiman, Head Department of Pharmacology for her kind help on giving the plant material and giving permission to utilize the equipments for extraction of plant. I thank Mrs. Halijah for helping me in plant extraction process and also thank to Ms. Ezumi given me references of *Labisia pumila*.

I am grateful to Dr. Roslin Hassan, Department of Hematology for giving permission to use hematology machine also thank to Mrs. Narishah Shariff and all the staff in hematology department in USM because doing the test during proceeding.

I thank to Mr. Mohd. Zaki Selamat for helping me was doing the blood count. I would like to thank the staff in animal house in USM for their help during the proceedings especially to Mr. Mustakim.

Last, but not least, I would love to thank my family for their support and encouragement and also thank my entire fellow friend especially to Nurul Azuar because helping me handling the rats.

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Abstract

The extract of *Labisia Pumila* was screened for its effects on stress induced changes in leukocyte count and organ weight of albino rats (Wistar strain). The total leukocyte count in rats was significantly reduced after exposure to noise stress. The neutrophil percentage was increased significantly but, there was significant decrease in the percentage of eosinophils and lymphocytes after exposure to noise stress. Noise stress caused significant increase in the weight of adrenal glands whereas weight of thymus and spleen were decreased significantly.

Pretreatment of rats with the extract of *Labisia Pumila* daily (100 mg/ kg body weight) for one week, prevented the changes in total leukocyte count, differential leukocyte count and organ weight induced by acute noise stress. The results of this study indicate that the plant, *Labisia pumila* has an antistressor affect against noise.

Introduction

Labisia pumila (Kacip Fatimah) is a herb that has been traditionally used by Malay women in parturition. The water decoction of the root or whole plant given to a pregnant woman between one and two months before delivery time is believed to induce and expedite labour. *Labisia pumila* is one of the most popular and potent ingredients used in traditional herbal preparations or 'jamus' for after birth care. It is administered by almost all mid-wives and traditional healers in both urban and rural areas including the natives in the jungle. There has so far been no or very little attempt to cultivate this plant. They are normally collected from the wild.

The plant is also used in post-partum medication as mixed preparation to help contraction of birth channel, to delay fertility and regain body strength. This herb is also traditionally used to treat ailments such as flatulence, dysentery, dysmenorrhoea, gonorrhea and bones disorders (Jamia *et al.*, 1999). *Labisia pumila* has three different varieties and the varieties are differentiated based on the characters of the petioles (Jamia *et al.*, 2000b). The local market demand for *Labisia pumila* products is very great giving to its use as an after birth tonic (Jamia *et al.*, 1998).Currently there are a number of local products of *Labisia pumila* in the market like Kacip Fatimah Power Root, Pearl Kacip Fatimah and Sri Rapat Kacip Fatimah.

The pharmacological actions of the different extracts from various parts of the plant have been studied extensively. However, studies reporting the effect of this plant on stress induced changes are not reported. Hence, it is proposed to elucidate

the effect of this plant on stress-induced changes in total leukocyte count, differential leukocyte count and the weight of adrenal glands, spleen, and thymus in this study.

The stressor agent that was used in this study is noise. Noise is generally considered as a common environmental stress factor and short term exposure to loud noise can lead to detrimental health hazards. This problem is more pertinent in the occupational environment where short term exposure to loud noise is unavoidable (Sembulingam *et al.*, 1999). Generally, noise can be defined as a random signal, which contains all the frequencies in the audio spectrum. There are many types of noise. In this study, it is decided to use white noise. White noise is the result of random signals, and contains all frequencies of sound at varying levels. A characteristic feature of white noise is that it is louder in the high frequency region. The sound produced by showers, rain, machines, vehicles etc are examples of white noise.

Review of literature

Labisia pumila

Labisia pumila (LP) is a sub herbaceous plant belonging to the family Myrsinaceae and the genus *Labisia Lindl.* Three different species of *Labisia pumila* are identified. These varieties are differentiated on the basis of characteristic features of the petioles. The three varieties of *Labisia pumila* are *L. pumila var. pumila, L. pumila var. alata and L. pumila var lanceolate.* This plant grows wild in the tropical rain forests about 80-100 meters above sea-levels in Malaysia, Thailand, Indo-China, Philippines and New Guinea.

Plant thrives in shady places. Open sunlight or even partial shade may be harmful to the establishment and growth of this plant. This is a relatively slow growing herb. It is normally propagated through seeds in the nursery under high humidity and shade. It thrives on rich (plenty of humus) moist sandy loam soil. It needs liberal fertilizing and does well with NPK (Nitrogen, Phosphate and Kalium) blue special and cow dung.

Though *Labisia pumila* is commonly called "Kacip Fatimah", it has many names locally such as Selusoh Fatimah, Rumput Siti Fatimah, Akar Fatimah, Tadah Matahari, Bunga Belangkas Hutan and Pokok Pinggang (Benjamin, 1988).

Morphology of *Labisia pumila*

Labisia pumila is a small herbaceous under shrub, rooting from the stem. The leaves are usually 4-12 in number pointing upwards. The leaves are elliptic-lanceolate and acuminate, tip pointed and base tapered or rather broad-rounded. The whole leaf is about is about 5-35cm long and 2-8cm wide, dark green on adaxial and lighter green on abaxial. The petiole of the leaf is usually 2-8 cm but may reach 12 cm long. The leaf-blade runs down to form a broad or narrow wing, or often absent. Flowers of *Labisia pumila* are very small, pink or white in colour and appear in spike like panicle of small cluster. The flowers are 6-30 cm long having sepals, petals and stamens (Figure 1). The petals of the flowers are wrapped round and enclose the stamens (Zhari *et al.*, 1999).

Figure 1: Herbarium picture of *Labisia pumila.*

Components isolated from *Labisia pumila*

The novel benzoquinoid compounds 1, 2 were isolated as major components from *L. pumila var. alata* (LPA) leaves and roots and their structure determined by spectroscopic method. The plant also is reported to contain large quantity of metals, particularly tin, and aluminium (Houghton *et al.*, 1999). An appreciable quantity of iron was detected in the leaves and roots of *L. pumila var. alata* (Jamia and Houghton, 2000a).

Medicinal uses of *Labisia Pumila*

Labisia pumila is used effectively to treat dysentery, rheumatism and women's ailment associated with childbirth. The decoction of the root or whole plant is administered as a post partum medication to help contract the birth channel and help the mother to regain body strength. The decoction is also used for firming and toning of abdominal muscles of the mother after parturition. The plant material is also found to assist women to achieve fuller and firmer breast and tighten vaginal muscles (Houghton *et al.*, 1999). The extracts of *Labisia pumila var. alata* contain iron. So, the administration of the decoction of the plant before childbirth may indicate that the plant might prevent anemia (Jamia and Houghton, 2000a).

Pharmacological actions of *Labisia pumila*

Labisia pumila prevents painful or difficult menstruation. It is being used by local women to induce childbirth or post-partum, the possibility of it being a phytoestrogen (plant estrogen) is highly likely. The phytoestrogen are present that act as abortifacients or contraceptives. When the estrogen level is high at later stage of pregnancy, it may help to sustain energy in difficult or prolonged labour. It is also believed that estrogen mediates increase in oxcytocin receptors in myometrium. The presence of compounds having estrogenic activity was investigated on hot water and ethanolic extracts of both varieties of *L. pumila* by performing an *in vitro* estrogen bioassay (Jamia *et al.*, 1998).

The leaf and root of *L. pumila var. alata* can cause contact dermatitis to hypersensitive individuals. The allergic reaction could be due to the presence of either alkenyl resorcinals or metals such as nickel. Therefore, sensitive individuals must avoid direct contact with *L. pumila* to prevent the occurrence of potential side effects. Oral consumption of this plant may also induce skin allergy (Jamia *et al.*, 2000b). No other reports are available regarding the pharmacological studies of the plant.

Noise stress

Noise is a strong stress factor for both human beings and animals. In public places and occupational environments, it is considered as a health hazard. This problem is more pertinent particularly in occupational environments where exposure to noise is unavoidable.

The auditory effects of noise are well known. But, the non auditory effects of noise stress are studied less extensively. The information available on the non auditory effects does not lead to definite conclusions. According to previous studies the exposure to continuous noise stress could lead to detrimental physiological function. The spleen and thymus lost weight after exposure to noise stress (Sembulingam et al., 1998; Sembulingam et al., 2000).

The stress animals also showed a reduction in total leukocyte count. The neutrophil percentage was increased but, there was reduction in percentage of

eosinophils and lymphocytes. The plasma corticosterone level was significantly elevated after the exposure to acute noise (Sembulingam *et al.*, 1996).

Lacunae in the literature

1. Perusal of literature reveals that *Labisia pumila* has not been studied yet for its anti stressor properties.

2. In addition, its actions on leukocytes or different organs are also not studied so far.

Objectives

The objectives of this study are:

1. To determine the effect of *Labisia pumila var. alata* on organ weight and leukocyte count
2. To determine the effect of Labisia *pumila var. alata* on changes in organ weight and leukocyte count induced by acute noise stress.

Materials and methods

The protocol of the study was approved by the Animal Ethical Committee of The University.

Extraction of plant material

A fresh *Labisia pumila var. alata* plant was collected from Gua Musang, Kelantan. The plants were completely dried under sunlight.

The aqueous extract of the plant was used for the study. The plant of LP that consists of the stem, leave and root was used. The plant was washed and dried in an oven at 40-42°C for a few days until it is dried completely. The dried parts were then ground into small pieces using a blender. The aqueous extraction of the plant material was obtained by sohlet apparatus. The extraction procedures were carried out for three days. The extract was transferred into a clean container until it cool. After that, the extract was rotavaporised until the volume in the container was reduced to about 30 ml and was allowed to cool down further. The extract was then kept in the freezer at -20°C and freeze-dried. The final product was stored at 4°C – 6°C until it was used for experimental purpose.

Fosage of *Labisia Pumila*

The product of LP extract was dissolved in distilled water at the dilution of 10g/100ml and the resultant suspension was used without filtration. For experimental use, this preparation was administered orally at a dose of 100mg/kg body weight. The dose of the extract was determined in view of the results of a pilot study, and the previous studies. Animals were administered with the extract orally by gavage method.

Stress procedure

The rats were exposed to stress, that is white noise for 30 minutes. The rats were placed in a noise stress chamber. Machinery was recorded in an audio cassette. This white noise were amplified and fed into a loud speaker fixed at the roof of the chamber. The intensity of the noise was fixed between 100 to 110 dB using digital sound level meter.

Experimental Animals

Twenty four male Albino rats of the Wistar strain were used for this study. All the animals were of the same age group with the body weight ranging between 180 and 200g. All animals were maintained under standard laboratory conditions. Food and water were freely available to all the animals. One week before the onset of the experiment, each rat was tested for auditory sense by observing its behavioral response to sound (Dickens, 1973). The rats were randomly divided into four equal groups, each group consisting of six rats.

Group 1: Control Group

The rats were sacrificed without the administration of the extract and without exposure to noise.

Group 2: Stress Group

The rats of this group were exposed to 30 minutes white noise and then sacrificed immediately.

Group 3: Stress with LP extract

The rats of this group were administered daily with extract of LP orally for 7 days. On the 8^{th} day, these rats were exposed to noise stress for 30 minutes and sacrificed immediately.

Group 4: LP extract

The animals of this group were administered with extract of *Labisia pumila var. alata* daily for 7 days. On 8^{th} day, these rats were sacrificed.

All the animals were given mild ether anesthesia before sacrificing. The blood sample was collected using the technique of Feldmen and Conforti (1980) to avoid further stress. The blood was collected directly from the heart, mixed with EDTA and used for haematological studies. Then the organs, adrenal glands, spleen and thymus were removed.

WBC count

The total count and differential count of leukocytes were enumerated from the blood sample. Enumeration of total leukocyte count was done by using computerized cell counter (SYSMEX – KX – 21N model). For differential count, blood smears were taken and the smear was stained by Wright's stain. Since the basophils and monocytes were in negligible number, only the percentage of neutrophils, eosinophils and lymphocytes were considered in the study.

Organ weight

All the organs were blotted dry and weighed using a digital electronic balance. The relative organ weight/body weight was expressed in mg/g of the body weight.

Parameters

1. Total WBC count
2. WBC differential count
    a) Neutrophil percentage
    b) Eosinophil percentage
    c) Lymphocyte percentage
3. Adrenal weight/body weight ratio
4. Thymus weight/body weight ratio
5. Spleen weight/body weight ratio

Statistical analysis

The data of result was analyzed using computerized statistical software SPSS programme. The result of each parameter was analyzed by applying the "One Way Analysis of Variance" (ANOVA).

This was followed by a post-hoc range test, Tukey's Multiple Comparison test, to determine the significant difference of means between the groups for each parameter. The significance level was fixed at $p<0.05$.

Results

The results of total leukocyte count, percentage of neutrophil, eosinophil and lymphocyte and the organ weight/ body weight are given in separate tables and figures. The three tables are used to describe each parameter. The first table shows Mean ± Standard error of mean (SEM), the F-test ratio and its significance. The second table shows the results of Tukey's Multiple Comparison Test and the third table shows the summary of the result. The data of result for each parameter are presented in a bar diagram. The bar diagram is used to differentiate result of each group for each parameter.

Total WBC count

The total WBC count (TLC) in rats was decreased significantly ($p < 0.001$) after exposure to acute stress. According to Tukey's multiple comparison test, there was a significant difference between the stressed animals and all the other groups.

The extract of LP caused the prevention of this decrease in total WBC count induced by noise. The multiple comparison test showed that administration of LP for seven days did not alter the TLC from the control values. (Table 1, 2, 3; figure 2)

Neutrophil percentage

The result of ANOVA test revealed that there is significant increase in the neutrophil count after exposure to noise. The Tukey's multiple comparison test showed alteration in neutrophil count in rats exposed to acute noise.

The pretreatment of rats with LP prevented the increase of neutrophil count in group 3 where the rats exposed by noise stress (Table 4, 5, 6; figure 3).

Eosinophil percentage

The eosinophil count was significantly ($p< 0.001$) decreased in animals that were subjected to stress as shown by ANOVA. The Tukey's test also showed significant decrease in eosinophil count.

Pretreatment of animals with the extract of LP prevented the decrease in eosinophil count induced by stress. The multiple comparison test showed that administration of LP for seven days did not alter the eosinophil count from value of control group (group 1) (Table 7, 8, 9; figure 4).

Lymphocyte percentage

The acute noise stress caused a significant ($p < 0.001$) reduction in lymphocyte count of rats. Tukey's test also showed significance between control and stressed rats.

Pretreatment of rats with LP extract for seven days before the exposure to noise stress (Group 3) prevented the changes in lymphocyte count after exposure to acute noise. (Table 10, 11, 12; figure 5)

Table 1: Total WBC Count

Group		Total WBC Count (per cu.mm)
1. Control	(6)	12,167 ± 522
2. Stress	(6)	4,450 ± 443
3. Stress with LP	(6)	11,650 ± 814
4. LP	(6)	9,483 ± 853
F – Test Ratio (3 , 20) =		26.657
Significance		p < 0.001

Values are expressed as Mean ± SEM
Number shown in the parenthesis indicates the number of animals used

Table 2: Tukey's Multiple Comparison Test for Total WBC Count

Dependent Variable: TC
Tukey HSD

(I) GROUP	(J) GROUP	Mean Difference (I-J)	Sig.
Control Group	Stress Group	7717*	.000
	Stress + Extract Group	517	.949
	Extract Group	2683	.052
Stress Group	Control Group	- 7717*	.000
	Stress + Extract Group	- 7200*	.000
	Extract Group	- 5033*	.000
Stress + Extract Group	Control Group	- 517	.949
	Stress Group	7200*	.000
	Extract	2167	.145
Extract Group	Control Group	- 2683	.052
	Stress Group	5033*	.000
	Stress + Extract Group	- 2167	.145

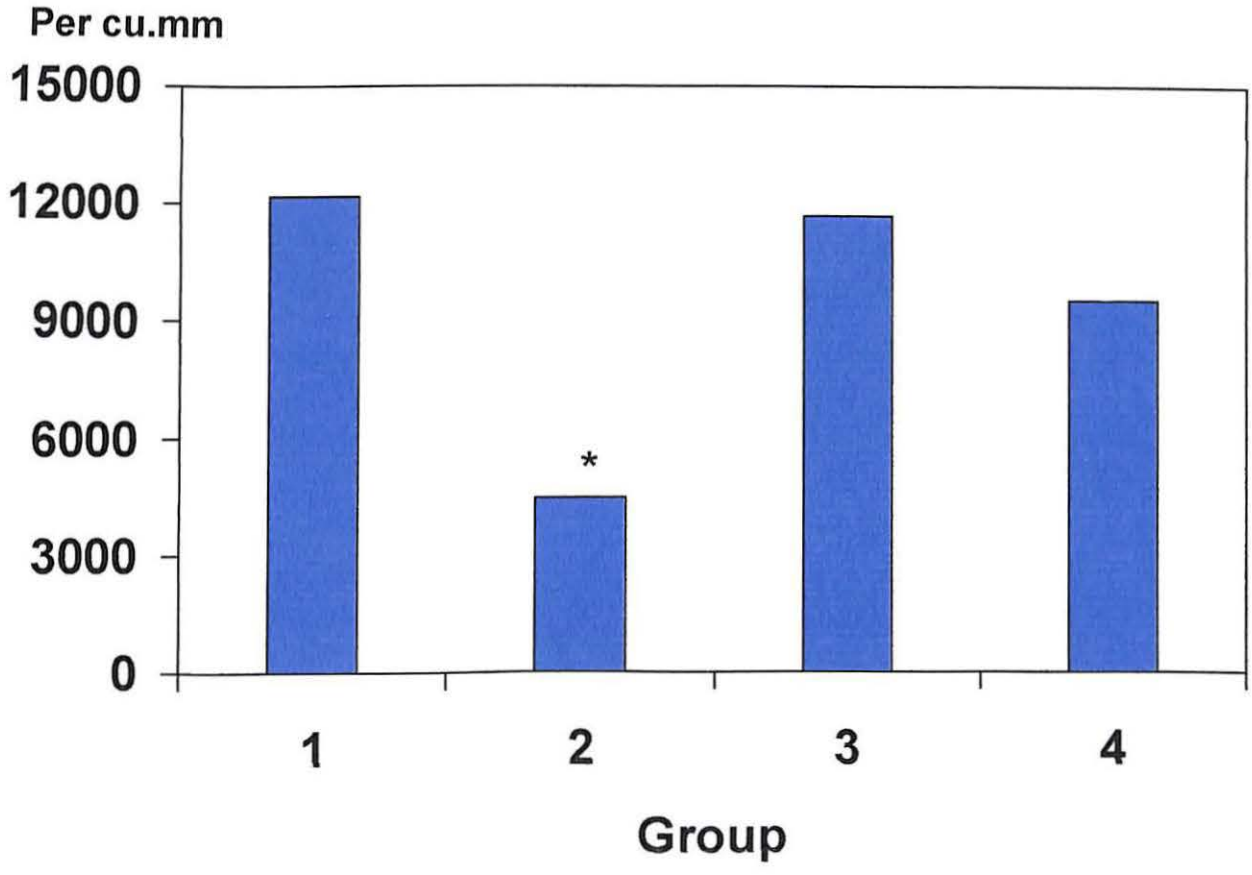
* The mean difference is significant at the .05 level

Table 3: Summary of Tukey's Multiple Comparison Test for Total WBC Count

Group	1. Control	2. Stress	3. Stress with LP	4. LP
1. Control	-	S	NS	NS
2. Stress	S	-	S	S
3. Stress with LP	NS	S	-	NS
4. LP	NS	S	NS	-

S : Significance
NS : No Significance
Significance level = $p < 0.05$

Figure 2: Total WBC Count



* Significance level $p < 0.05$

Group 1 : Control Group
Group 2 : Stress Group
Group 3 : Stress with LP Group
Group 4 : LP Group

Table 4: Neutrophil Percentage

Group		Neutrophil (%)
1. Control	(6)	31.50 ± 1.232
2. Stress	(6)	45.83 ± 1.939
3. Stress with LP	(6)	32.33 ± 0.989
4. LP	(6)	33.83 ± 1.249
F – Test Ratio (3 , 20) =		23.031
Significance		$p < 0.001$

Values are expressed as Mean ± SEM
Number shown in the parenthesis indicates the number of animals used

Table 5: Tukey's Multiple Comparison Test for Neutrophil Percentage

Dependent Variable: NEUTROPH
Tukey HSD

(I) GROUP	(J) GROUP	Mean Difference (I-J)	Sig.
Control Group	Stress Group	-14.33*	.000
	Stress + Extract Group	-.83	.974
	Extract Group	-2.33	.646
Stress Group	Control Group	14.33*	.000
	Stress + Extract Group	13.50*	.000
	Extract Group	12.00*	.000
Stress + Extract Group	Control Group	.83	.974
	Stress Group	-13.50*	.000
	Extract Group	-1.50	.872
Extract Group	Control Group	2.33	.646
	Stress Group	-12.00*	.000
	Stress + Extract Group	1.50	.872

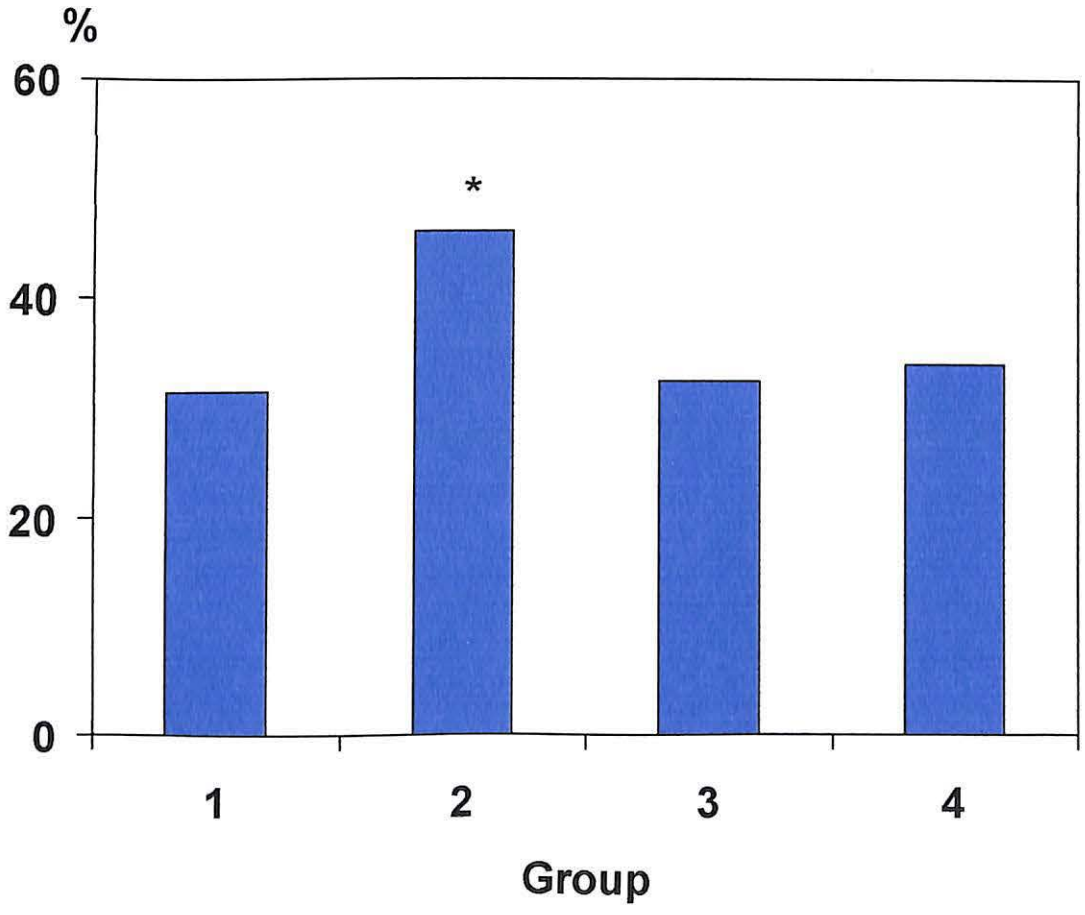
*. The mean difference is significant at the .05 level.

Table 6: Summary of Tukey's Multiple Comparison Test for Neutrophil Percentage

Group	1. Control	2. Stress	3. Stress with LP	4. LP
1. Control	-	S	NS	NS
2. Stress	S	-	S	S
3. Stress with LP	NS	S	-	NS
4. LP	NS	S	NS	-

S : Significance
NS : No Significance
Significance level = $p < 0.05$

Figure 3: Neutrophil Percentage



* Significance level $p < 0.05$

Group 1 : Control Group
Group 2 : Stress Group
Group 3 : Stress with LP Group
Group 4 : LP Group

Table 7: Eosinophil Percentage

Group		Eosinophil (%)
1. Control	(6)	2.33 ± 0.211
2. Stress	(6)	0.33 ± 0.211
3. Stress with LP	(6)	2.00 ± 0.258
4. LP	(6)	2.17 ± 0.167
F – Test Ratio (3 , 20) =		18.737
Significance		$p < 0.001$

Values are expressed as Mean ± SEM
Number shown in the parenthesis indicates the number of animals used

Table 8: Tukey's Multiple Comparison Test for Eosinophil Percentage

Dependent Variable: EOSINOPH
Tukey HSD

(I) GROUP	(J) GROUP	Mean Difference (I-J)	Sig.
Control Group	Stress Group	2.00*	.000
	Stress + Extract Group	.33	.693
	Extract Group	.17	.945
Stress Group	Control Group	-2.00*	.000
	Stress + Extract Group	-1.67*	.000
	Extract Group	-1.83*	.000
Stress + Extract Group	Control Group	-.33	.693
	Stress Group	1.67*	.000
	Extract Group	-.17	.945
Extract Group	Control Group	-.17	.945
	Stress Group	1.83*	.000
	Stress + Extract Group	.17	.945

*. The mean difference is significant at the .05 level.