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Rutin And Vitamin C Ameliorated Monosodium Glutamate And Alcohol Induced Neurotoxicity Via Inhibition Of Oxidative Stress, Neuro-Inflammation And Suppression Of TNF Pathway In Rats.

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Abstract

Addressing environmental problems that contribute to neurotoxicity underscores the urgent need to investigate adverse effects of MSG and Alcohol. The present study attempts to determine the neuro-protective effects of rutin and vitamin C on monosodium glutamate and alcohol induced neurotoxicity in male Wistar rats. 50 male Wistar rats were randomly distributed into ten (10) groups with each group having five animals (n=5). Neurotoxicity was induced with an oral single daily administration of 30% alcohol and 1.5g/kg of monosodium glutamate for 28 days consecutively in all rat groups except group1. The animals were subsequently grouped as follows: Group 1: Control. Group 2: MSG only (1.5g/kg). Group 3: Alcohol only (30% alcohol). Group 4: MSG + Alcohol. Group 5: MSG + Alcohol + Rutin. Group 6: MSG + Alcohol + Vitamin C. Group 7: MSG + Rutin. Group 8: MSG + Vitamin C. Group 9: Alcohol + Rutin. Group 10: Alcohol + Vitamin C. All administrations were orally carried out once daily in the morning hours with the aid of a cannula for 28 days. The rats were later anesthetized, and brain tissues harvested, homogenized in sucrose solution and centrifuged with cold centrifuge at 10,000rpm for 10 minutes. Supernatant was removed and used for antioxidant (MDA, SOD, GST and CAT) and inflammatory parameters (IL-6, TNF-Alpha, NO and MPO) analysis. Expectedly, significantly lower values ($p < 0.05$) of SOD, CAT and GST but higher values of MDA, NO, MPO, IL-6 and TNF-alpha were observed amongst groups 2 (MSG only) and 3 (Alcohol only) rats treated with monosodium glutamate and alcohol when compared to group 1 (control) rats. Suggesting a possible neurotoxic effect of MSG and alcohol. Surprisingly, significantly higher values ($p < 0.05$) of SOD, CAT and GST but lower values in inflammatory markers were observed amongst groups 9 (Alcohol + rutin) and 10 (Alcohol + Vit C) rats when compared to groups 2 (MSG only), 3 (Alcohol only) and 4 (MSG + Alcohol) rats. Suggesting a possible beneficial effect of rutin and Vit C in male Wistar rats. Our study outcome suggests a possible beneficial function of rutin and vitamin C on oxidative stress and inflammation.

Keywords: *Rutin, vitamin C, Monosodium Glutamate, Alcohol, Neurotoxicity*

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I. Introduction

The frequent use of monosodium glutamate (MSG) and alcohol by caterers and food sellers as preservatives and regular drinks, in our environment underscores the urgent need to investigate effective strategies for mitigating their adverse effects on the nervous system (neuro-protective potentials of Rutin and Vitamin C on monosodium glutamate and alcohol lipopolysaccharide induced neurotoxicity in male Wistar rats (Brick, (2024): Gbaranor *et al.,* (2024). Addressing environmental problems that contribute to neurotoxicity requires a multifaceted approach that includes research into natural neuro-protective agents like Rutin and vitamin C (Nkpaa et al 2019; Alwan, 2020;Araujo *et al.,* (2021). Evaluating their efficacy against MSG and Alcohol-induced neurotoxicity can contribute to the development of interventions aimed at preserving neurological health amidst environmental challenges (Banerjee *et al*., (2023).

In modern society, environmental factors pose significant challenges to neurological health, necessitating research into potential protective measures. Among these challenges are foods additives like monosodium glutamate and alcohol used as preservatives which induce neurotoxicity and contribute to cognitive impairments (Abd-Elkareem *et al.,* (2022): Sarawagi *et al.,* (2021): Carey, (2023). These

environmental stressors are exacerbated by dietary habits characterized by processed foods and low antioxidant intake, highlighting the importance of exploring natural neuroprotective agents (Haber & Schneidman, 2022). Hence, the need to evaluate the neuro-protective potentials of Rutin and Vitamin C on monosodium glutamate and alcohol lipopolysaccharide induced neurotoxicity in male Wistar rats.

II. Materials And Methods

Purchase of Experimental Animals and Ethical Approval:

Forty Male Wistar rats weighing between 120 to 150g were procured from the Department of Physiology animal house PAMO University of Medical Sciences, Nigeria and were housed and treated under standard laboratory conditions with 12 hours light and dark cycle. They were fed with standard laboratory animal chow and had unhindered access to water. The animals were acclimatized for two weeks and were subsequently grouped for the study. The present study was approved by the Research Ethics Committee of our University

Procurement of Drugs and Neurotoxicity Induction:

Vitamin C was sourced from a local pharmaceutical store while MSG (Ajinomotto) was obtained from a standard market in the city of Port Harcourt, Alcohol (Chelsea dry gin) was purchased from a Port Harcourt Supermarket. Furthermore, Rutin was purchased from a chemical store located in Choba, Rivers State, Nigeria. Neurotoxicity was induced with an oral single daily administration of 30% alcohol and 1.5g/kg body weight of Monosodium glutamate for 28 days consecutively in all rat groups except group1**.**

Experimental Design

After two weeks of acclimatization, the animals were randomly selected into ten (10) groups with each group having five animals each (n=5). Neurotoxicity was induced with an oral single daily administration of 30% alcohol and 1.5g/kg body weight of Monosodium glutamate for 28 days consecutively in all rat groups except group1**.** The animals were subsequently grouped as follows:

Group 1: Control; animals in this group had free access to water and laboratory rat chow. **Group 2:** MSG only; animals in this group received no additional treatment after induction of neurotoxicity with a single daily administration of 1.5g/kg of MSG. **Group 3:** Alcohol only; animals in this group received no additional treatment after induction of neurotoxicity with a single daily administration of 30% alcohol. **Group 4**: MSG + Alcohol; animals in this group got no treatment after the induction of neurotoxicity with a single daily administration of 1.5g/kg of MSG and 30% alcohol. **Group 5:** MSG + Alcohol + Rutin; animals in this group received 30mg/kg of rutin following induction of neurotoxicity. **Group 6:** MSG + Alcohol + Vitamin C; animals in this group received 200mg/kg of vitamin C following induction of neurotoxicity. **Group 7:** MSG + Rutin; animals in this group received 30mg/kg of rutin following induction of neurotoxicity. **Group 8:** MSG + Vitamin C; animals in this group received 200mg/kg of rutin following induction of neurotoxicity. **Group 9:** Alcohol + Rutin; animals in this group received 30mg/kg of rutin following induction of neurotoxicity. **Group 10:** Alcohol + Vitamin C; animals in this group received 200mg/kg of Vitamin C following induction of neurotoxicity. All administrations were orally carried out once daily in the morning hours with the aid of a cannula for 28 days. The rats were later anesthetized using diethyl ether and brain tissues were harvested, homogenized in sucrose solution and centrifuged with cold centrifuge at 10,000rpm for 10 minutes. Supernatant was removed and used for antioxidant (MDA, SOD, GST and CAT) and inflammatory parameters (IL-6, TNF-Alpha, NO and MPO) analysis.

Oxidative Stress and Inflammatory Markers Analysis

Antioxidant parameters were analyzed as previously described by Sun *et al., (*1988), Aebi, (1974) and Ezebuiro *et al.,* (2020). Inflammatory indices were assayed using standard procedures.

Statistical Analysis

Data was expressed as Mean + standard error of mean. All data was analyzed using one way analysis of variance (ANOVA) and comparison of the groups was performed with post hoc Newman-Keuls test using Graphpad prism 7.0 (Graphpad Software, San Diego, CA, USA) and a $P < 0.05$ was considered statistically significant.

III. Results

Figure 1 shows significantly lower values ($p < 0.05$) in superoxide dismutase (SOD) amongst groups 2 (MSG only) and 3 (Alcohol only) rats treated with monosodium glutamate and alcohol when compared to group 1 (control) rats. Suggesting a possible neurotoxic effect of MSG and alcohol. Similar results were observed amongst group 4 (MSG + alcohol) rats co-treated with both MSG and alcohol; however, the difference was

apparently not significant. Significantly higher values ($p < 0.05$) of SOD were observed amongst groups 9 (Alcohol + rutin) and 10 (Alcohol + Vit C) rats when compared to groups 2 (MSG only), 3 (Alcohol only) and 4 (MSG + Alcohol) rats. Suggesting a possible beneficial effect of rutin and Vit C in male Wistar rats.

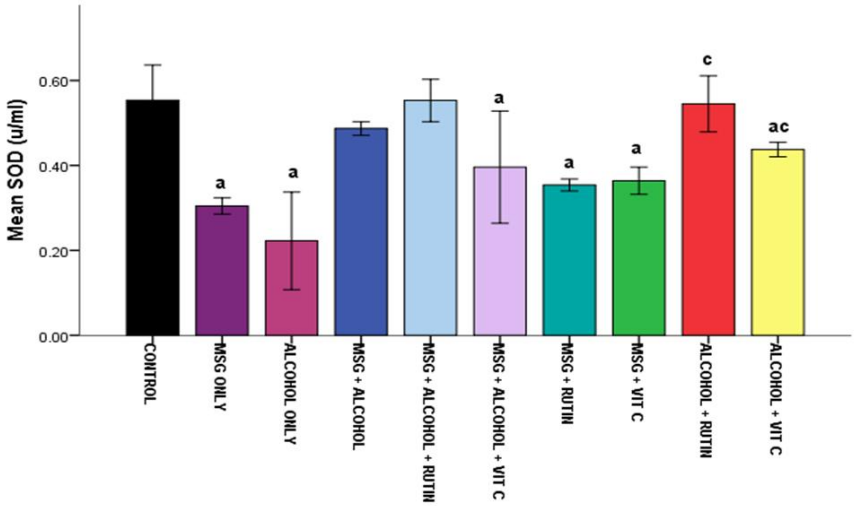

Figure 1: Shows the effect of Rutin and Vitamin C on super oxidize dismutase (SOD) in monosodium glutamate (MSG) and Alcohol induced neurotoxicity in male Wistar rats.

In figure 2, a non-significant decrease ($p < 0.05$) in catalase activity was observed in groups 2 (MSG only) and 3 (Alcohol only) rats compared to group 1 (control) rats. This is indicative of a potential harmful effect of MSG and alcohol. However, upon administration of rutin and Vit C to groups 9 (Alcohol + rutin) and 10 (Alcohol + Vit C) rats, there was a significant ($p < 0.05$) reversal of CAT level when compared to groups 1 (Control) and 3 (Alcohol only) rats. These findings suggestive a possible reversibility effect of rutin and Vic C in MSG and alcohol induced neurotoxicity.

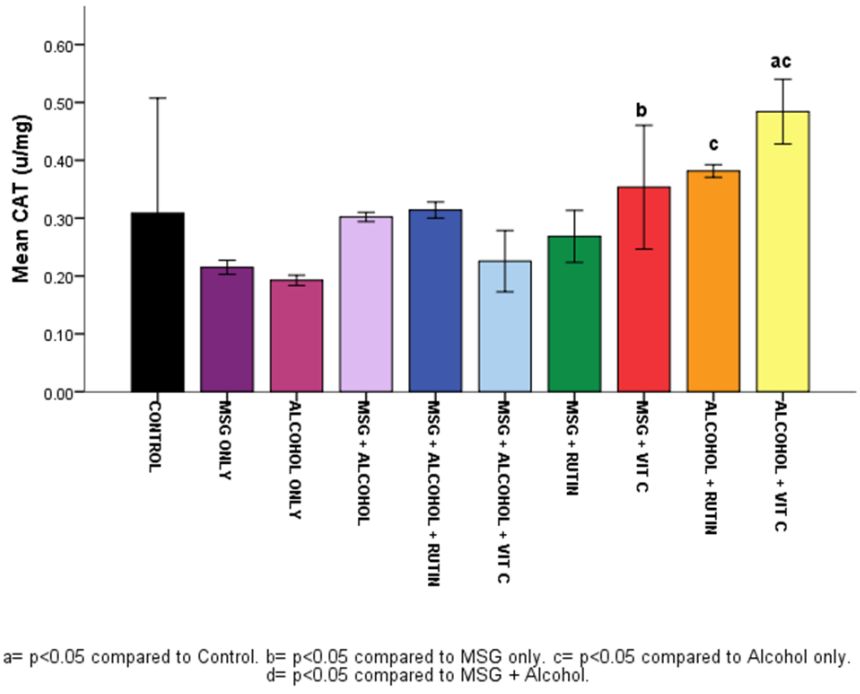

Figure 2: Shows the effect of Rutin and Vitamin C on catalase in MSG and Alcohol induced neurotoxicity in male Wistar rats.

Figure 3 shows significant increase ($p < 0.05$) in MDA activity amongst groups 2 (MSG only) and 3 (Alcohol only) rats. Suggesting a potential pro-peroxidative effect of MSG and alcohol at the administered doses. Administration of rutin and Vit C amongst groups 9 (rutin + alcohol) and 10 (Vit C + alcohol) rats demonstrated a corresponding significant reduction ($p < 0.05$) in MDA level when compared to group 1 (control) rats. Indicating a likely free radical scavenging activity of both rutin and Vitamin C (Vit C).

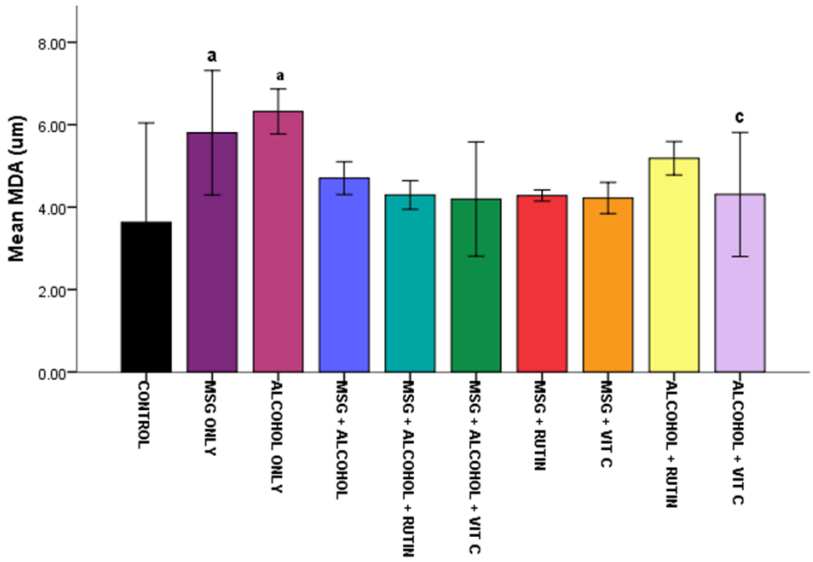

Figure 3: Shows the effect of Rutin and Vitamin C on MDA in MSG and Alcohol induced neurotoxicity in male Wistar rats.

Administration of MSG and Alcohol amongst groups 2 (MSG only) and 3 (Alcohol only) rats demonstrated a significant decrease ($p < 0.05$) in GST activity when compared to group 1 (control) rats as seen in figure 4. This indicates a likely deleterious effect of MSG and Alcohol. Treatment with Rutin and Vitamin C show a non-significant increase ($p < 0.05$) in GST activity compared to groups 2 (MSG only) and 3 (Alcohol only) rats. Suggesting a possible correction of the harmful effect of MSG and Alcohol induced toxicity.

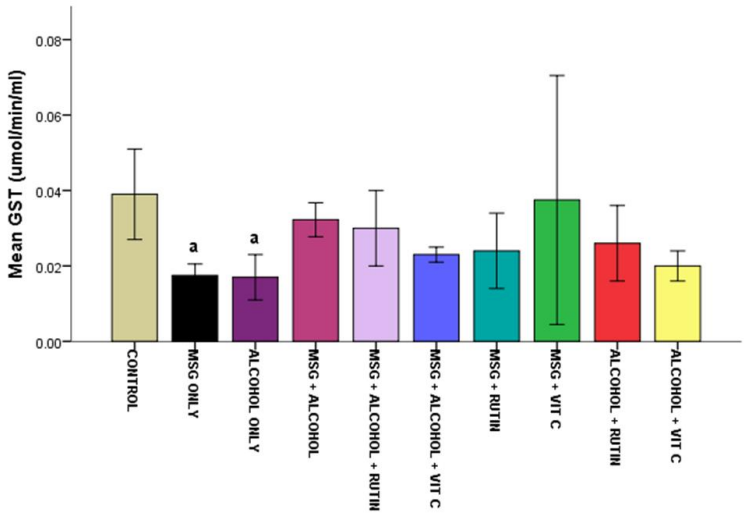

Figure 4: Shows the effect of Rutin and Vitamin C on GST in MSG and Alcohol induced neurotoxicity in male Wistar rats.

Figure 5 shows a significant decrease in NO activity in group 2 (MSG only) rats but an increase in Group 3 (Alcohol only) rats was observed when compared to group 1 (control) rats. Rutin and Vitamin C treatment amongst groups 9 and 10 rats tend to normalize NO activity when compared to group 2 (MSG only) rats.

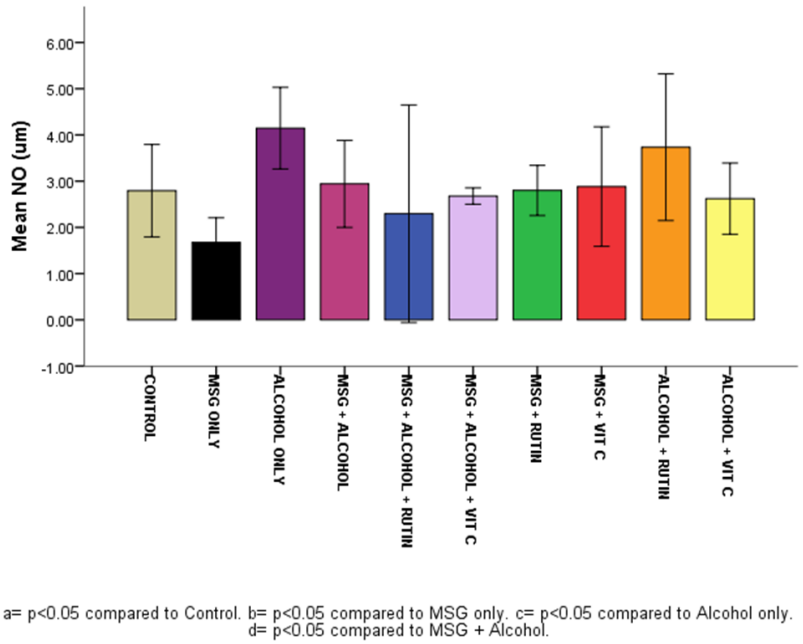

Figure 5: Shows the effect of Rutin and Vitamin C on NO in MSG and Alcohol induced neurotoxicity in male Wistar rats.

A significant increase ($p < 0.05$) in MPO activity was observed in MSG and Alcohol only treated rats groups when compared with control as shown in figure 6. Rutin and Vitamin C treatment groups demonstrated a moderate decrease in MPO activity when compared to MSG only treated group.

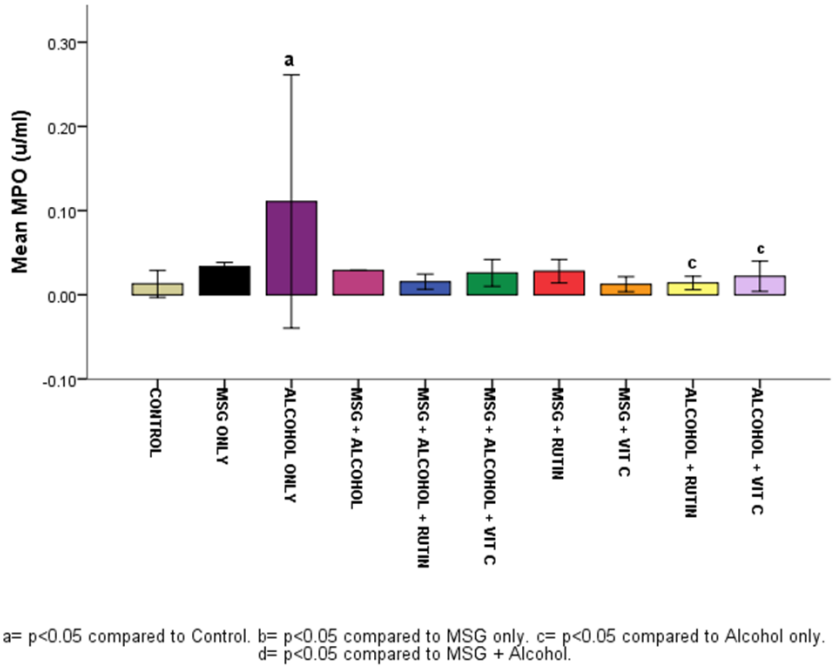

Figure 6: Shows the effect of Rutin and Vitamin C on MPO in MSG and Alcohol induced neurotoxicity in male Wistar rats.

When compared to group 1 (control) rats, Figure 7 reveals a significant increase ($p < 0.05$) in IL-6 levels amongst groups 2 (MSG only), 3 (Alcohol only) and 4 (MSG + Alcohol) rats. Indicating a likely pro-inflammatory effect of MSG and Alcohol. However, groups 9 and 10 rats treated with Rutin and Vitamin C show moderate decrease in IL-6 activity when compared to MSG only, Alcohol only and both MSG + Alcohol treated rats. Suggesting a possible anti-inflammatory activity of rutin and vitamin C.

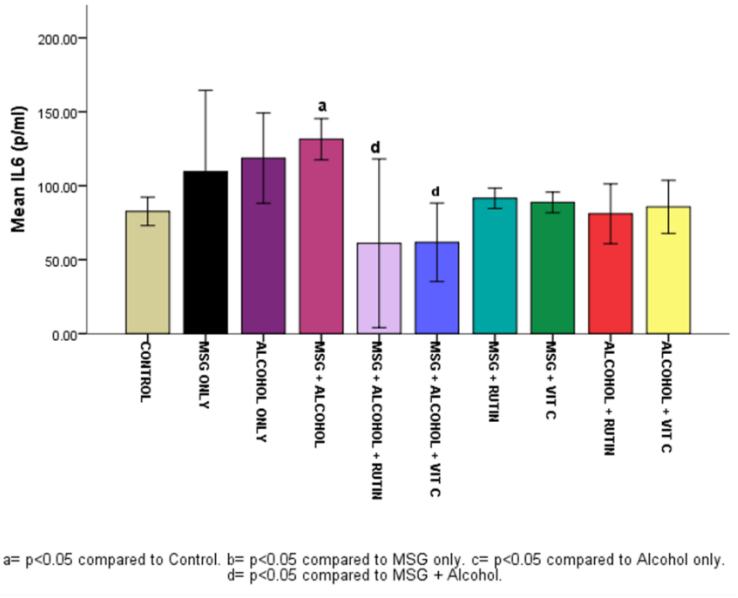

Figure 7: Shows the effect of Rutin and Vitamin C on IL-6 in MSG and Alcohol induced neurotoxicity in male Wistar rats.

There was a significant increase ($p < 0.05$) in TNF-a levels amongst groups 2 (MSG only), 3 (Alcohol only) and 4 (MSG + Alcohol) rats when compared to group 1 (control) rats as seen in figure 8. The elevation is suggestive of a possible harmful effect. Rutin and Vitamin C treatment observed amongst groups 9 and 10 rats show a moderate remarkable decrease in TNF-a activity when compared to groups 2 (MSG only), 3 (Alcohol only) and 4 (MSG + Alcohol) rats. This indicates a likely beneficial effect.

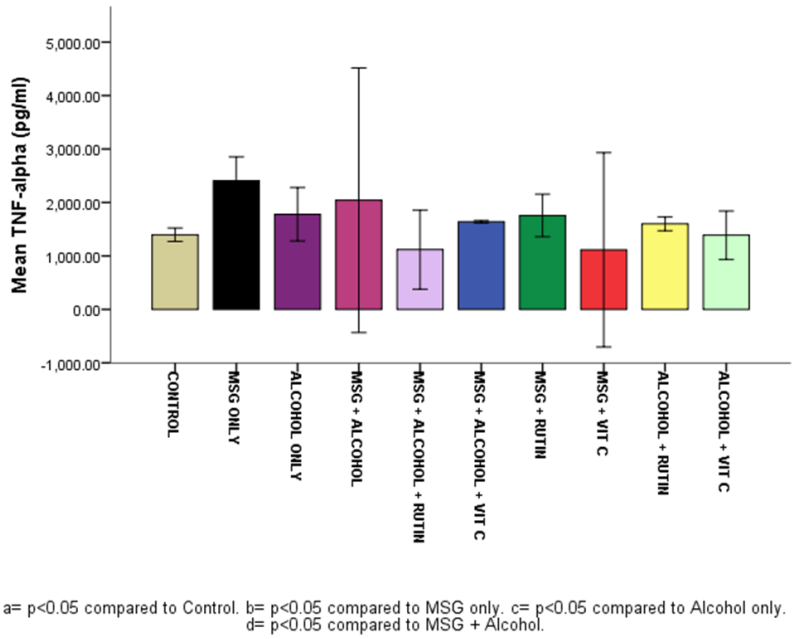

Figure 8: Shows the effect of Rutin and Vitamin C on TNF- a in MSG and Alcohol induced neurotoxicity in male Wistar rats.

IV. Discussion

The present study investigated the neuroprotective effects of Rutin and Vitamin C on Monosodium glutamate (MSG) and Alcohol induced neurotoxicity in male Wistar rats. Expectedly, available results emanating from our study suggest that MSG and Alcohol induced neurotoxicity when administered singly or in combined state as previously reported by Gonzales & Jaworski, (1997). A further review of literature shows that N-methyl-D-aspartic acid (NMDA) receptors are activated in the presence of excess glutamate or glycine which leads to excitotoxicity (Daşdelen *et al.,* (2022); Olney, (1989).

Effect of Rutin and Vitamin C on antioxidant parameters in MSG and Alcohol induced neurotoxicity in male Wistar rats.

The presence of antioxidants helps to mitigate free radicals in the body. Super Oxide Dismutase (SOD), Catalyse (CAT), Malonaldehyde (MDA), Glutathione Transferase (GST) are all important antioxidant markers, and these indices help to confirm the stress levels via the measurement of free radicals concentration. Tissue lysate of the Brain was analyzed, and obtained results show a decline in SOD, CAT and GST values and an increase in MDA in MSG and alcohol treated rats groups. These findings are consistent with previous studies in which similar interventions were administered to Wistar rats (Vilioglu *et al.,* (1998), Oloyede and Afolabi (2012). Though, observable increases were seen in SOD, CAT and GST values with a corresponding decrease in MDA values in rutin and vitamin C treated rats indicating a possible reduced stress level and free radicals. These changes were obvious as it showed a reversal of the effects of MSG and Alcohol. This agrees with Vilioglu *et al.,* (1998).

Effect of Rutin and Vitamin C on inflammatory markers in MSG and Alcohol induced neurotoxicity in male Wistar rats.

Inflammatory markers like Tumor Necrosis Factor-alpha (TNF-a), IL-6, NO, MPO are used to visualize specific proteins in tissues allowing for the assessment of neuroinflammatory and oxidative damage (Saronee *et al.,* 2024): Germolec *et al.,* (2018). Tumor Necrosis Factor-alpha (TNF-a) is a pro-inflammatory cytokine that plays a crucial role in neuro-inflammation and apoptosis. Treatment with MSG and alcohol causes an increase in the level of TNF-a in the brain. However, Rutin and Vitamin C administration reduces TNF-a expression, therefore reversing the effect of MSG and Alcohol treatment.

Interleukin-6 is another pro-inflammatory cytokine that is elevated in neuroinflammation. Obtained results from the present study show that MSG and Alcohol treatment exacerbates neuronal damage thereby increasing the IL-6 levels. Treatment with Rutin and Vitamin C lowers IL-6 levels thereby reducing inflammatory damage caused by MSG and Alcohol. Similar results were reported by Saronee *et al.,* (2024) and Rose-John, (2018).

Nitric Oxide levels are increased in neurotoxicity. From this study, NO levels were elevated in MSG and Alcohol treatment while MSG showed a decrease in NO levels. Treatment with Rutin and Vitamin C lowers NO levels thereby reducing inflammatory damage caused by MSG and Alcohol (Germolec *et al.,* 2018).

Myeloperoxidase (MPO) contributes to oxidative stress during inflammation (Rose-John, 2018). In our study, MPO levels were elevated in MSG and Alcohol treated groups but treatment with Rutin and Vitamin C lowers MPO levels thereby reducing oxidative stress caused by MSG and Alcohol.

V. Conclusion

The present study reports that administration of rutin and vitamin C caused significant increase in superoxide dismutase, catalase, GST but decrease malondialdehyde, interleukin 6, tumour necrosis factor alpha levels, NO and MPO concentration following MSG and alcohol induced neurotoxicity in male Wistar rats. Our study outcome suggests a beneficial function of rutin and vitamin C on oxidative stress and inflammation.

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