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Effect of Eugenol on Restraint Stress-Induced WBC'S and Corticosterone disturbance in rats

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ABSTRACT:

Background and objectives: The effect of eugenol on white blood cells (WBCs) and corticosterone is known. However, there is a paucity of data regarding the effect of eugenol on restraint stress-induced white blood cells and corticosterone disturbances in rats. The study aimed to evaluate the role of eugenol in the restraint stress-induced white blood cells and corticosterone disturbances in rats. **Methods:** Female Wistar albino rats (n = 30) weighing (150–220 g)were divided into five groups with six animals in each group, Group I:normal control; II: vehicle polyglycerol treated (PG); III: eugenol treated alone -TA (150 mg/kg/i.p for 15 days); IV: stress-induced(SA-stress alone), and V :stress followed by treated (T/S) with . Blood samples were collected at the end of the study and total leucocyte count (TLC), differentiation leucocyte count (DLC), platelet count (PC) and concentration of corticosterone were estimated. **Results:** The TA group showed significant (pwhen compared with other groups, whereas, no significant differences were observed between basophils, monocytes, and eosinophils of T/S when compared with SA group. No significant differences were observed in corticosterone levels in all groups. Interpretation and conclusion: Acute restraint stress-induced distribution of WBC was not significantly attenuated by eugenol and no changes were observed in corticosterone level. However, eugenol increases platelet aggregation.

Keywords: Stress, Eugenol, Total leukocyte count, Differentiation leukocyte count, Platelets, Corticosterone.

I. INTRODUCTION:

The controlled movement of immune cells within various body compartments is crucial for efficient immune monitoring and overall immune health. The bloodstream serves as a vital conduit through which immune cells must circulate to uphold their routine surveillance routes and to promptly access locations of new immune responses, such as injury, antigen exposure, or pathogen invasion. Consequently, the quantity and ratios of white blood cells in the bloodstream serve as a significant reflection of the distribution of white blood cells throughout the body and the status of immune system activity. Multiple investigations have demonstrated that stress can have a substantial impact on the distribution and operation of immune cells.[1, 2] Stress can be described as a series of interconnected events, beginning with a stimulus (referred to as a stressor) that triggers a response in the brain (known as stress perception). This, in turn, sets off the activation of the body's physiological fight-or-flight systems (referred to as the physiological stress response). [3] Depending on the nature, timing, and intensity of the stimulus it receives, stress can induce a wide array of effects on the body. These effects span from disruptions in normal bodily equilibrium to severe consequences, potentially even fatal ones. Stress frequently plays a role in the development of health issues, and individuals exposed to stressful conditions, such as those working or residing in high-stress environments, are at a greater risk of various disorders. Stress can function as a catalyst or exacerbating factor in numerous diseases and pathological states.[4] It's crucial to acknowledge that a stressor's impact on the brain or body solely occurs through the induction of biological changes within the organism. This underscores the vital role played by stress hormones and the physiological stress response. While chronic or prolonged stress can be detrimental, it's worth noting that a stress response often brings about beneficial and adaptive effects in the short term. [4, 5]

It is widely recognized that acute or brief stress triggers a significant repositioning of immune cells within the body. This repositioning pattern is notably consistent across various species, including humans, implying its evolutionary

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significance and its potential role in conferring adaptive benefits. Given the swift onset and substantial scale of immune cell redistribution induced by stress, these stress-related impacts are crucial to consider when assessing immune function, administrating stress hormones for therapeutic purposes, and when collecting, examining, and interpreting both experimental and clinical data. Despite its importance, it's somewhat surprising that immune cell redistribution due to stress has not received as much recognition and remains relatively untapped in clinical applications.[6, 7] The primary stress hormones—norepinephrine (NE), epinephrine (EPI), and corticosterone (CORT)—play a pivotal role in orchestrating the broad-ranging physiological responses of an acute stress reaction. Furthermore, these hormones serve as the primary endocrine mediators governing the distinct phases and distribution patterns of leukocyte subpopulations in response to various stressors, be they psychological or physical (such as exercise). [8, 9] The exploration of the collective impacts of stress hormones is crucial for comprehending the varying roles of NE, EPI, and CORT. These roles may manifest differently based on the specific concentrations and combinations of these hormones elicited in distinct stress scenarios. Corticosterone is a key player in the body's stress response and can have various physiological effects. Norepinephrine (NE) and epinephrine (EPI), rapidly released in response to stress, trigger the movement of immune cells into the bloodstream. In contrast, corticosterone (CORT) reduces the number of immune cells in both the blood and various tissues. [10] The activation of the hypothalamus-pituitary-adrenal (HPA) axis leads to the release of glucocorticoid hormones, namely cortisol or corticosterone (hereafter referred to as CORT), depending on the species. At typical concentrations, CORT plays a role in regulating metabolic processes, activity levels, and feeding behavior. However, when confronted with higher concentrations induced by a stressor, CORT becomes a vital component of the stress response in vertebrates.[11, 12] During this stress response, CORT aids animals in mobilizing energy reserves, enhancing specific components of the immune system, and promoting behaviors related to escape and self-preservation. [13, 14] Nevertheless, persistent elevation of CORT levels due to frequent or prolonged exposure to stressors can result in various stress-related health issues. These may include the suppression of reproductive functions and the immune system, disruptions in metabolic regulation, and cognitive impairment. Chronic stress is believed to occur when CORT and other physiological elements of the stress response shift from being beneficial to being detrimental, hindering the restoration of balance (homeostasis) and the normal activities associated with a particular stage in an animal's life cycle. [15-17] The study examines levels of corticosterone, a stress hormone produced by the adrenal glands, in response to restraint stress. Eugenol (2-methoxy-4-(Prop-2-en-1-yl) is a prevalent essential oil found in abundance within clove oil, nutmeg, cinnamon, and bay leaf. Eugenol finds application in the formulation of skincare products, cosmetics, and is used as a flavouring agent in dental and pharmaceutical products due to its antiseptic and antispasmodic properties.[18]

In traditional medicine, eugenol has been employed for addressing issues like flatulence, colic, chronic diarrhoea, and various gastrointestinal disorders. Eugenol exhibits a range of pharmacological activities, including antioxidant, antibacterial, anti-inflammatory, and antipyretic effects. Studies have shown that eugenol can enhance motor coordination and reduce plasma corticosterone levels in Wistar rats subjected to immobilization stress. Additionally, in vitro experiments have revealed that eugenol can impede leukocyte migration, thus aiding in the inflammatory process. [19-22] The use of eugenol in the study determines whether this compound has a modulating effect on the stress-induced changes in white blood cell counts and corticosterone levels. Eugenol's reported anti-inflammatory and stress-reducing properties make it a candidate for potentially mitigating the adverse effects of stress on the immune system and hormonal balance. [23, 24] This study contributes to the understanding of how eugenol, a natural compound, may offer therapeutic benefits in managing stress-related physiological disturbances, stress management, and immune system support in rats.

II. MATERIAL & METHODS:

a) Chemicals:

Analytic grade eugenol ($C_{10}H_{12}O_2$), a clear to pale yellow oily liquid extracted from certain essential oils especially from clove oil and cinnamon, was purchased from Sigma Chemical Industry.

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b) Animals:

Female Wistar albino rats weighing between 150 – 220 g were part of this study and housed according to the standard conditions. The study 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 study protocol was approved by the Institute's Animal Ethics Committee. Methodology Restraint stress was induced by immobilizing rats in a suitable restraint device for a defined period, as described in the relevant literature or based on specific experimental requirements.

c) Experimental Procedure:

Animals were divided into five groups with six animals in each group. The reported LD50 of pure eugenol was 1.8ml (1.93gm/kg) and 150 mg/kg/ip dose of eugenol for the study. A detailed experimental procedure is illustrated as follows: Group I (normal control) - received a standard diet. Group II - administrated with the vehicle used to emulsify eugenol that was polyglycerol (PG) intraperitoneal for 15 days. Group III - administrated with Eugenol (treated alone TA) 150 mg/kg/i.p body weight for 15 days.Group IV – animals were subjected to immobilization stress alone (SA) for 15 days (6 hr/day) Group V - animals were subjected to stress and immediately treated with Eugenol 150 mg/kg/i.p body weight for 15 days.

d) Immobilization Stress Induction Procedure [25]

Rats were subjected to restraint stress in a wire mesh restrainer for 6 hours per day for 15 days. The wire mesh restrainer (length: 8 cm, breadth: 4 cm, and height: 4 cm) has a wooden base and stainless-steel wire mesh restrainer hinged to the base. A padlock and latch helped to secure the rat in the restrainer.

e) Blood Sample Collection [26]

The blood sample was collected at the end of the study; blood will be collected from the ventral/dorsal artery or lateral tail vein by nicking the vessel and cannulation was done to minimize contamination of the samples. Precaution was taken to avoid the haemostasis.

f) Determination Of Haematological Indices

WBC count was performed by the Dacie and Lewis method.[27] Turk's fluid was used for TLC (1:20) and cell count was done by using a Neubauer counting chamber under a light microscope. DLC was performed using the method of Mathers RA et al.[28]

g) Assay Of Corticosterone

The assay was conducted with a minor adaptation of the method described by Singh and Verman.[29] It relies on the oxidation of corticosteroids using ferric iron (III) in an acidic environment, followed by the formation of a complex with ferrous iron (II) and potassium hexacyanoferrate. To execute the assay, 0.5 µl of plasma samples were mixed withappropriate volumes of working solutions of corticosteroids and transferred into a series of 10 ml volumetric flasks. Subsequently, 2 ml of 4N sulfuric acid and 2 ml of 0.5% ferric chloride were added to each flask, followed by the addition of 0.5 ml of potassium hexacyanoferrate (III) solution. The mixture was then subjected to heating in a water bath, maintaining a temperature of 70±2ºC for 30 minutes, with intermittent shaking. Afterward, the solution was diluted to the 5 ml mark using distilled water, and the absorbance was measured at 780 nm against the reagent blank.

h) Statistical Analysis

Data was analyzed by ANOVA and Tukey's multiple comparison tests using SPSS 20 software.

III. RESULTS

a) Effect Of Eugenol On Wbc Count

The mean WBC count of control and PG group animals was found to be 9.08±0.25x103 /mm3 and 11.02±0.71x103 /mm3 respectively. Whereas in TA, SA, and T/S group animals it was found to be 12.21± 0.28x103 /mm3 , 13.22± 0.63x103 /mm3 , and 12.20±0.48x103 /mm3 respectively (figure 1).

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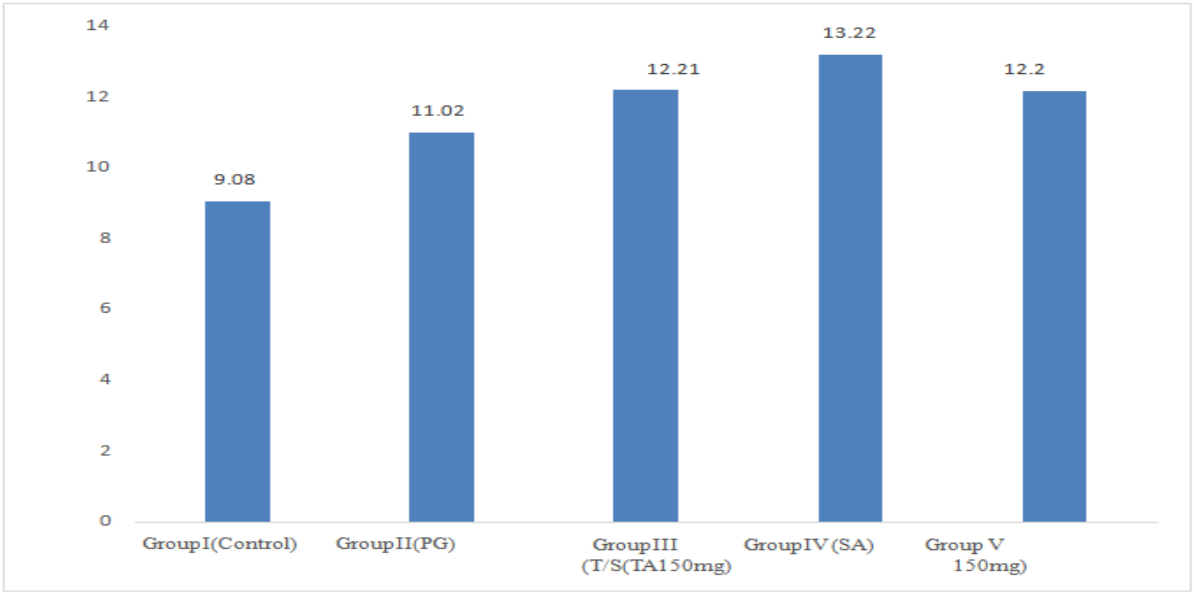

Figure1.Effect of Eugenolon WBC

b) Effect Of Eugenol On Platelets Count

The mean platelets count of control and PG group animals was found to be 274.75±11.92x106 /mm3 and 263.89±6.69x106 /mm3 respectively. Whereas in TA, SA, and T/S group animals it was found to be 193.31± 2.88x106 /mm3 , 66.62± 5.16x106 /mm3 , and 225.41±12.69x106 /mm3 respectively (figure 2).

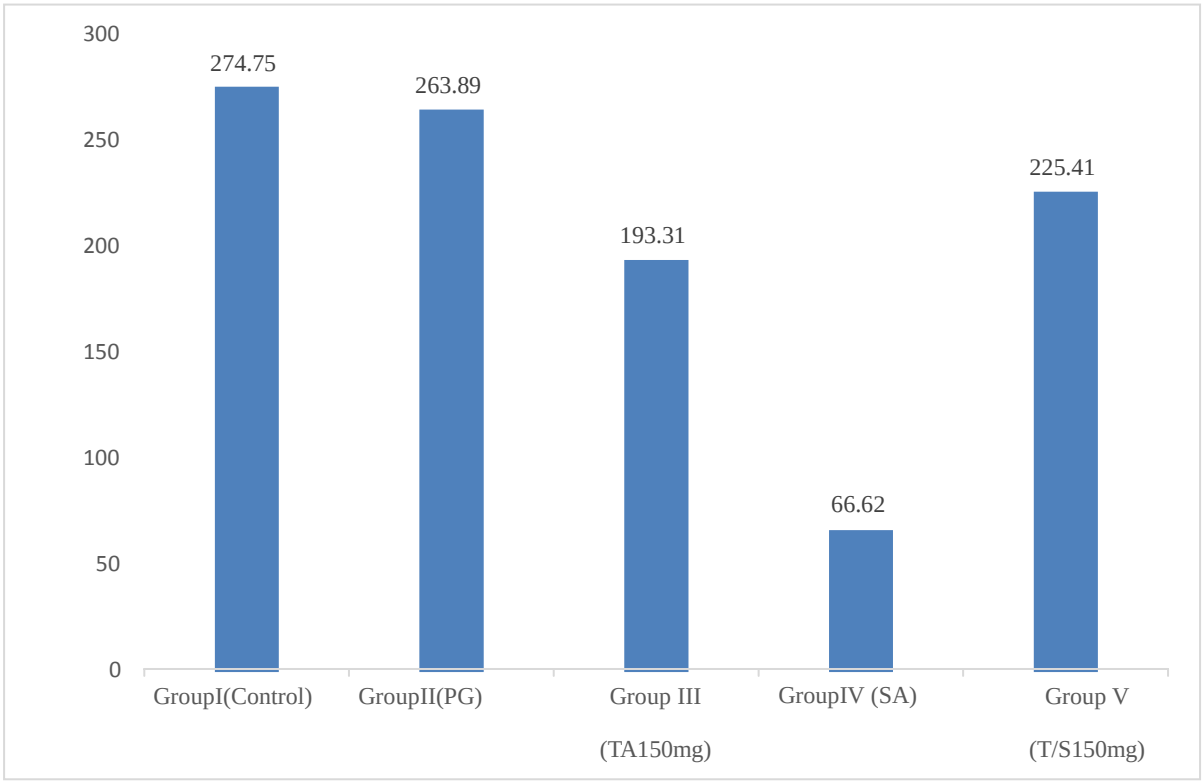

Figure2.Effect of Eugenol on Platelets Count

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c) Effect Of Eugenol On Differentiation Leucocyte Count(Table1)

The differential leucocyte count according to groups is depicted as follows:

a. Comparison Of Neutrophil Count According To Groups

The differential leukocyte count of all groups indicates significantly decreased counts of neutrophils in the SA group when compared with PG, TA, and T/S ($p<0.05$).

b. Comparison Of Lymphocyte Countaccording To Groups

A significantly decreased lymphocyte count was observed in TA when compared with control, SA ($p<0.05$) and PG ($p<0.001$).

c. Comparison Of Basophils Count According To Groups

Basophil count was found to be significantly decreased in the SA group compared to control group animals ($P<0.05$).

d. Comparison Of Monocytes Count According To Groups

Monocytes count was found to be significantly decreased in SA group compared to TA group animals ($P<0.05$).

e. Comparison Of Eosinophils Countaccording To Groups

Eosinophils count was found to be significantly decreased in SA group compared toTA group animals ($P<0.05$).

Groups	Neutrophils, mean, ($\times10^3$/mcL) Pvalue	Lymphocyte, mean, ($\times10^3$/mcL) Pvalue	Basophil, mean, ($\times10^3$/mcL) Pvalue	Monocyte, mean, ($\times10^3$/mcL) Pvalue	Eosinophil, mean, ($\times10^3$/mcL) Pvalue
PGvs Control	0.33	0.45	0.26	0.98	0.63
SAvs Control	0.62	0.95	0.02*	0.75	0.01*
TAvs Control	0.21	0.02*	0.67	0.47	0.99
T/S vs Control	0.17	0.91	0.60	0.99	0.19
SA vs PG	0.01*	0.071	0.55	0.51	0.001*
TA vs PG	0.99	0.000***	0.98	0.38	0.68
T/S vs PG	0.96	0.88	0.98	0.99	0.70
TA vs SA	0.04*	0.008*	0.17	0.02*	0.02*
T/S vs SA	0.02*	0.94	0.1	0.99	0.70
T/S vs TA	0.94	0.001*	1	0.23	0.29

P-pvalue, PG-polyglycerol, TA-treatedalone, SA-stressalone, T/S-stress+treatment,'***'P<0.001, '**' P<0.01,'*' P<0.05

Table1.Comparison of Neutrophils, Lymphocyte, Basophils, Monocytes and Eosinophils count according to groups.

d) Effect Of Eugenol On Corticosterone

The mean plasma corticosterone in group control and PG group animals was found to be1.82±0.05 and 1.81±0.04 respectively. Whereas in TA, SA, and T/S group animals it was found to be 1.61±0.04, 2.12±0.14, and 2.80±0.06 respectively (figure 3).However, no significant differences were observed in the mean plasma CORT concentration of groups when compared with each other.

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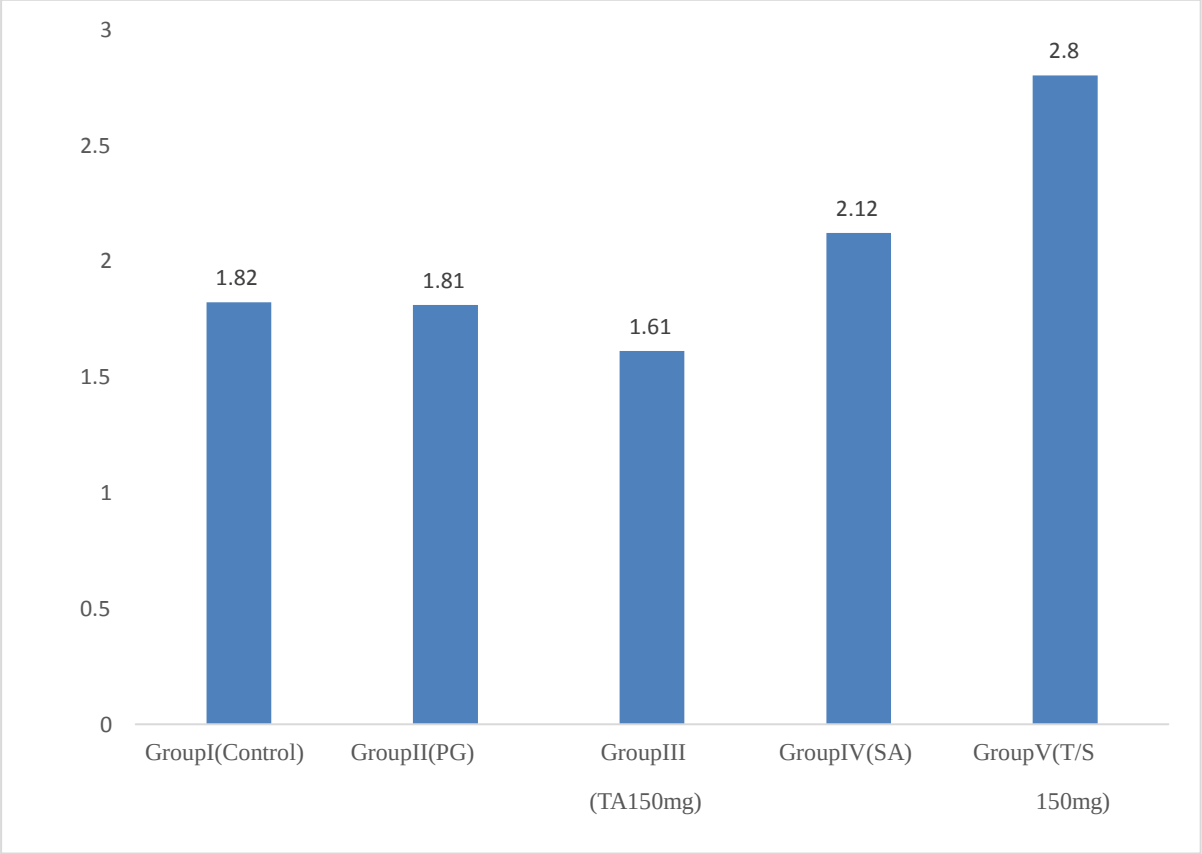

Figure3.Effectofeugenoloncorticosterone

IV. DISCUSSION

The present study was under taken to evaluate the role of eugenol on restraint stress-induced white blood cells and corticosterone disturbances in rats. Stress, whether originating from psychological or physical sources, triggers the release of nor epinephrine and epinephrine. These stress hormones, in turn, lead to an increased release of blood lymphocytes, monocytes, and neutrophils. Conversely, corticosterone has the opposite effect by reducing the presence of immune cells in both the bloodstream and tissues.[2] The accumulation of leukocytes in the blood stream serves to enhance the immune response, ensuring maximum presence of these cells at the site of activation.[7,30]In humans, acute stress leads to an elevation in the distribution of immune cells in the bloodstream compared to a resting state. In contrast, rodents exhibit a reduction in immune cell presence in the blood under acute stress conditions. This disparity may be attributed to the initial gathering of immune cells and may reflect the movement or trafficking of these cells.[2] According to the research conducted by Rosenberger etal., exposure to stress leads to a swift migration of leukocytes within the bloodstream, resulting in a decrease in the counts of monocytes and lymphocytes, alongside an increase in the number of neutrophils. [31] In the current study, the rewash a decrease in leukocyte concentration in rats exposed to stress, which could possibly be attributed to the initial aggregation of these cells and might indicate the movement of immune cells within the body. However, the group treated with eugenol did not exhibit any notable changes in leukocyte counts. Conversely, a temporary reduction in the number of leukocytes in the blood stream suggests that these cells are being directed out of the blood and towards target organs, as observed in previous research.[2] Similarly, in this study, stress-induced animals showed decreased levels of monocytes and neutrophils. These findings are similar to the findings of Pandian M and Desai PR.[10]

The research conducted by Malyszko etal.[32] and Takeda etal.[33] elucidated the effects of acute water immersion restraint and acute cold-restraint stress, respectively, which led to a

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reduction in collagen-induced aggregation in whole blood and ADP-induced aggregation in platelet-rich plasma. Similarly, in our study, we observed a decrease in platelet count in animals subjected to acute restraint stress. However, we observed an increase in platelet count in the groups of animals treated with eugenol and exposed to stress. These findings align with the results reported by Hata et al. and Pandian M. and Desai PR.[34, 10] These findings suggested that eugenol at tenuates stress-induced platelet disturbance. In the research conducted by Pitman etal. [35], it was noted that restrain stress-induced rats exhibited an increase in their baseline corticosterone (CORT) levels on days 2 and 3.However, from days 4 to 6, these levels did not show a significant increase, possibly due to the animals habituating to the stressor. Similarly, the study by Sadler et al. [36] suggested that there was no significant elevation in CORT levels in mice subjected to 14 days of restraint, which could be attributed to their adaptation to the stressor. This indicates that the hypothalamic-pituitary-adrenal (HPA) axis becomes less responsive to repeated restraint stress after 8 or 14 days, as previously noted [37, 38]. In this study, we did not observe anotable rise in CORT levels across the animal groups. This lack of significant increase could potentially be attributed to the animals becoming habituated to the stressor. These findings are similar to the findings of Pandian M and Desai PR. [10] In contrast to the findings of our current study, the research conducted by Pandian etal.[7] reported anotable and significant elevation in plasma CORT levels among the groups of animals subjected to 15 days ofrestraintstresslastingfor6 hours when compared to the control group. However, in our study, the group exposed to both stress and eugenol treatment demonstrated a significant decrease in CORT levels compared to the group subjected to stress alone.[25] The difference in the results may be due to the difference in methods used to assess CORT, type of strain used, etc.

In this study, the introduction of eugenol at a dosage of 150 mg/kg in both the T/S and TA groups over a 15-day period resulted in an increase in total leukocyte count (TLC) when compared to the control group of rats. However, the influence of eugenol on the differential leukocyte count (DLC) did not exhibit a significant difference in terms of neutrophil and lymphocyte counts in the T/S groups when compared to the other groups. Furthermore, nonotable distinctions were observed between basophils, monocytes, and eosinophils in the T/S group compared to the SA group. These findings are similar to the findings of Pandian M and Desai PR. [10] The strength of the study was the adequate sample size and uniform application of protocol. The current study investigated the impact of eugenol on WBC counts and corticosterone levels in rats subjected to sub-acute restraint stress. This study sheds light on the potential therapeutic utility of eugenol in ameliorating stress-induced disturbances in corticosterone levels and WBC counts. The main limitation of the study was blood samples were collected at the end of the study, which could have led to variable results. Therefore, additional research is warranted to assess the influence of eugenol on WBC counts and corticosterone levels at regular intervals and to examine its impact on other stress-related hormones.

V. CONCLUSION

Eugenol did not significantly mitigate the stress-induced redistribution of white blood cells (WBCs), as evident in the group of rats subjected to both stress and eugenol treatment .Moreover, there were no observable alterations in corticosterone (CORT) levels across all animal groups. Consequently, further investigations are necessary to understand how eugenol affects the chemical mediators released during stress, which play a role in the distribution of WBCs and platelets in the bloodstream.

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