IOSR Journal of Dental and Medical Sciences (IOSR-JDMS)
e-ISSN: 2279-0853, p-ISSN: 2279-0861.Volume 17, Issue 12 Ver. 7 (December. 2018), PP 81-85
www.iosrjournals.org

Effect of 4-Allyl-2-Methoxyphenol (Eugenol) On Red Blood Cells In Subacute Restraint Stress Induced Wistar Albino Rats

Pandian M[1], Padmaja R Desai[2], Ravindran Rajan[3]
[1,2]*Department of Physiology, D.Y. Patil Medical College, Kolhapur*
[3]*Department of Physiology, IBMS, University of Madras, Chennai*
Corresponding Author:Padmaja R. Desai

Abstract:
Objective*: This study aims to evaluate the effect of 4-Allyl-2-Methoxyphenol (Eugenol)on Red Blood cells in restraint stress induced wistar albino rats.*
Methods:*For each test, respective animal models were used. Animals were divided into five groups of six animals each as Group I Negative control (Unstressed, untreated), Group II Positive control (received vehicle alone Polyglycerol {PG}), Group III treated alone(TA) with Eugenol (150 mg/kg b.w.), Group IV Restraint stress alone (SA), Group V treated with Eugenol and restraint stress (T/S) (150 mg/kg b.w.). The treatment was given for 15 days.At the end of the 15thday,the hematological parameters (RBC and relative parameters)were measured.*
Results: *The TA group (**6.40±0.43**×10^{6}/mm^{3})**, SA group(**3.89±0.16**×10^{6}/mm^{3})** and T/S **(5.51 ± 0.77**×10^{6}/mm^{3})**group had a significantly (P<.05) less RBC count compared with the control**(7.62 ± 0.08×10^{6}/mm^{3})** and PG **(7.46 ± 0.25**×10^{6}/mm^{3})**groups. The PCV of theTA (150mg), SA and T/S (150mg) group was significantly higher than that of the control (P<.001).The SA group (**10.5±0.52** g/dl) had a significantly (P<.001) less Hb concentration than the control (**15.16±0.71** g/dl) and treated groups, (**14.83±0.71** g/dl in TA group and **14.66±0.77****g/dl in T/S group). The MCV of the T/S150mg group was significantly higher than that of the TA 150mg group. However, the MCV,MCH and MCHC values of the control, T/S150mg and TA 150mg groups were not significantly different from that of the control group.*
Conclusion:*The extract (dose 150mg) of Eugenol does not make any changes in erythrocyte count. On intraperitoneal administration of Eugenol, normal red blood cell count, packed cell volume, and hemoglobin level were increased in treated animal with 150mg doses.*

Keywords:*Eugenol, Hematological Parameter,Wistar Albino Rats.*

Date of Submission: 07-12-2018 Date of acceptance: 22-12-2018

I. Introduction

Eugenol (4-allyl-2-methoxyphenol), is one such phenolic compound present in clove, basil, tulsi, cinnamon and nutmegincluding Eugenia caryophyllus, Oscimum sanctum Linn and Myristica fragrans. It is widely used as a flavoring agent in baked foods, sweets, beverages, frozen dairy products and in cosmetics. It is a pale yellow color liquid and is a major component of essential oils isolated fromEugenia caryophyllata (Myrtaceae). Eugenolis used in dentistry as a filling material with zinc oxide and a pulp-capping agent or as a sedative agent(Markowitz et al., 1992)[1]. In traditional medicine, Eugenolhas been used in the treatment of flatulence, cholic, chronic diarrhea and other gastrointestinal disorders (Pruthi, 1976)[2]. It is considered non-mutagenic, non-carcinogenic and generally recognized as safe (GRAS) by Food and Drug Administration. Eugenol is well known antioxidant and anti-inflammatory activities.

Eugenol ameliorates gamma-radiation induced clastrogenic effects (Tiku et al., 2004)[3] and genotoxin-induced DNA damage (Abraham, 2001)[4] in vivo.In addition, the hepatoprotective effect of eugenol on carbon tetrachloride-induced liver damage has also been proved (Kumaravelu et al., 1995)[5].These rats had been selected for their ability to remain on the resolving bar for a period. After the administration of Eugenol (150 mg/kg, Intraperitoneal) each rat was placed on hematology lab to check their Blood indices andhematological parameters. Although many scientists have subjected Eugenol to such vast research, the exact mechanism for its mode of neuro-protection against eugenol and motor coordination remains unclear.

Thus, the current study isdesigned to investigate the effects of Immobilization stress and stress-associated hormonal changes andHematological parameters in female Wistar albino rats.

II. Aims And Objectives

1. To investigate the protective potential of Eugenol in restraint stressinduced Wistar albino female rats.
2. Evaluate the effect of 4-Allyl-2-Methoxyphenol (Eugenol) on Red Blood Cells in female Wistar albino rats.

III. Materials And Methods

The Institute's Animal Ethical Committee (IAEC no. 01 /17 /2015) and the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA) approved the study. Experimental animals were all healthy adult female wistar albino rats, weighing 150 – 220 g. All the animals experimentation involved in this work was done in accordance with national and institutional guidelines for the protection of animal welfare.

Experimental Groups:

In this study, five groups randomly divided and each group consisted of 6 animals. Eugenol was used for this study. (2-Methoxy-4-[2-propenyl])phenol ($C_{10}H_{12}O_2$) was purchased from Sigma Chemical Industry). Group I animals were Control. Group II animals were administrated with vehicle polyglycerol (PG) IP for 15 days and Group III animals were administrated with Eugenol (treated alone - TA) 150 mg/kg.b.wt for 15 days. Group IV subjected to immobilization stress alone (SA) for 15 days (6 hr/day) and immobilization stress induced changes were observed in this group. Group V animals were treated with Eugenol 150 mg/kg.b.wt along with immobilization stress (treated with stress T/S) for15 days. The doses were selected according to the lethal dose (LD_{50}). Eugenol was administered Intraperitoneally. Blood samples were collected from the animal through Tail vein into EDTA bottles after anaesthetizing the animals with chloroform at the 15^{th}day of the experiment for hematological studies.

Blood sample collection

Lateral Tail Vein or Ventral/Dorsal Artery Sampling:

Sample collection were performed easily by nicking the vessel. Sample collection using a needle (cannulation) minimizes contamination of the sample.Adequate volumes were obtained by cannulation or nicking: medium to large size artery. The vein small in general arterial sampling produces larger volumes and is faster, but precaution must be taken for adequate hemostasis (Christensen SD et al .2009)[6]. Hence, for larger volumes for blood sample artery shouldbe used. This procedure can be used for multiple small samples in case of serial testing(Kurawattimath V et al .2012)[7]. To collect adequate blood volume the tailed is warmed with the help of circulating warm water or warm compresses. Cannulation and tail nicking procedureis routinely done without anesthesia, although effective restraint is required.

Hematology:

Hematological parameters were measured in 30Wistar albinorats (erythrocyte count, hemoglobin levels, means cell volume, mean cell hemoglobin levels, mean cellhemoglobin concentrations).

Determination of hematological indices

Red blood cell (RBC) count was done using the conventional method of Dacie and Lewis (2001). Hayem's fluid was used for red blood cell counting; blood was diluted to 1:200 andTurk's fluid was used for total white blood cell count in a ratio of 1:20then counted with an improved Neubauer counting chamber under a light microscope.The conventional method (Sahli's hemoglobin-meter) was employed for estimation of hemoglobin (Hb) content of the blood, and packed cell volume (PCV) was done using the macro-hematocrit method (Dacie and Lewis, 2001)[8].

Eugenol

Eugenol, Allyl chain-substituted guaiacol (2-methoxyphenol), is a clear to pale yellow oily liquid extracted from certain essential oils especially from clove oil and cinnamon.

Assay of Corticosterone:

This method was carried out with slight modification from (Singh &Verman 2008)[9] and is based on the oxidation of corticosteroids with ferric iron (III) in acidic medium and subsequent complex with ferrous iron (II) and potassium hexacyanoferrate. 0.5µl of Plasma samples were mixed with appropriate volumes of working solutions of corticosterone and then were transferred into a series of 10 ml volumetric flasks. Sulphuric acid (4N, 2ml) and ferric chloride (0.5%, 2 ml) were added to each followed by potassium hexacyanoferrate (III) solution (0.5%, 0.5 ml). The mixture was heated in a water-bathwith occasional shaking. It maintained at 70±2ºC for 30 minutes and diluted to the 5ml mark with distilled water. The absorbance was measured at 780 nm against the reagent blank.

Statistical Analysis:

The statistical software package SPSS 20 version for windows 7 was used to analyze the data. Statistical analysis was undertaken by using ANOVA Tukey's multiple comparison tests. P<0.05 was considered statistically significant. From this we can understand, control is significant with stressed and treated animals.

IV. Results And Discussion

1) Effect of 4-Allyl-2-Methoxyphenol (Eugenol) on RBC in rats:

The mean RBC count of the Control, PG, TA *(*150mg*)* SA and T/S 150mg, groups were **7.62 ± 0.08**,**7.46 ± 0.25, 6.40 ± 0.43, 3.89 ± 0.16*** and **5.51 ± 0.77******×10^{6}/mm^{3}** respectively. The TA *(*150mg*)*, SA and T/S *(*150mg*)* group had a significantly (P<.05) less RBC count compared with the control and PG groups. The mean PCV was **86.6±0.77**% in the control group, **81.6 ±1.40**% in the PG group, **83.3±0.49%** in the TA (150mg), **60±0.85***% in the SA group, and **76.6±0.77****% in the T/S *(*150mg) group. The PCV of the TA *(*150mg*)*, SA and T/S *(*150mg*)* group was significantly higher than that of the control (P<.001).The mean Hb concentrations were control **15.16±0.71** g/dl, PG **14.83±0.71** g/dl, **14.83±0.71** TA (150mg), **10.5±0.52*** SA and **14.66±0.77**** g/dl T/S(150mg) for the respective groups. The SA group had a significantly (P<.001) less Hb concentration than the control and treated groups. (Table 1)

Table*:* **1** Effect of different doses of 4-Allyl-2-Methoxyphenol (Eugenol) on hematological parameters:

Haematological Indices	Group I Control	Group II PG	Group III TA150mg	Group IV SA	Group V T/S 150 mg
RBC (×10^{6}/mm^{3})	7.62±0.08	7.46±0.25	6.40±0.43	3.89±0.16*	5.51±0.77**
PCV (%)	38.3±3.4*	38.16±1.4	48.1±1.4*	44.8±2.6*	41.6±4.6
Hb (g/dl)	15.16±0.71	14.83±0.71	14.83±0.71	10.5±0.52*	14.66±0.77**
MCV (fl)	50.29±4.05	51.01 ±2.62	75.69 ± 7.36	-----	76.66±12.91**
MCH (pg)	20.14±1.15	19.87±1.17	23.23±1.44	26.99±1.54*	27.09 ±4.77**
MCHC (g/dl)	39.83±4.15	38.87±1.64*	30.80 ±2.10*	23.48±1.94*	35.66 ±5.3**

MCV: Mean corpuscular volume; MCH: Mean corpuscular hemoglobin; MCHC: Mean corpuscular hemoglobin concentrationLang,P.L.[10] , Johnson-Delaney [11]

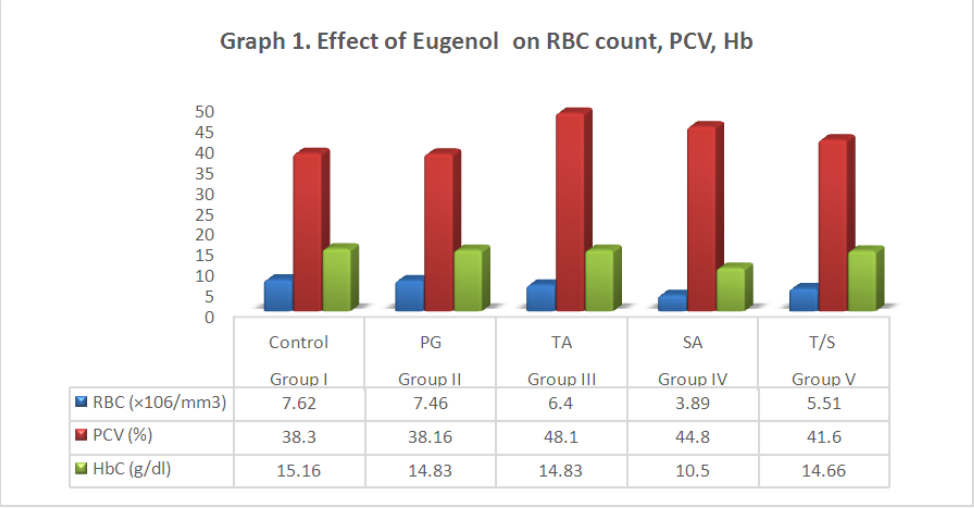
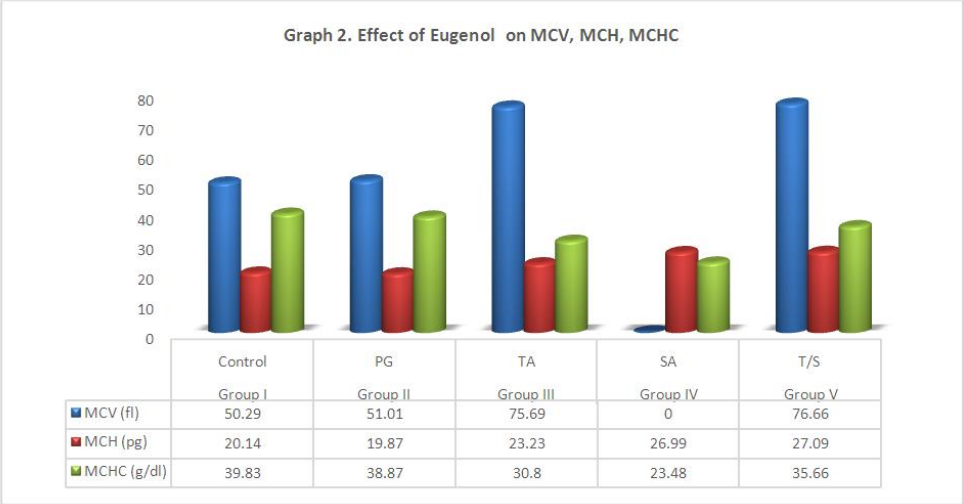


Figure – 1.1

1) Effect of 4-Allyl-2-Methoxyphenol (Eugenol) on RBC indices in rats:

The MCV for the control group was **50.29±4.05** fl, while the values for the TA 150mg and T/S150mg were **75.69 ± 7.36** fl and **76.66±12.91** fl, respectively. The MCV of the T/S150mg group was significantly (P<.01) higher than that of the TA 150mg group. However, the MCV values of the T/S150mg and TA 150mg groups were not significantly different from that of the control group. The MCH and MCHC of the control, TA150mg, and T/S150mg groups were not significantly different from one another. (Table 1)



V. Discussion

The effect of eugenol on hematological parameters in rats was investigated in this study. The extract (dose 150mg) of Eugenol does not make any changes in erythrocyte count. The normal range of hematocrit (PCV) confirmed this.The percentage of Hb decreased in the TA and T/S recipient group. The total WBC (leucocyte) counts were not significantlyaltered following extract administration.Similar findings were seen in a study by **OE Ofem et al[12],** they found that the HD (High dose) group had significantly (P<.05) higher red blood cell (RBC) counts, packed cell volume (PCV), hemoglobin (Hb), and platelet counts as compared with the control and LD (Low dose) groups. The mean corpuscular volume (MCV) was significantly (P<.05) lower in the HD group than in control. No significant changes were observed in levels ofmean corpuscular hemoglobin (MCH) and mean corpuscular hemoglobin concentration (MCHC) among the groups. Oral administration of Ogratissimum increases RBC, PCV and Hb. Our study is at variance with the earlier study by **Jimoh *et al.*[13]**who reported decrease in these same hematological parameters following administration of *O. gratissimum* in Wistar rats. They attributed the decrease to the presence of saponins in the extract.

VI. Conclusion

On intraperitoneal administration of Eugenol, there was normal red blood cell count, packed cell volume but hemoglobin level decreased in treated animal with 150 mg doses / kg of b.w.This study tells us that the dose that we administrated did not significantly affect the hematological parameter except hemoglobin level. Therefore, further study is needed to determine the effect of eugenol with increased dose with LD50.

Acknowledgement

I express my profound thanks to my professor Dr. R. Ravindran, Ph.D., Assistant Professor and Malathy Dept. of Physiology, Dr.A.L.M.PG IBMS, University of Madras, without him this work would not have completed successfully. I wish to express my gratitude for this constant encouragement, valuable suggestion and advise throughout the work. I express my heartfelt thanks to all teaching, research scholars and non-teaching staffs of Dept of Physiology Dr.A.L.M.PG IBMS, University of Madras.

Reference

[1]. Markowitz K, Moynihan M, Liu M, Kim S (1992) Biologic properties of eugenol and zinc oxide-eugenol. A clinically oriented review. Oral Surg Oral Med Oral Pathol 73: 729-737.

[2]. Pruthi J.S. Species and Condiments, 1st ed, National Book Trust: New Delhi, India, 1976; 91–98

[3]. Tiku AB, Abraham SK, Kale RK. Eugenol as an in vivo radioprotective agent. J Radiat Res. 2004 Sep;45(3):435-40.

[4]. Abraham RT. Cell cycle checkpoint signaling through the ATM and ATR kinases. Genes Dev. 2001;15:2177–2196.

[5]. Kumaravelu P, Subramaniyam S, Dakshinamoorthy DP, Devaraj NS. The antioxidant effect of eugenol on CCl4 -induced erythrocyte damage in rats. J Nutr Biochem. 1995;7:23–8.

[6]. Christensen SD, Mikkelsen LF, Fels JJ, Bodvarsdottir TB and Hansen AK. Quality of plasma sampled by different methods for multiple blood sampling in mice. Laboroatory Animals (2009) 43: 65-71.

[7]. Kurawattimath V, Pocha K, Mariappan TT, Trivedi RK and Mandlekar S. A modfied serial blood sampling technique and utility of dried-blood spot technique in estimation of blood concentration: application in mouse pharmacokinetics. Eur J Drug Metab Pharmacokinet (2012) 37: 23-30.

[8]. Dacie JV, Lewis SM (2001). Practical Haematology. 11th ed, Longman Group.Ltd. Hong Kong, pp. 11-17

[9]. Singh D. K.,&Verman R. Spectrophotometric Determination of Corticosteroids and Its Application in Pharmaceutical Formulation.Iranian Journal of Pharmacology & Therapeutics. 2008; 7: 61-65.
[10]. Lang P.L., serum chemistry parameters for the Crl:CD*BR Rat, Charles River Laboratories, 1993.
[11]. Exotic Animal Companion Medicine Handbook for Veterinarians, Johnson-Delaney, C., 1996, Zoological Education Network.
[12]. Ofem OE, Ani EJ, Eno AE. Effect of aqueous leaves extract of Ocimum gratissimum on hematological parameters in rats. Int J App Basic Med Res 2012;2:38-42.
[13]. Jimoh OR, Olaore J, Olayaki LA, Olawepo AO, Biliaminu SA. Effects of aqueous extract of ocimum gratissimum on haematological parametersof Wistar rats. Biokemistri 2008;20:33-7.
[14]. Obianime AW, Aprioku JS, Esomonu C. The effects of aqueous Ocimum gratissimum leaf extract on some biochemical and hematological parameters in male mice. Asian J Biol Sci 2011;4:44-52.

Pandian M. "Effect of 4-Allyl-2-Methoxyphenol (Eugenol) On Red Blood Cells In Subacute Restraint Stress Induced Wistar Albino Rats" IOSR Journal of Dental and Medical Sciences (IOSR-JDMS), vol. 17, no. 12, 2018, pp 81-85.