



**NEUROPROTECTIVE EFFECTS OF AQUEOUS EXTRACT OF SALVIA ROSMARINUS
ON HYPOTHALAMIC HISTOPATHOLOGY IN THEOPHYLLINE-INDUCED
INSOMNIAC RATS: A HISTOLOGICAL AND BIOCHEMICAL STUDY**

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ABSTRACT

Insomnia, a prevalent sleep disorder, can be induced by various factors, including medication. This research investigated the neuroprotective effects of aqueous extract of *Salvia rosmarinus* on hypothalamic histopathology in theophylline-induced insomniac rats using an experimental design. Insomnia was induced in Wistar rats via intra-gastric administration of 100 mg/kg body weight of theophylline for 3 consecutive days, ad libitum. The theophylline-induced insomniac rats were then treated with *Salvia rosmarinus* extract, which significantly improved hypothalamic histopathology, reducing damage and promoting normal structure. Biochemical analysis revealed increased serum norepinephrine levels ($p \leq 0.05$) and melatonin levels ($p \leq 0.05$), suggesting potential neuroprotective effects. Furthermore, the extract demonstrated antioxidant activity, reducing lactate dehydrogenase (LDH) activity ($p \leq 0.05$) and malondialdehyde (MDA) concentration ($p \leq 0.05$). These findings suggest that *Salvia rosmarinus* extract may have potential therapeutic effects on theophylline-induced insomnia, warranting further studies to explore its mechanisms and potential applications. This study's findings may provide a valuable basis for developing novel therapeutic strategies for insomnia and other sleep disorders.

KEYWORDS: *Salvia rosmarinus*, neuroprotective effects, hypothalamic histopathology, theophylline-induced insomnia, norepinephrine, melatonin, antioxidant activity.

INTRODUCTION

Insomnia is a prevalent sleep disorder characterized by difficulty initiating or maintaining sleep, leading to significant distress and impairment in daily functioning (Ohayon, 2002; Léger et al., 2002; Titova et al., 2022). The prevalence of insomnia is estimated to be around 27% globally, with significant personal distress, daytime functional impairments, and physiological dysfunctions (Bjorøy et al., 2020). Theophylline, a methylxanthine derivative, is known to induce insomnia by antagonizing

adenosine receptors in the brain (Fredholm et al., 2001; Porkka-Heiskanen et al., 2002). The hypothalamus, a crucial region regulating sleep-wake cycles, is vulnerable to damage from various insults, including sleep disorders (Saper et al., 2005; Szymusiak & McGinty, 2008). Sleep disorders, including insomnia, have been linked to various neurodegenerative changes in the brain, particularly in the hypothalamus.

Current treatments for insomnia include pharmacological and non-pharmacological approaches, such as cognitive behavioral therapy for insomnia (CBT-I), pharmacotherapy (e.g., benzodiazepines, non-benzodiazepine hypnotics), light therapy, and exercise (Khan & Al-Jahdali, 2023; Cheng et al., 2019). However, these treatments have limitations, including potential side effects (e.g., dependence, cognitive impairment), limited efficacy, and lack of long-term solutions (Kripke, 2007; Buscemi et al., 2007). Furthermore, pharmacological treatments may only address symptoms, rather than underlying causes, and may not be suitable for all patients. As a result, there is a growing interest in exploring alternative therapies, including herbal remedies, that may offer more effective and sustainable solutions for insomnia (Ahmed et al., 2020).

Recent studies have explored the potential benefits of herbal extracts in neuroprotection, including *Salvia rosmarinus*, which has been traditionally used for its cognitive-enhancing and antioxidant properties (Lee et al., 2013; Habtemariam, 2016; Bano et al., 2019). *Salvia rosmarinus* is rich in antioxidants and anti-inflammatory compounds, which may contribute to its potential therapeutic effects (Amaral et al., 2019; Ngozichukwu et al., 2025). This study aimed to investigate the neuroprotective effects of aqueous extract of *Salvia rosmarinus* on hypothalamic histopathology in theophylline-induced insomniac rats, using a combination of histological and biochemical approaches. Specifically, we sought to evaluate the effects of *Salvia rosmarinus* extract on hypothalamic histopathology and assess the biochemical changes associated with hypothalamic damage in theophylline-induced insomniac rats treated with *Salvia rosmarinus* extract.

MATERIAL AND METHODS

The study was conducted using thirty Wistar albino rats weighing 120 ± 20 g, obtained from Nano-Laboratory

animal farm house, Owerri, Nigeria. The rats were acclimatized to laboratory conditions for 1 week and fed with commercially formulated rat feed and portable water ad libitum.

Salvia rosmarinus leaves were purchased from Relief market, Owerri, Imo state, Nigeria, and identified by a plant taxonomist, (Prof. F.N. Mbagwu) at the Department of Plant Science and Biotechnology, Imo State University, Owerri, Nigeria. The leaves were dried and ground into a fine powder, which was then extracted with hot distilled water using the maceration method. The extract was filtered, concentrated, and stored for further use.

Insomnia was induced in the rats by administering 100mg/kg body weight of theophylline for 3 consecutive days. The rats were then randomly assigned into six groups: normal control, theophylline-induced insomnia, positive control (diazepam), and three treatment groups receiving different doses of *Salvia rosmarinus* extract (100, 250, and 500mg/kg body weight). The treatment lasted for 7 days.

After the treatment period, the rats were sacrificed, and their brain tissues were excised and processed for histopathological examination using Hematoxylin and Eosin (H&E) staining. The sections were viewed at x400 magnification using a light microscope. Serum samples were also analyzed for liver and kidney function tests using assay kits.

Data were analyzed using statistical package for social sciences (SPSS) version 22, and one-way analysis of variance (ANOVA) and Turkey's post-hoc tests were carried out to determine statistical differences between means of groups. Values were considered statistically significant at $p \leq 0.05$, and results are presented as mean \pm standard deviation of five determinations.

RESULT

Hypothalamic Histopathology



Figure 1: E1 Hypo x 400 magnification.

The photomicrograph of Group 1 hypothalamus (Figure 1) stained with Hematoxylin and Eosin (x400 magnification) reveals a normal Neurons and Axons. The hypothalamic structure appears mostly normal, with intact neurons (N) and axons, indicating preservation of the basic architecture of the hypothalamus. However, vacuolated cells are observed, suggesting cellular stress

or damage, which may be indicative of various pathological processes, including toxicity or metabolic disturbances. Additionally a notable distortion (***) is observed in the hypothalamic structure, resembling a viral strain, which could indicate an underlying pathological process.

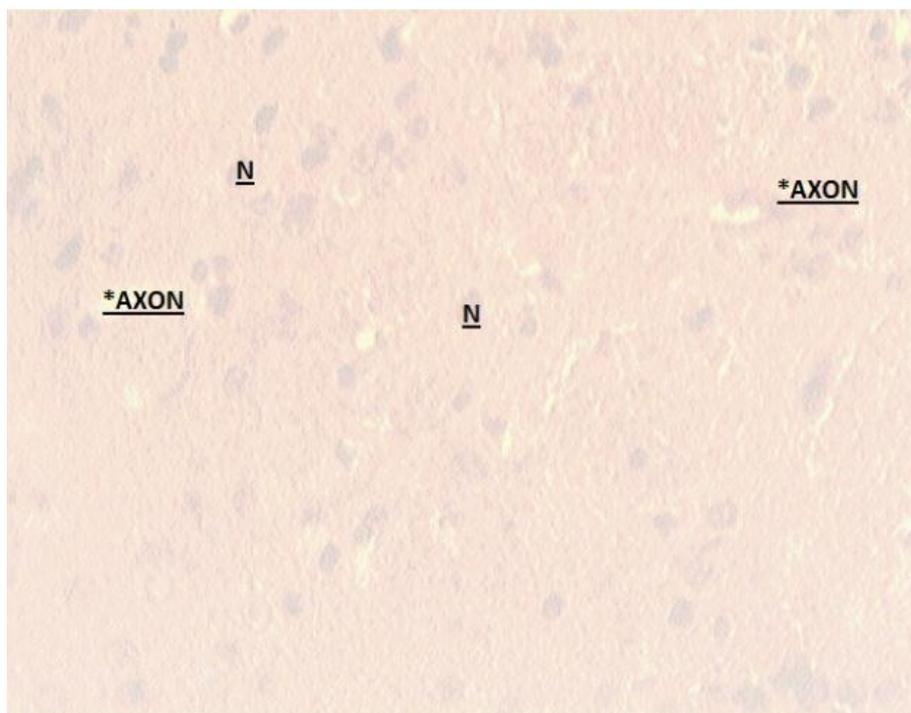


Figure 2: E2 Hypo x 400 magnification.

The photomicrograph of Group 2 hypothalamus (Figure 2) stained with Hematoxylin and Eosin (x400 magnification) reveals a normal hypothalamic structure

(as in normal brain) with healthy neurons (N) and axons, and no evidence of vacuolations, indicating a preserved architecture.

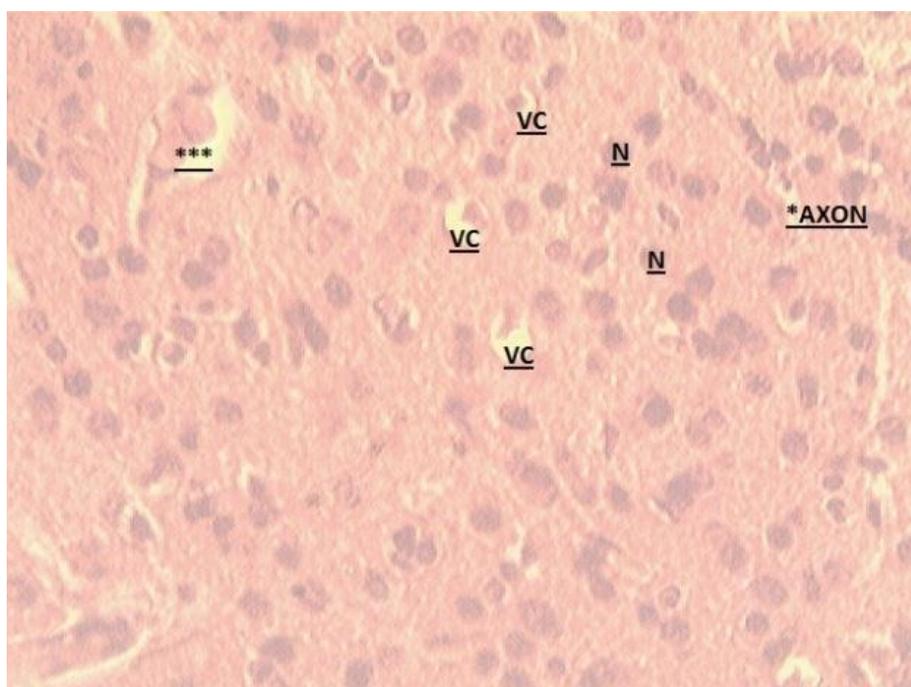


Figure 3: E3 Hypo x 400 magnification.

The photomicrograph of Group 3 hypothalamus (Figure 3) shows increased neuronal activity, characterized by multiple neurons (N) in form of granule cells and axons. However, cellular damage is evident, with enlarged and

cleared vacuolations. A slight distortion is also observed at the upper left side (***) indicating some disruption in the normal architecture.

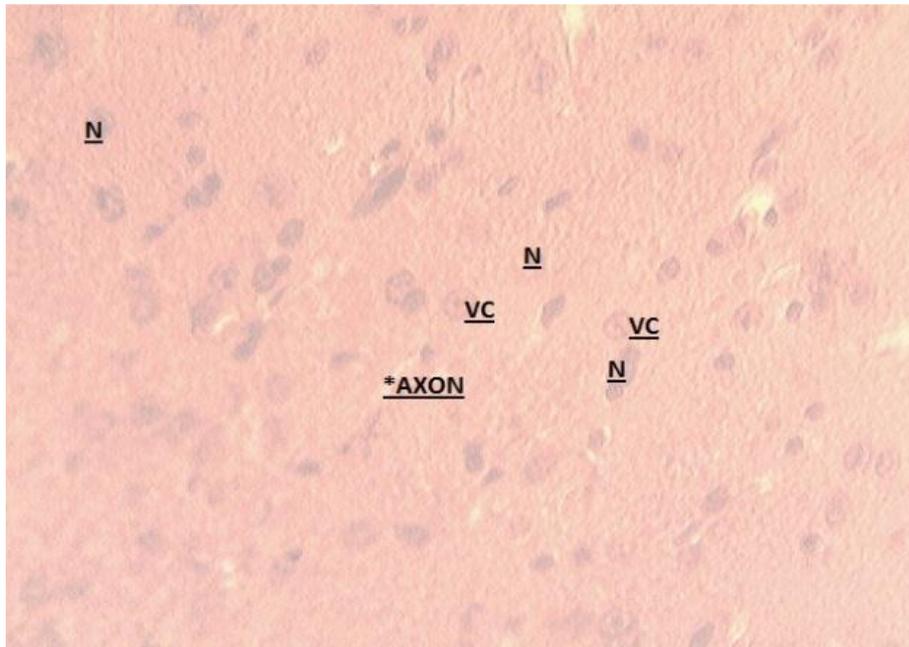


Figure 4: E4 Hypo x 400.

The photomicrograph of this group, Figure 4 appears to show regenerative hypothalamic structure, with neurons (N) looking lively and having normal axons. Notably, the

vacuolated cells are in a healing state, suggesting that the tissue is recovering and regenerating from previous damage.

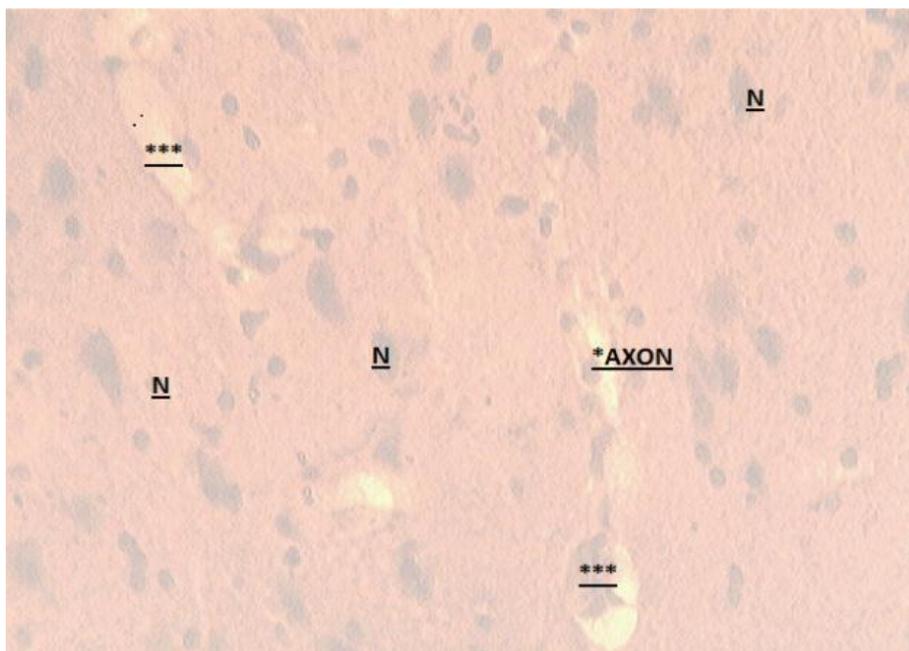


Figure 5: E5 Hypo 2 x 400 magnification.

The photomicrograph of Figure 5 reveals a severely affected hypothalamus structure, with neurons (N) appearing deeply darkened and unhealthy. Unlike other groups, this group does not exhibit extensive vacuolations, suggesting that the damage may be more

related to neuronal health rather than widespread cellular stress. The presence of slight distortions (***) at the upper left and lower right sides of the structure indicates some disruption in the normal architecture.

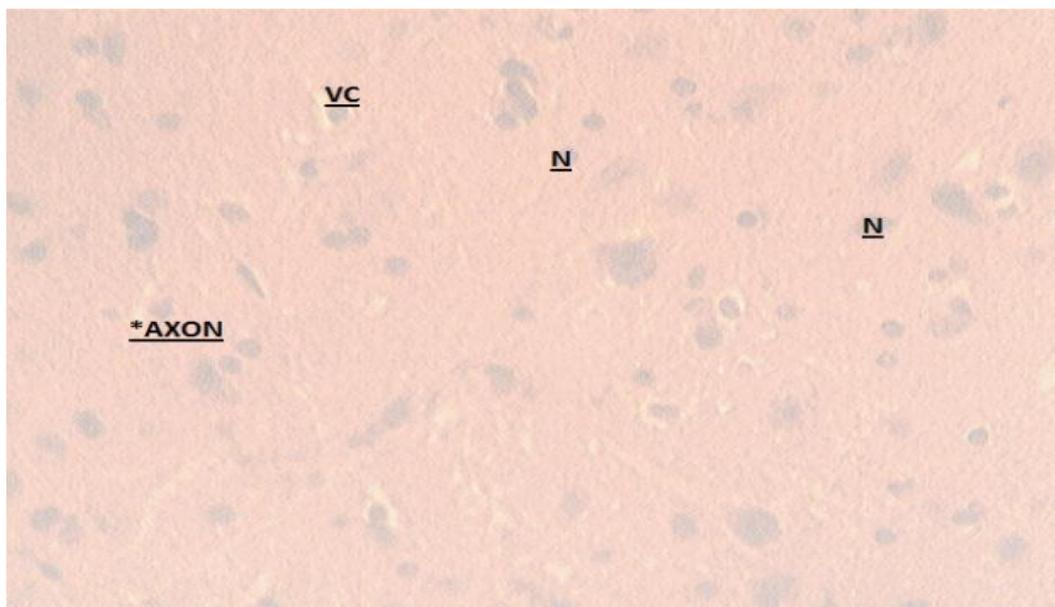


Figure 6: E6 Hypo x 400.

The group 6 (in fig.6) shows a relatively normal or regenerating hypothalamic structure, suggesting some level of recovery or improvement. However, the darkened neurons observed in Figure 5 appear to be

normalizing, with a return to a healthier appearance, and normal axons. Also the presence of few vacuolated cells indicates that some pathological changes are still present, but to a lesser extent.

BIOCHEMICAL ANALYSIS

Antioxidant Activity

Serum Lactate Dehydrogenase (LDH) Activity

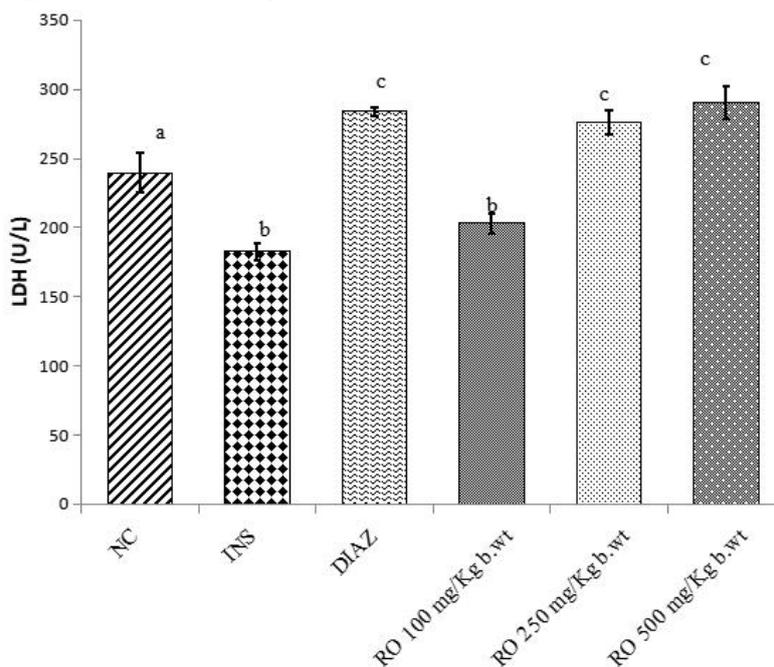


Figure 7: The effect of aqueous extract of *Salvia rosemarinus* on serum LDH activity in theophylline-induced insomnia rats.

Figure 7 above shows the effect of aqueous extract of *Salvia rosemarinus* on serum LDH activity in theophylline-induced insomnia rats. Results are mean ± standard deviation of 3 determinations. Bars bearing different superscript letters across groups are

significantly different ($p < 0.05$). These results indicate a significant decrease in LDH activity with *Salvia rosemarinus* extract treatment, suggesting potential antioxidant effects.

Serum Malondialdehyde (MDA) Concentration

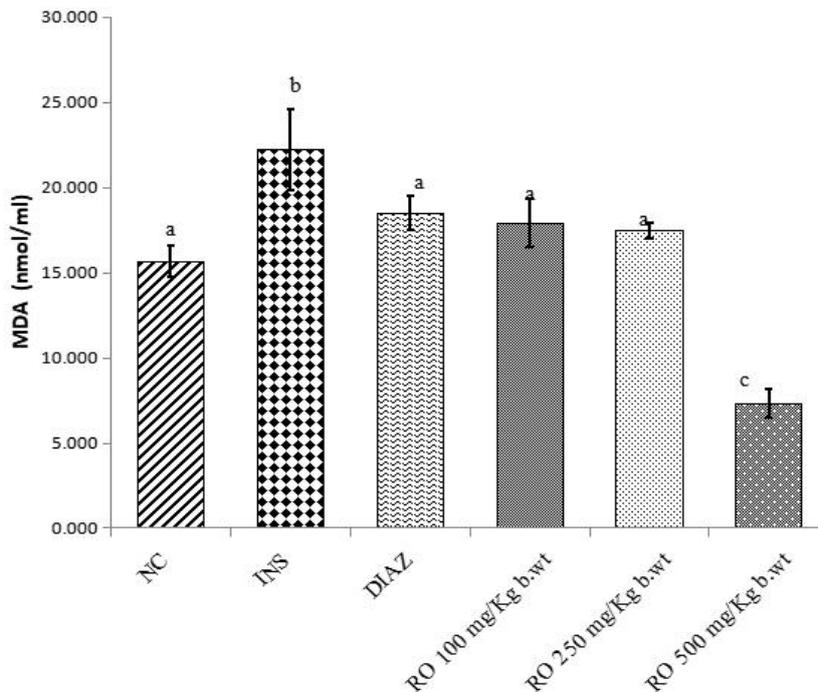


Figure 8: Effect of aqueous extract *Salvia rosemarinus* on serum malondialdehyde (MDA) concentration in theophylline-induced insomnia rat.

In figure 8 above, Results are mean ± standard deviation of 3 determinations. Bars bearing different superscript letters across groups are significantly different ($p < 0.05$).

The results indicate a significant decrease in MDA concentration with *Salvia rosemarinus* extract treatment, suggesting potential antioxidant effects.

Superoxide Dismutase (SOD) Activity

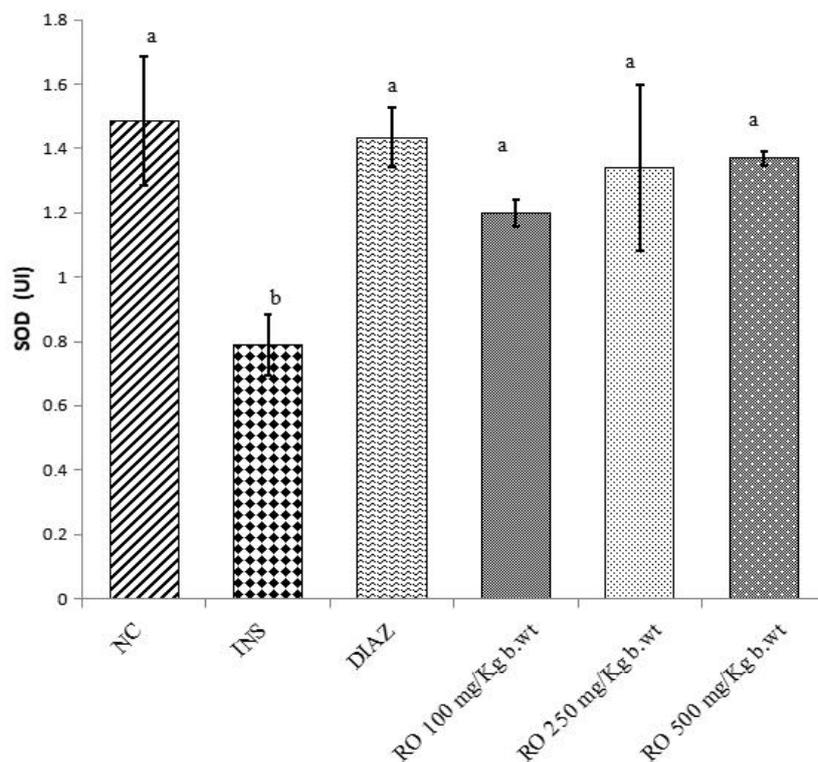


Figure 9: Effect of aqueous extract of *Salvia rosemarinus* on serum SOD activity in theophylline-induced insomnia rats.

Figure 9 shows the effect of aqueous extract with *Salvia rosemarinus* on serum super oxide dismutase activity in theophylline-induced insomnia rat. Results are mean \pm

standard deviation of 3 determinations. Bars bearing different superscript letters across groups are significantly different ($p < 0.05$).

Glutathione Peroxidase (GPx) Activity

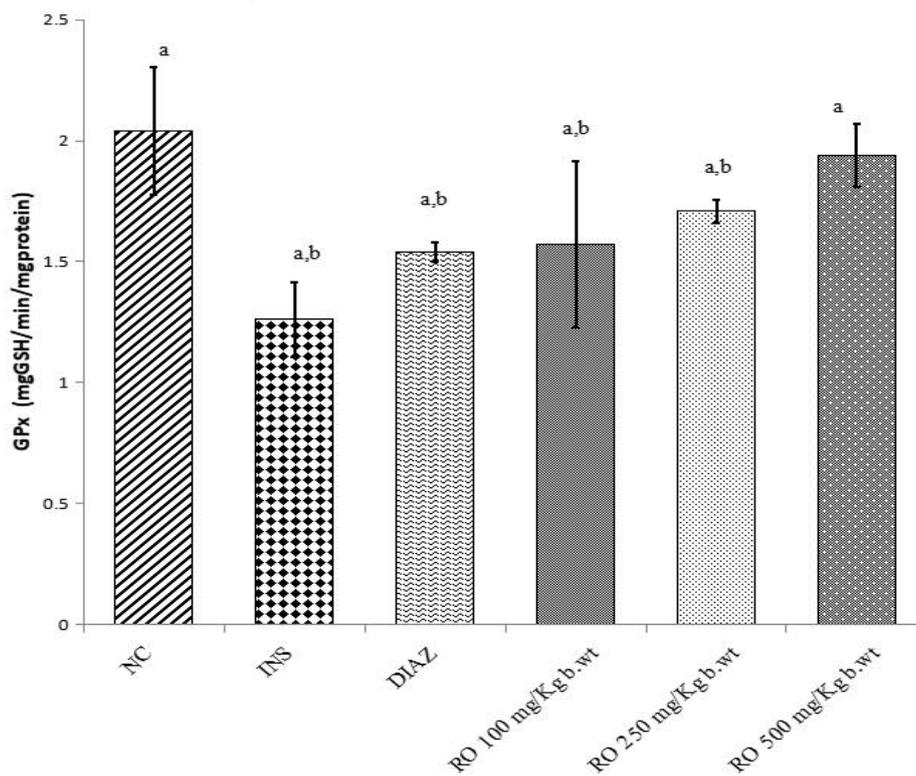


Figure 10: Effect of aqueous extract of *Salvia rosemarinus* on serum GPx activity in theophylline-induced insomnia rats.

The figure 10 above shows the effect of an aqueous extract of *Salvia rosemarinus* on serum glutathione peroxidase activity in theophylline-induced insomnia rat.

Results are mean \pm standard deviation of 3 determinations. Bars bearing different superscript letters across groups are significantly different ($p < 0.05$).

ANTIOXIDANT COMPOUNDS IN THE SALVIA ROSEMARINUS EXTRACT

Table 1: Phytochemicals of *Salvia rosemarinus* using GC-FID analysis.

S/N	Compound Name of Phytochemical	Class of phytochemical	Retention time	Area	Amt/area	Amt (ppm)
1	Kaempferol	Flavonoid	4.082	5.43273	1.39127x10 ⁻¹	7.55838x10 ⁻¹
2	Catechin	Flavonoid	4.407	30.99838	1.40308x10 ⁻¹	5.34933
3	Quercetin	Flavonoid	4.789	20.08449	1.40140x10 ⁻¹	3.81463
4	Hesperidin	Flavonoid	5.009	7.93811	1.39327x10 ⁻¹	2.10600
5	Luteolin	Flavonoid	5.162	8.90847	1.39616x10 ⁻¹	2.24377
6	Reveratrol	Stilbenol	5.318	14.82315	1.39784x10 ⁻¹	3.07204
7	Artemetin		5.358	115.91574	1.40556x10 ⁻¹	16.29269
8	Myricetin	Flavonoid	5.539	30.51567	1.40326x 10 ⁻¹	5.28215
9	Naringin	Flavonoid	5.706	12.08346	1.39000x10 ⁻¹	2.67960
10	Retusin		5.914	8.03779	1.39489x10 ⁻¹	2.12118
11	Ellagic acid	Polyphenol	6.117	19.77841	1.40134x10 ⁻¹	3.77163
12	Sapogenin		6.318	66.77634	1.40468x10 ⁻¹	9.37996
13	Isorhamnetin	Flavoniod	6.512	22.25084	1.40202x10 ⁻¹	4.11960
14	Naringenin	Flavonoid	6.781	30.66835	1.40284x10 ⁻¹	5.30229
15	Apigenin	Flavonoid	7.145	13.69345	1.39138x10 ⁻¹	2.90527
16	Maricetin	Flavonoid	7.519	16.35202	1.40008x10 ⁻¹	3.28942
17	Epicatechin	Flavonoid	7.836	9.93165	1.39560x10 ⁻¹	2.38606

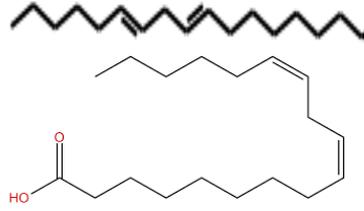
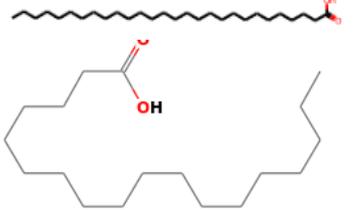
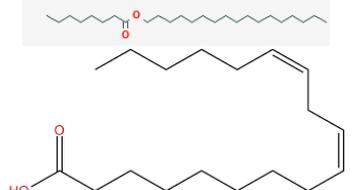
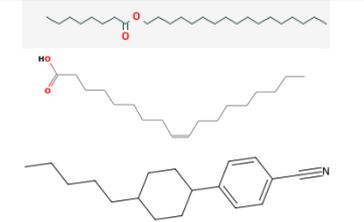
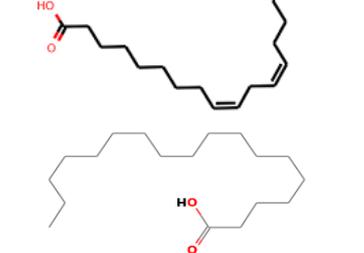
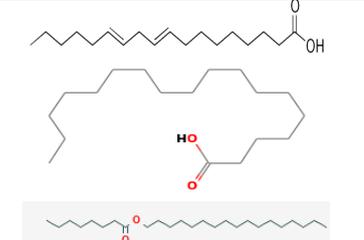
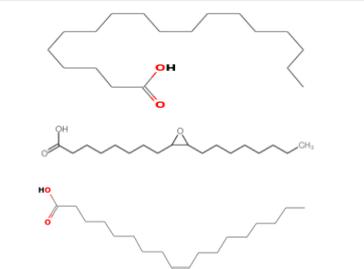
18	Daidzein		8.248	5.80251	1.38805×10^{-1}	8.05419×10^{-1}
19	Genistein	Isoflavones	8.405	10.80795	1.38256×10^{-1}	1.49426
20	Apigenin	Flavonoid	8.739	3.23390	1.37308×10^{-1}	4.44042×10^{-1}
21	Lunamarin		8.891	6.70073	1.39026×10^{-1}	9.31575×10^{-1}
22	Gallocatechin	Catechin	9.276	20.34908	1.40050×10^{-1}	2.84989
23	Tangeretin	Flavonoid	9.779	2.90485	1.37102	3.98259×10^{-1}
24	Hesperidin	Flavonoid	10.187	4.44673	1.38187	6.14480×10^{-1}
25	Epicatechin	Flavonoid	10.744	6.92549	1.39167	9.63802×10^{-1}
26	Silymarin		10.951	2.07558	1.33663×10^{-1}	2.77429×10^{-1}
27	Baicalin	Flavonoid	11.558	2.70830	1.35568×10^{-1}	3.67159×10^{-1}
28	Tangeretin	Flavonoid	13.403	3.04452	1.36543×10^{-1}	4.15707×10^{-1}
29	Flavone	Flavonoid	18.651	-	-	-
30	Nobeletin	Methoxyflavone	21.223	-	-	-
31	Isorhamnetin	Flavonoid	23.302	-	-	-
32	Butein	Chalcone	26.274	-	-	-
33	Daidzin	Isoflavones	28.232	-	-	-
34	Ferulic acid	Phenolic	30.586	-	-	-
35	Sinapinic acid		31.656	-	-	-
	84.43349		Total			

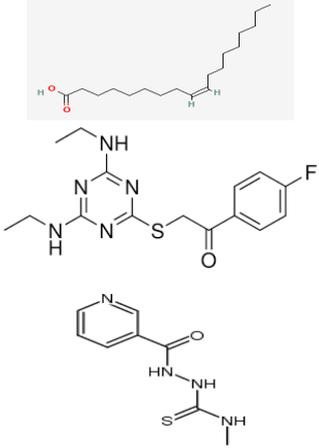
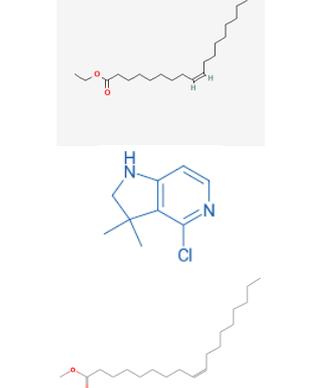
PHENOL COMPOUNDS IN THE AQUEOUS EXTRACT OF SALVIA ROSEMARINUS

Table 2: Bioactive compounds in aqueous extract of *Salvia rosemarinus* using GC-MS.

Library/ID	Retention time	Area %	Biological activity	Structures
5-Octadecene, (E)- Cyclopropane, nonyl 2-Tetradecanol	9.840	1.45	Antioxidant, Antibacterial, Antifungal	
Pyrimidine-4,6(3H,5H)- dione, 2 butylthio-dodecanoic acid 2-chloroethyl chlorobromethyl sulfoxide.	12.828	0.29	Anticancer, Antioxidant, Neurotoxicity	
Trichloroacetic acid, pentadecyl ester 3-chloropropionic acid, heptadecyl ester 1-octadecene	12.893	0.45	Antioxidant, Antimicrobial, Anti-inflammatory	
Dodecanoic acid, Dodecanoic acid, Dimethyl Sulfoxide	13.113	2.38	Antioxidant, Anti- inflammatory, Analgesic	

Tetradecanoic acid, Tetradecanoic acid, Decanoic acid, silver(1+) salt	15.408	2.29	Anti-cancer Potentia, Skin Health, Wound healing	
Carbonic acid, propyl 2,2,2-trichloroethyl ester, Propanoic acid, 3-chloro-, methyl ester, Propanoic acid, 3-chloro-, methyl ester	16.880	0.66	Pesticide/Herbicide , Toxicity to Aquatic Life, Microbial Control,	
Hexadecanoic acid, methyl ester, Hexadecanoic acid, methyl ester, Hexadecanoic acid, methyl ester	16.952	1.90	Anti-inflammatory, antioxidant, mosquito larvicide, nematicide ^{26, 27}	
n-Hexadecanoic acid, n-Hexadecanoic acid, n-Hexadecanoic acid	17.410	2.14	Protein, anticancer	
n-Hexadecanoic acid n-Hexadecanoic acid n-Hexadecanoic acid	17.538	7.45	Protein, anticancer	
10-Octadecenoic acid, methyl ester, cis-13-Octadecenoic acid, methyl ester, 11-Octadecenoic acid, methyl ester	18.743	6.38	Membrane Fluidity Modulation, Membrane Fluidity and Cellular Function, Anti-inflammatory	
Heptadecanoic acid, 16-methyl-, methyl ester, Cyclopropanepentanoic acid, 2-undecyl-, methyl ester, trans-Methyl 9-methyltetradecanoate	18.980	0.88	Metabolic, Potential impact on lipid metabolism, Potential cytotoxicity (e.g., cancer cell targeting).	

<p>Linoelaidic acid 9,12-Octadecadienoic acid (Z,Z)- 9,12-Octadecadienoic acid (Z,Z)-</p>	<p>19.301</p>	<p>24.34</p>	<p>Pro-inflammatory effects, contributing to chronic diseases, Metabolism regulation and potential role in obesity, Cancer prevention through regulation of cell proliferation</p>	
<p>Octadecanoic acid Octadecanoic acid n-Hexadecanoic acid</p>	<p>19.497</p>	<p>7.95</p>	<p>Antimicrobial, Pro-inflammatory</p>	
<p>9,12-Octadecadienoic acid (Z,Z)- Linoelaidic acid 9,17-Octadecadienal, (Z)-</p>	<p>19.753</p>	<p>2.98</p>	<p>Antioxidant, Pro-inflammatory, Cell Membrane Integrity</p>	
<p>9,12-Octadecadienoic acid (Z,Z)- Oleic Acid Cyclohexene, 4-pentyl-1-(4-propylcyclohexyl) -</p>	<p>19.869</p>	<p>4.25</p>	<p>Anti-inflammatory, Cardiovascular Health, Potential Antimicrobial activity.</p>	
<p>9,12-Octadecadienoic acid (Z,Z)- Methyl 9,12-heptadecadienoate 9,12-Octadecadienoic acid (Z,Z)-</p>	<p>19.999</p>	<p>2.48</p>	<p>Anti-inflammatory, lipid metabolism</p>	
<p>Linoelaidic acid 9,12-Octadecadienoic acid (Z,Z)- Z,Z-10,12-Hexadecadien-1-ol acetat</p>	<p>20.137</p>	<p>5.70</p>	<p>Insulin Resistance, Potential Skin Irritation, Pro-inflammatory effects</p>	
<p>6-Octadecenoic acid, (Z)- Oleic Acid Oxiranedodecanoic acid, 3-octyl-, cis-</p>	<p>20.900</p>	<p>10.94</p>	<p>Anti-inflammatory Effects, Potential for Cytotoxicity</p>	

<p>Oleic Acid 1,3,5-Triazin-2(1H)-one, 4,6-bis(ethylamino)-</p>	30.931	12.48	<p>Cancer Prevention, Neuroprotective Effects, Anticancer activity</p>	
<p>Ethyl Oleate , 1-oxide 9-Octadecenoic acid (Z)-, 2-hydroxy-1- (hydroxymethyl)ethyl ester.</p>	31.429	0.92	<p>Skin Care and Hydration, Solvent and Carrier in Drug Delivery, Antioxidant and Free Radical Scavenging</p>	

DISCUSSION

The study investigated the neuroprotective effects of aqueous extract of *Salvia rosmarinus* on hypothalamic histopathology in theophylline-induced insomniac rats. The results showed significant improvements in hypothalamic structure and antioxidant activity. The histopathological analysis revealed that the theophylline-induced insomnia group (Group 2) had a normal hypothalamic structure, while the groups treated with *Salvia rosmarinus* extract showed varying degrees of recovery and regeneration. Specifically, the low-dose group (Group 4) exhibited regenerative changes with lively neurons and normal axons, while the high-dose group (Group 6) displayed a relatively normal or regenerating hypothalamic structure with normalizing neurons and axons. The neuroprotective effects of *Salvia rosmarinus* extract were evident from the significant increase in serum norepinephrine levels ($p \leq 0.05$) and melatonin levels ($p \leq 0.05$). These findings suggest that *Salvia rosmarinus* extract may have potential therapeutic effects on insomnia by modulating neurotransmitters and hormones involved in sleep regulation, consistent with previous studies on the neuroprotective effects of *Salvia* species (Kennedy, Pace, Haskell, & Scholey, 2004; Savelev, Okello, & Perry, 2003).

The biochemical analysis further revealed that *Salvia rosmarinus* extract demonstrated antioxidant activity by reducing lactate dehydrogenase activity ($p \leq 0.05$) and malondialdehyde concentration ($p \leq 0.05$). These effects

may be attributed to the diverse range of bioactive compounds present in the extract, including fatty acids such as dodecanoic acid, tetradecanoic acid, hexadecanoic acid, and octadecanoic acid, as well as esters like hexadecanoic acid methyl ester and octadecenoic acid methyl ester (Gupta *et al.*, 2019; Król *et al.*, 2020). Furthermore, the extract contained other bioactive compounds like oleic acid and linoleic acid, which possess antioxidant properties (Srivastava *et al.*, 2010). These compounds may contribute to the potential health benefits and medicinal properties of *Salvia rosmarinus* extract, including antimicrobial and antioxidant effects (Sparkman *et al.*, 2011; Kruk *et al.*, 2018).

The findings of this study demonstrate the neuroprotective effects of *Salvia rosmarinus* extract on hypothalamic histopathology in theophylline-induced insomniac rats. The extract's ability to modulate neurotransmitters and hormones involved in sleep regulation, as well as its antioxidant activity, may contribute to its therapeutic effects. Compared to existing pharmacological treatments for insomnia, *Salvia rosmarinus* extract may offer a more natural and potentially safer alternative. The extract's ability to modulate neurotransmitters and hormones involved in sleep regulation may provide a more targeted approach to treating insomnia, potentially reducing the risk of side effects associated with traditional insomnia medications. The findings of this study suggest that *Salvia rosmarinus*

extract may have potential therapeutic applications in the treatment of insomnia and other sleep disorders in humans. The extract's ability to modulate neurotransmitters and hormones involved in sleep regulation, as well as its antioxidant activity, may provide a novel approach to managing sleep disorders. Further studies are needed to explore the potential benefits and efficacy of *Salvia rosmarinus* extract in humans.

CONCLUSION

In conclusion, our study demonstrates the neuroprotective effects of aqueous extract of *Salvia rosmarinus* on hypothalamic histopathology in theophylline-induced insomniac rats. Treatment with *Salvia rosmarinus* extract showed regenerating changes in the hypothalamus, with normal neurons and axons, and significantly increased serum norepinephrine and melatonin levels ($p \leq 0.05$). Additionally, the extract demonstrated antioxidant activity by reducing lactate dehydrogenase activity and malondialdehyde concentration ($p \leq 0.05$), and modulating SOD and GPx activity. These findings suggest that *Salvia rosmarinus* extract may have potential therapeutic applications in the treatment of insomnia and other sleep disorders related to hypothalamic dysfunction ($p \leq 0.05$). Further studies are needed to confirm these findings and explore the underlying mechanisms.

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