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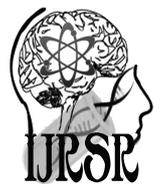
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## Research Article

### EFFECT OF NIGELLA SATIVA OIL AGAINST MONOSODIUM GLUTAMATE - INDUCED TOXICITY ON HEMATOLOGICAL PARAMETERS IN RATS

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MSG, *Nigella sativa* oil, WBC, Neutrophil, Lymphocyte, RBC, PCV, Hb, MCV, MCH, MCHC, Platelet Count, Immunity, Anaemia.

#### ABSTRACT

The present study was undertaken to evaluate the protective effect of *Nigella sativa* oil (NSO) in preventing the alteration in hematological parameters induced by toxic effect of Monosodium Glutamate (MSG) in pubertal male rats. Twenty four pubertal male albino rats were divided into four groups consisting of six each. Rats of groups 2, 3 and 4 were treated with MSG (0.8g/Kg b.wt), NSO (1ml/Kg b.wt) and Co-administered with MSG+NSO orally for 28 days, respectively. The blood samples were analysed for hematological parameters. Administration of MSG (Group- 2) caused a significant ( $P<0.05$ ) decrease in Hemoglobin (Hb) concentration, Red blood cells (RBCs), White blood cells (WBCs), Neutrophil count and Packed Cell Volume (PCV). However, there was a significant ( $P<0.05$ ) increase in Lymphocytes, Platelet, Mean cell volume (MCV), Mean corpuscular hemoglobin (MCH) and Mean corpuscular haemoglobin concentration (MCHC) in MSG treated rats compared to control group (Group 1). On the other hand, NSO (Group-4) significantly increased the content of Hb, RBCs, WBCs, Neutrophil, Lymphocyte and PCV. However Platelet count, MCV, MCH and MCHC were significantly ( $P<0.05$ ) decreased when compared to MSG treated rats (Group-2). The results indicate the protective effect of NSO against MSG induced toxicity on hematological parameters.

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## INTRODUCTION

Monosodium glutamate, the sodium salt of glutamic acid is a non-essential amino acid which is a food additive used as a flavouring agent for enhancing taste, various processed and prepared foods such as traditional seasoning sauce and certain restaurant foods contain significant levels of MSG (Eskes, 2008).

In recent years, studies have shown possible adverse effects of MSG. It is believed to be the cause of Chinese restaurant syndrome which is characterized by headache, flushing, numbness, muscle tightness, generalized weakness and broncho-constriction in asthmatics (Freeman, 2006). Researchers have also reported that MSG is a neurotoxic, killing brain cells, causing retinal degeneration, endocrinal disorders besides being associated with number of pathological conditions such as stroke, epilepsy, brain trauma, schizophrenia, anxiety, Parkinson disease and Huntington's disease (Mozes and Sefcikova, 2004; Cortese and Phan, 2005; Eweka and Om'Iniabohs, 2007; He *et al.*, 2008). It has also

been shown that the ingestion of MSG can alter normal range of hematological parameters (Ashaolu *et al.*, 2011).

*Nigella sativa* Linn is an annual plant belongs to the botanical family of *Ranunculaceae* (Saad, 1975) and commonly grows in Europe, Middle East and Western Asia. *Nigella sativa* known as black cumin is usually used as a traditional medicine in Arabian countries, Indian sub-continent and Europe (Lautenbacher,1997) for a wide range of illnesses including bronchial asthma, headache, dysentery, infections, back pain, hypertension and gastrointestinal problems (Salem,2005; Cheikh-Rouhou *et al.*, 2007). The beneficial medicinal effects of *Nigella sativa* oil (NSO) have been attributed to their pharmaceutical uses, such as acting as an antioxidant, carminative, anticancerogenic, diuretic, anti-hyperlipidemic, hypocholesterolemic and anti-inflammatory agent (Ali and Blunden, 2003; Badary *et al.*,2003; Yaman and Balıkcı, 2010 ).

Recent findings from our laboratory have shown that NSO has a protective effect on MSG induced toxicity on dyslipidemia in pubertal rats (Binu and Kumaran, 2015). Although the reports are available to suggest the beneficial effects of NSO, its effect

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on the MSG induced toxicity is limited. Therefore, the present investigation is aimed to study the effect of NSO on the MSG induced alteration on the haematological parameters.

## MATERIALS AND METHODS

### Chemicals

Monosodium glutamate was purchased from the Local available market, Nigella Sativa oil was purchased from Greenish (India) trades Pvt. Ltd and All other chemicals used were of analytical grade.

### Animals and Treatment

Pubertal male rats (Wistar), 135-150g in body weight was procured from Tamil Nadu Veterinary and Animal Science University (TANUVAS), Madhavaram, Chennai, India. The animals were housed in clean polypropylene cages lined with paddy husk with a temperature-controlled environment of 25°C ± 1°C, 50±10% humidity and an automatically controlled cycle of 12/12 h light and dark. The animals were fed with standard commercial diet pellets (M/s Hindustan Foods Ltd., Bangalore, India) *ad libitum*. The animals were maintained and handled as per the guidelines given by the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA), Government of India (845/GO/ac/04/ 2004/CPCSEA) and Institutional Animal Ethic Committee (IAEC).

### Experimental Design

The experimental design, selection of the dose of MSG, NSO and the route of administration were followed as per the method of Binu and Kumaran, (2015).

The Pubertal male rats were divided into four groups consisting of six rats each.

**Group 1:** The rats were given distilled water as vehicle orally, daily for 28 days (Control rats).

**Group 2:** The rats were treated with 0.8g/Kg b.wt of MSG orally for 28 days.

**Group 3:** The rats were treated with 1ml/Kg b.wt of Nigella sativa oil (NSO) for 28 days.

**Group 4:** The rats were Co-administered with MSG (0.8g/Kg b.wt) and NSO (1ml/Kg b.wt) for 28 days.

At the end of the experimental period the blood samples were collected into EDTA coated tubes via cardiac puncture for hematological indices.

### Hematological Analysis

The collected blood is analysed for the following parameters: Hb concentration, RBCs, Platelets, WBCs (Lymphocyte, Neutrophil, Monocyte, Eosinophil and Basophils) counts, PCV, MCV, MCH and MCHC. The hematologic parameters were analysed by automatic hematology analyser -Sysmex XT-2000iV and all analytical reagents and consumables for the Sysmex XT-2000iV were supplied by Sysmex Milton Keynes, UK (Mathers et al., 2005; Weissenbacher, 2009).

### Statistical Analysis

IBM SPSS 20.0 statistical software was used to analyse the data for Single way Analysis of Variance (ANOVA).

## RESULTS

The data obtained in the present study has been presented in Table-1 and 2. In the present study there was a significant decrease in Hb content, number of RBCs, WBCs, Neutrophil and PCV in MSG treated rats (Group-2) when compared to control group (Group-1). The level of MCV, MCH, MCHC, platelet and lymphocytes counts were significantly (P<0.05) increased in MSG (Group-2) treated rats. Interestingly, NSO (Group-3) had no significant effect on Hb, RBCs, WBC, Neutrophil, lymphocytes and platelet count, PCV, MCV, MCH, MCHC when compared to the control rats (Group-1).

**Table 1** Effect of administration of NSO (1ml/Kg b.wt) in MSG (0.8g/Kg.b.wt) induced alteration on hematological parameters (RBC, Hb, PCV, MCV, MCH, MCHC, Platelet) in rats.

Parameters	Group-1 Control	Group-2 MSG	Group-3 NSO	Group-4 MSG+NSO
RBC (x10 <sup>6</sup> /mm <sup>3</sup> )	7.28±0.23	5.96±0.11 <sup>a</sup>	7.41±0.24	6.79±0.23 <sup>b</sup>
Haemoglobin (g/dL)	13.6±0.45	10.2±0.33 <sup>a</sup>	14.2±0.47	12.8±0.41 <sup>b</sup>
PCV (%)	43.34±1.68	34.4±1.43 <sup>a</sup>	45.41±1.73	42±1.62 <sup>b</sup>
MCV (ft)	50.9±1.96	60.32±2.09 <sup>a</sup>	49.81±1.88	52.62±1.99 <sup>b</sup>
MCH (Pg)	15.9±0.61	20.41±0.86 <sup>a</sup>	14.9±0.57	16.9±0.71 <sup>b</sup>
MCHC (g/dL)	31.3±1.17	37.86±1.18 <sup>a</sup>	28.9±0.88	32.89±1.08 <sup>b</sup>
Platelet (10 <sup>3</sup> /mm <sup>3</sup> )	532±11.02	589±11.83 <sup>a</sup>	524±10.61	544±10.20 <sup>b</sup>

MSG=Monosodium glutamate, NSO=Nigella sativa oil, RBC = red blood cells; PCV = packed cell volume; MCV = mean cell volume; MCH = mean cell hemoglobin; MCHC = mean cell hemoglobin concentration. Each value is Mean± SEM of 6 Animals. <sup>a</sup>and <sup>b</sup> represent statistical significant at P<0.05 Compared with Control and MSG respectively. Control Vs other Groups ; MSG Vs MSG + NSO.

**Table 2** Effect of administration of NSO (1ml/Kg b.wt) in MSG (0.8g / Kg.b.wt) induced alteration on hematological parameters (WBC, Neutrophil, Lymphocyte, Monocyte, Eosinophil, Basophils) in rats.

Parameters	Group-1 Control	Group-2 MSG	Group-3 NSO	Group-4 MSG+NSO
WBC (x10 <sup>3</sup> /mm <sup>3</sup> )	7.92±0.28	5.84±0.26 <sup>a</sup>	7.98±0.26	6.96±0.28 <sup>b</sup>
Neutrophil (%)	35.6±1.31	21.8±0.83 <sup>a</sup>	37.4±1.39	32.2±1.26 <sup>b</sup>
Lymphocyte (%)	56.7±2.03	68.7±2.15 <sup>a</sup>	53.9±1.80	59.8±2.04 <sup>b</sup>
Monocyte (%)	5.4±0.12	6.5±0.15	3.9±0.09	5.9±0.13
Eosinophil (%)	1.6±0.04	2.1±0.06	1.4±0.03	1.9±0.05
Basophils (%)	0.22±0.01	0.43±0.32	0.18±0.01	0.37±0.02

MSG=Monosodium glutamate, NSO=Nigella sativa oil, WBC = white blood cells. Each value is Mean± SEM of 6 Animals. <sup>a</sup>and <sup>b</sup> represent statistical significant at P<0.05 Compared with Control and MSG respectively. Control Vs other Groups; MSG Vs MSG + NSO.

Co-administration of MSG and NSO (Group- 4) showed significant (P<0.05) increase in Hb, RBCs, WBC and Neutrophil count, PCV when compared to MSG treated rats (Group-2). The level of MCV, MCH, MCHC, platelet and lymphocytes count were significantly (P<0.05) decreased. However, in the present study it is interesting to observe that the monocyte, eosinophil and basophil counts were unaltered in all the treated groups (Group2-4) when compared to control rats.

## DISCUSSION

The results obtained from the present study showed that the administration of MSG (0.8g/Kg.b.wt) and NSO (25mg/Kg b.wt) orally for 28 days had a significant effect on the hematological parameters. Decreased in the number of RBC's count, PCV and Hb concentration in the MSG treated group might be mediated through deleterious effect of MSG on the

hemopoietic stem cells in the bone marrow, decrease in the RBC count in the MSG treated rats suggests that it probably reduces the life span of red blood cells in the blood which might be as a result of direct toxicity of MSG. MSG might have increased oxidative stress in the tissues of the animals and significantly induced the formation of micro nucleated polychromatic erythrocytes (MNPCEs) (Eweka, 2007; Elphick *et al.*, 2008). Decrease in PCV level in MSG treated rats might be due to renal cortical necrosis induced by MSG (Eweka, 2007) since PCV is generated by kidney and considering the endocrine role of the kidney in erythropoiesis (Donnelly, 2005) which clearly indicates decreased PCV level in MSG treated rats. In addition, it has been reported that effect of MSG on the gastro-intestinal tract lining may cause distortions in the regulated absorption of minerals and vitamins (Eweka and Om'Iniabohs *et al.*, 2007) which are important for erythropoiesis (Guyton and Hall., 2006; Sembulingham and Sembulingham, 2010). This clearly states that MSG can induce anaemia since PCV is an important diagnostic tool used in determining blood loss, health status and anaemia.

In the present study, Co-administration of MSG+NSO showed the protective effect of NSO against MSG induced toxicity by significantly increasing the RBC's count, PCV and Hb concentration when compared to MSG treated rats increased RBC count by NSO could be due to the lowered lipid peroxide level in RBC membrane leading to a decreased susceptibility of RBC to hemolysis (Meral *et al.* 2001). Previous studies in mice and rats have shown that treatment with *Nigella sativa* significantly protects from cisplatin-induced falls in hemoglobin level and PCV or hematocrit increase (El-Daly, 1998). It has been reported that the administration of NSO increased the fall of PCV level induced by streptozotocin (MariemYusuksawad and Narongsak Chaibutr, 2012). Our current finding (Table-1) further supports the safety of NSO with no negative effect on hematological parameters.

The levels of MCV, MCH and MCHC were observed to be significantly increased ( $P < 0.05$ ) in MSG treated rats (group-2) when compared to control group. It has been reported that there is an increase in the level of MCV in pernicious anaemia and megaloblastic anaemia (Richards, 1993) likewise increase in MCH level is observed in macrocytic anaemia (Sembulingham, 2005), hence increased level of MCV, MCH and MCHC indicates the deleterious effect of MSG thereby inducing anaemic status in rats. The present study reveals that the NSO had no significant effect on MCV, MCH and MCHC levels which clearly suggests the safety of NSO with no negative effect on MCV, MCH and MCHC, our findings are in agreement with the study of Zaoui *et al* (2002). Co-administration of NSO with MSG (group-4) shows a significant decrease ( $P < 0.05$ ) in the MCV, MCH and MCHC counts which reflects the ameliorative effect of NSO against MSG by preventing anaemic status in rats.

The present study showed that MSG significantly increased ( $P < 0.05$ ) the platelet cell counts of rats (Table 1) which indicates that MSG treatment may lead to thrombocytosis or thrombocythemia, our study is consistent with the earlier report of Meraiyebu Ajibola *et al* (2012) who observed the MSG induced thrombocytosis in rats. It was also suggested that the increase in platelet cell count might be due intake of one of MSG component which includes sodium and glutamate that

affect blood and body fluid compartments and water balance of the body. However, the mechanism of action of MSG by altering the platelet cells, in the body is yet to be fully elucidated (Eweka, 2007). In the present study, Co-administration of MSG+NSO, shows protective effect against MSG induced toxicity by significantly decreasing the platelet count when compared to MSG treated rats, which indicates the ameliorative effect of NSO by preventing MSG induced thrombocythemia.

The present study reveals that the increased number of lymphocytes in MSG group could be due to interaction between MSG and gastrointestinal macrophages (Sembulingham, 2005), Macrophages serve as antigen presenting cell and the antigenic products (polypeptides) to the helper T cells and the B lymphocytes bringing about their activation (Sembulingham, 2005). Interleukin-1/cytokines are also secreted by Macrophages, which brings about the activation, proliferation and increase in the lymphocyte count (Sembulingham, 2005; Barrett *et al.*, 2010). In the present study, co-administered rats with MSG+NSO (Group 4) shows protective effect against MSG induced toxicity by significant decrease ( $P < 0.05$ ) in the lymphocyte count.

MSG treated rats shows significant decrease ( $P < 0.05$ ) on WBC count when compared to control group as shown in table- 2, WBCs are the defensive cells of the body. According to Douglass and Jane (2010), their levels have implications for immune responses and the ability of the animal to fight infection. Species with higher levels of WBC will be able to fight infection more effectively than other species. Significant reduction in the WBC count occurs due to the metabolic changes induced by MSG, reducing immunologic function and increasing the risk of infection (Hellen Maluly *et al.*, 2013). The decreased WBC count noted in MSG treated rats in the present study is in agreement with the results obtained by Magda *et al.*, (2010) and Hellen Maluly *et al* (2013). However, in the present study Co-administration of MSG+NSO (Group 4) shows protective effect against MSG induced toxicity by significant ( $P < 0.05$ ) increase in the WBC count, Our studies are in line with the earlier reports (Meral *et al.*, 2001; El-Daly, 1998).

The present study shows that MSG had a significant effect on Neutrophil by decreasing the count in rats. Ashaolu *et al* (2011) described that MSG has a toxic effect on neutrophils in the blood and also it has a deleterious effect on blood production in the bone marrow, especially on the progenitor cells (aplasia). Neutrophils along with monocytes provide the body's first line of defence against invading microorganism, toxic substances, and foreign substances emphasizing the important role of neutrophils play in the body defence (Hall, 2011; Ganong, 1991). This might be an indicative of the deterioration of immune status in the MSG treated rat group in response to the toxic effect of MSG. However, in the present study co-administered groups MSG+NSO shows protective effect of NSO against MSG induced toxicity. This result indicates that NSO treatment might also increase the defence mechanism of the body against infections in MSG treated rats. Meral *et al* (2001) demonstrated that *Nigella sativa* increased the lowered neutrophil percentage of WBC to normal level in the alloxan-induced diabetic rabbits. Interestingly, the present study reveals

that the Monocyte, Eosinophil and Basophils counts were not affected by MSG.

## CONCLUSION

The present study suggests that MSG (0.8g /Kg b.wt) administration has an adverse effect on Hematological parameters on (Hb, RBC, PCV, MCV, MCH and MCHC) which are indicative of anaemic conditions in the treated rats. Further, the lowered number of the neutrophil and lymphocyte count indicates the weakened immune status and poisoning respectively in the MSG treated animals. Further, NSO (1ml/Kg b.wt) prevents the alteration on hematological parameters induced by MSG.

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