AMELIORATIVE EFFECT OF RESVERATROL ON TRAMADOL INDUCED NEUROTOXICITY IN ADULT ALBINO RATS

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ABSTRACT

Background: Neurotoxicity of tramadol which is used as a potent analgesic considers a significant public health concern. Resveratrol has antioxidants, anti- inflammatory and anti-apoptotic characters, therefore elicits cerebral oxidative disturbances, it offers neural protection. **Aim:** this experimental study aimed at assessing the tramadol's toxic impacts on brain of adult albino rat behaviorally, biochemically, histopathological, and immuno-histochemical, and to explore the potential ameliorative effect of resveratrol. **Materials & Methods:** fifty rats weighing 180-200 gm were used and parted into four groups: two Control groups [Negative, and Solvent], resveratrol (20mg/kg B.W./day) group, tramadol group (50mg/kg/day), tramadol+ resveratrol group. for 28 days. **Results:** Resveratrol relieved the cognitive deficit and oxidative stress (CAT, & GPx concentrations raised significantly ($P<0.05$) after resveratrol was taken with tramadol in comparison with tramadol taken alone. Tramadol with resveratrol intake diminished brain MDA level versus tramadol taken alone.). It also reduced neuronal inflammation (caspase3, and TNF-α) and damage. The administration of Resveratrol with tramadol declined significantly ($P<0.05$) the changes in brain 5-HT and NOR in contrast to Tramadol treated rats **Conclusion:** Resveratrol owns neuroprotective role on tramadol

Key words: resveratrol, tramadol, neurotoxicity, rats.

INTRODUCTION

Substance abuse is a huge problem in a lot of societies, as it can give rise to serious damage physically and mentally. Opioids e.g., Morphine are noticed because of their dangerous impacts. For example, Morphine has a potent ability as a pain killer, but it has serious dangers like dependence and addiction. Recently, tramadol used as an analgesic 10 times less than morphine and more safely **(*Aghajanpour* et al. 2020).**

Tramadol is synthetic opioid that works centrally. It is used as analgesic for treatment of different types of pain. Its mechanism is not only as agonist of mu- opioid receptor but also as inhibitor of norepinephrine and serotonin reuptake **(Abou elnaga et al., 2018).**

Central nervous system (CNS) is the most rustable system for affection by tramadol. Tramadol abuse can seriously affect brain structure and function; it disrupts cerebral cortex so that tramadol abuse symptoms are due to cortical neurodamage. ***(Aghajanpour et al., 2020).***

Tramadol causes neurotoxic effects, especially with prolonged use or overdose **(Olaniyi et al., 2025)** moreover, dysregulation of signaling cascades as oxidative stress, apoptosis and inflammation caused its neurotoxic effects. **(*Aghajanpour* et al. 2020)**.

Black grapes contain resveratrol (3,5,4'-OHstilbene), which is a main non-flavonoid multiphenol, plants produced it in response to stress. **(Jang et al., 2008)**

Traditional chinese and japanese medicine used resveratrol, it has biologically and pharmacologically significant activities, it exerts numerous vital activities against inflammation, oxidize stress, mutation, and tumor**. (Fuggetta et al., 2006)**

Neuroprotective effect of resveratrol has been observed in multiple studies, they shown that resveratrol has antiepileptic, anti-tumorigenic, antidepressant, anticonvulsant, anti-neurodegenerative, antinociceptive, and sedative effect **(Kennedy et al., 2010).** Resveratrol also can regulate multiple processes, as damage of DNA, apoptosis and metabolism **(Ma et al., 2020).**

Tramadol abuse found to be a more attention concern among the Egyptians. Unfortunately, tramadol widely utilized among Egyptian youth, for dealing with various impairments like premature ejaculation, and to expand sexual pleasure and orgasms. 83% of Egyptian adolescents who have disorders of substance abuse consume tramadol, so it reclassified from schedule three to one by the ministry of health of Egypt., The United Nations Office for Drugs and Crime (UNODC), in 2014, recorded that 5 billion tablets were acceptable in Cairo, where tramadol represented more utilized street drug ***(Ahmed et al., 2018).***

few studies were structured to recognize neuroprotective action of resveratrol against tramadol provoked noxious effects in rats. **(Wang qun et al., 2004).**

AIM OF THE WORK

This experiment is intended to uncover the toxic outcomes of tramadol on the adult albino rats' brains, and the possible neuroprotective capacity of resveratrol.

MATERIAL AND METHODS

Chemicals:

Tramadol 99% purity (tramadol HCL pill), resveratrol (99% purity), most reagents and chemicals were sourced from sigma company for chemicals (St. Louis, mo., USA).

Experimental design:

Fifty wistar rats (6-8 weeks old) weighing l80 - 200. Grams (gm) were used. They were from the Benha experimental animal center, faculty of veterinary medicine, Egypt. Benha clinical pharmacology department, faculty of medicine, Benha university, is the place where all animals are habituated for experimental interventions. Rats placed inside well-ventilated clean cages (12h-light and 12h-dark cycle) before and during the

experiment. Benha university hospital's incinerator received the remains of rats after experiment. Procedures related to the care and handling of animals were conducted in strict adherence to the animal care guidelines established by the National Institutes of Health (NIH).

The protocol of this experimental research has been permitted by the Research Ethics Committee at the Faculty Of Medicine, Benha University (REC-FOMBU), with permission code R.C .1.2.2026

The animals were parted randomly into four groups:

Group. I (Control -Group): which was sub-divided into:

Group IA (-ve control group): were left untreated.

Group IB (solvent control group): received oral 1 ml/day of saline 0.9% (dissolvent for tramadol and resveratrol) for 28 days **(Manawy, et al., 2024) (*Nafea et al. 2016)***

Group II (resveratrol -treated group):

A dose (20 mg/kg/day) of resveratrol were received orally through gastric gavage for 28 -days **(Yal-cin et al., 2024)**.

Group III (tramadol treated group): every rat fed 50 mg/kg/day of tramadol by gastric gavage for 28 -days. **(Soliman et al., 2017).**

Group. IV (tramadol + resveratrol treated group):

Tramadol taken at a dose of (50 mg/kg/day) with (20 mg/kg/day) of resveratrol by gastric gavage once for 28 days period.

Assessment of cognitive abilities

The test of Morris Water Maze (MWM) was employed to evaluate spatial navigation skills and memory. Morris described the water maze in 1984. The MWM is one of the most frequently used behavioral tasks for assessing spatial memory defects. **(Gaoy, et al., 2011)**

Procedures (Nafea et al., 2016) (Hindawy et al., 2024)

The rats were trained to navigate a water maze, which was a circular pool measuring 180cm diameter and 60cm height. The water depth of the pool was 40cm. The submersed platform is acube (10× 10×10cm). The water temperature was 28±1◦c and water was colored black with non- toxic food dye to hide the cube. The pool was parted equally into 4quadrants by 4starting points that were marked on its edge as follows: North(N), East(E), South(S)and West(W). The submersed platform was consistently positioned in the middle of the SW quadrant. The experimental animals were trained to find the submersed platform using visual cues (colored flags) put around the pool, which maintained fixed all through the research to help the rats in finding the submersed platform. On day20 of drug intake, the training commenced, it is consisting of 4 trials with a10-mins interval. We used various starting points in each trial. We recorded the time taken by every animal to find the platform the initial acquisition latency (IAL), with a maximum allowed duration was set at120s. This represented the maze acquisition phase(training). On days 21and28, we allow each animal to go from any of the starting points, facing the poolwall, and tested for the retention of the previously learnt task. We document the latency to reach

the submersed platform on both days(21and28), and we term as first and second retention latency (1st RL and 2nd RL), respectively. This represented the maze retention phase (testing for retention of the learned task).

At the appropriate time, animals were euthanized **(Gaertner et al. 2008).** All the rats were sacrificed 24 hours after the last treatment. Brain (right & left hemispheres) was dissected out.

Rat body weight and **relative brain weight (RBW%):** body weights of all groups were estimated and documented before drug intake and on 28th day of the experimental research by a sensitive balance. The rats' brains were separated, then cleaned in normal saline and we recorded weights as regards the shown equation:

Relative weight (%) =absolute organ weight (g)/ body weight on 28thday of the experiment (g)×100 ***(Khalil et al., 2022).***

Biochemical assays

Tissue homogenate preparation

The phosphate buffer at pH. 7.4 was used. For 20 min, the homogenates were centrifuged at 11,000×g. to obtain supernatant which used for the recognition of the assays.

Oxidant/antioxidant biomarkers assessment:

The attained supernatant was preserved at minus eighty degrees Celsius until assay. The pro-oxidative marker; cerebral malondialdehyde (**MDA**) level was recognized by spectrophotometry utilizing a bio-diagnostic kit (cat. No. Md 2529, bio diagnostic company, Doki, Giza, Egypt) for the Thio barbituric acid technique of **Ohkawa et al. (1979), concentration of cerebral** glutathione peroxidase (GPx) and catalase (CAT) antioxidant enzymes have been measured (biodiagnostic kits (cat. No. Ca 2517, Egypt) for CAT and (cat. No. Gp 2524, Egypt) for GPx), by methods of **Aebi (1984)** & **Paglia and Valentine (1967)**.

The proinflammatory, proapoptotic, anti-inflammatory markers

Brain proinflammatory cytokines, namely tumor necrosis factor-alpha (TNF-α), along with proapoptotic marker Cysteiny1 Aspartate Specific Protase3(Caspase3), Interleukin 10 (IL.10) as antinflammatory marker, and Follistatin (FST) were determined in brain homogenates with ELISA employing kits (ray biotech, USA)(TNF-α-cas 94948-59-1) (Casp3c, na.84) (IL10- 19160-1kt-f) (FST -e-el-h5574-).**(Pandey et al., 2015)**.

Assessment of neurotransmitters in brain tissues:

Rat ELISA kits (ray biotech, USA) were engaged to estimate the amounts of serotonin (**5–HT**) and noradrenaline (**NOR**). **(Baldo, 2021).**

Histological study:

Samples of cerebral cortical tissues are fixed for twenty four hours in ten percent neutral-buffered formalin, then utilising routine protocols, letting samples in flat molds later embedding them in paraffin., staining 5 micrometer sections with Hematoxylin with

Eosin (H&E). (***Bancroft, and Gamble, 2008)*** ***(Bancroft et al.,*** **2019*).***

Immunohistochemical study:

Immunohistochemical staining for caspase3 was employed to find cerebral apoptotic cell death. Paraffin sections were incubated with a rabbit monoclonal caspase3 antibody using a Vidin biotin peroxidase method ***(Bancroft et al.,*** **2018*).***

Histomorphometry assessment

Caspase 3 immunostaining percentage was quantified by estimating the mean area. This analysis was done in 10 distinct fields on each slide for every animal, using magnifications of ×100and×400. Image acquisition was performed with the Leica icc50w light electric microscope at the image analysis unit of the pathology department, faculty of medicine, Benha university, Benha, Egypt. Histomorphometry evaluation used the image j analyzer computer system developed by waynerasb and at NIH, Bethesda, Maryland, USA. Analysis of image included automatic calibration to chang pixels to µm units **(Faul et al., 2009).**

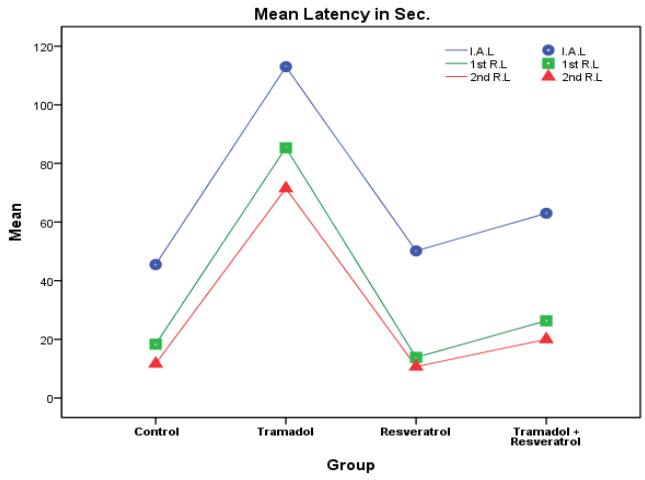
Data analysis

The normal distribution of values was known by performing Shapiro's test for normality. The data were analyzed by employing one-way analysis of variance (Anova) followed by Tukey's post-hoc tests for comparison between the groups (IBM spas statistics for windows, IBM Corp, version 22.0. Armonk, Nye, USA). All values were represented as mean ± standard deviation. If probability value p< 0.05, differences were regarded as significant.

RESULTS

Resveratrol got memory and learnings skills better

Tramadol has bad effects on memory and learning skills, as the initial acquisition latency (IAL) expanded in group treated with tramadol with statistically significant difference in comparison with Control and Resveratrol groups (*P*<0.001each). But concurrent Resveratrol with Tramadol reduced IAL significantly compared with Tramadol taken alone. Also, tramadol expanded 1stRL(day21) and 2ndRL(day28) latencies with statistically significant difference in comparison with Control and Resveratrol groups (*P*<0.001each). Simultaneous Resveratrol intake with Tramadol shorting both1stRL & 2ndRLlatencies significantly compared with tramadol group **(Fig.1)**.



(Fig.1) Linear plot graph with error bars showing effects of Resveratrol supplement on memory and learning skills in spatial navigation task of tramadol treated rats, Data are mean±standard deviation

Tramadol decreased body weight and RBW%

A statistically greatly significant reduction was found in the rats' body weight and RBW% of group taken tramadol as compared with control rats (P<0.001) however simultaneous resveratrol administration with tramadol raised the body weight statistically non-significant (P value >0.05) as compared with administration of tramadol alone. Resveratrol+tramadol group shown nonsignificant variance compared with control as shown in **Table (1).**

Table (1): Body Weight (gm) and RBW% in different studied groups

Groups/ parameters	Controls	resveratrol	tramadol	Resveratrol+ tramadol
Body Weight (gm)	312.3±12.5	312.5±11	215.5±11.9 [a]	276.7±21.2
RBW%	22.7±.46	21.06±2.3	15.6±1.2 [a]	18.9±1.3

Data are presented as mean± SD. RBW%: relative brain weight, [a]: significance between controls and tramadol treated group.

Table 2: Effects of tramadol, resveratrol, and their combination on brain oxidant/antioxidant parameters (MDA, CAT and GPx)

Group/ parameters	Control	resveratrol	tramadol	Resveratrol+ tramadol
Brain MDA (nmol/g. tissue)	0.50±0.05	0.51±0.06	17.88±4.11[a]	1.2±1.09*
CAT (U/g tissue)	42.21±5.08	42.99±4.67	19.65±4.07[a]	36.095±3.97 *
GPx (U/g.tissue)	150.48±7.03	149.80±5.03	44.01±3.05[a]	130.99±2.01*

Data are presented as mean± SD, MDA: malondialdehyde, CAT: Catalase, GPx: glutathione peroxidase. [a]: significance between controls and tramadol treated group, [*]: significance between tramadol treated group and resveratrol+tramadol group.

The outcomes of tramadol administration on oxidant/antioxidant parameters, including brain MDA, CAT, and GPx

Tramadol lowered brain CAT, and GPx than control with significant variance. The CAT, & GPx concentrations raised significantly after resveratrol was taken with tramadol in comparison with tramadol taken alone. The level of brain MDA increased significantly after tramadol intake as compared with control. Tramadol with resveratrol intake diminished brain MDA level versus tramadol taken alone. **(Table 2).**

Resveratrol improved brain inflammatory and apoptotic biomarkers induced by tramadol. There was a significant elevation in caspase3, and TNF-α of Tramadol -treated group in comparing with control, as well as a significant decline in IL10 and FST belonging to tramadol group versus control. The administration of Resveratrol with tramadol improved significantly the changes in caspase-3, TNF-α, IL10 and FST comparing with the tramadol intoxicated rats **(Table 3**).

Resveratrol improved brain neurotransmitters following tramadol administration.

A significant raise in brain Serotonin (5–HT) & Noradrenaline (NOR) of Tramadol group was noticed versus Control group. The administration of Resveratrol with tramadol

improved significantly the changes in brain 5-HT and NOR in contrast to Tramadol treated rats **(Table 4**).

Table 3: Effects of tramadol, resveratrol, and their combination on brain inflammatory and apoptotic parameters (TNF-α, Caspase-3, IL10 and FST).

Group\ parameters	Control	resveratrol	tramadol	Resveratrol+ tramadol
TNF-α (pg/ ml)	24.01 ±0.08	28.96±0.17	84.05 ±0.17[a]	32.05±0.02*
IL10 (pg/ ml)	200.03±20.08	210.11±15.67	90.55±7.3[a]	180.48±12.9*
Caspase-3 (ng/ml)	0.80 ± 0.07	1.01± 0.03	4.07 ± 0.07[a]	1.09 ±0.04*
FST (ng/ml)	1.55± 1.08	1.35± 0.90	0.48± 0.408[a]	1.092± 0.67*

Data are presented as mean± SD, tumor necrosis factor-alpha (TNF-α), Cysteinyl aspartate specific proteinase-3(caspase-3), interleukin 10 (IL-10), and Follistatin (FST), [a]: significance between controls and tramadol treated group, [*]: significance between tramadol treated group and tramadol-resveratrol treated group.

Table 4: Effects of tramadol, resveratrol, and their combination on brain neurotransmitters (5–HT, and NOR).

Group\ parameters	Control	resveratrol	tramadol	Resveratrol+ tramadol
5–HT (µg/mg. tissue)	0.88±0.051	0.92±0.041	21.99±3.5[a]	2.99±0.13*
NOR (pg-mg tissue)	75.7±8.6	78.9±9.4	226.9±31.1[a]	100.1±7*

Data are presented as mean± SD, 5-HT: serotonin, NOR: noradrenaline, [a]: significance between controls and tramadol treated group, [*]: significance between tramadol treated group and tramadol-resveratrol treated group.

Histological study:

Resveratrol ameliorated neuronal histological derangements in tramadol treated animals

H&E-stained sections of the cerebral cortical tissue from the control and Resveratrol groups demonstrated the normal six layers: layer I (molecular layer) was enriched in fibers and few cells. Layer II (outer granular layer) was represented by sets of cells with dominant, vesicular spherical nuclei. Layer III (pyramidal layer), exhibited pyramidal basophilic cells, vesicular dominant nuclei, and long dendrites at the apex. Plus, glial cells with dense tiny nuclei were demonstrated in layer III. Layer IV (inner granular layer) showed a lot of granular tiny cells, layer V (inner pyramidal layer) characterized by huge pyramidal cells, and layer VI (multiform layer) was filled with cells having variable size and shapes, **(Fig.2a–d)**. However, sections from the rats' brain taken tramadol exhibited severe neurodistortion and dilated congested bl.vessels. Neuronal cells showed apoptosis with diminished in their size and chromatin condensation. Degenerative vacuolization, wide intercellular spaces, separated layers, and inflammatory cellular collections in the cerebral cortex layers were observed **(Fig.3& 4).** Whereas cerebral sections from the tramadol+resveratrol group demonstrated improvement compared with the tramadol treated animals, with few vascular congestion of rats' brain still noticed (**Fig.5).**

Table (5): Effects of resveratrol supplement on caspase-3 immunoexpression area% in the brains' tissues of tramadol -treated rats.

Caspase 3	Group I	Group II	Group III	Group IV
Mean ± SD	2.6 ± 1.7	2.03 ± 1.4	25.8 ± 9.4	9 ± 3.7
Significance ≤ 0.05	With group III	With group III	With groups I, II & IV	With group III

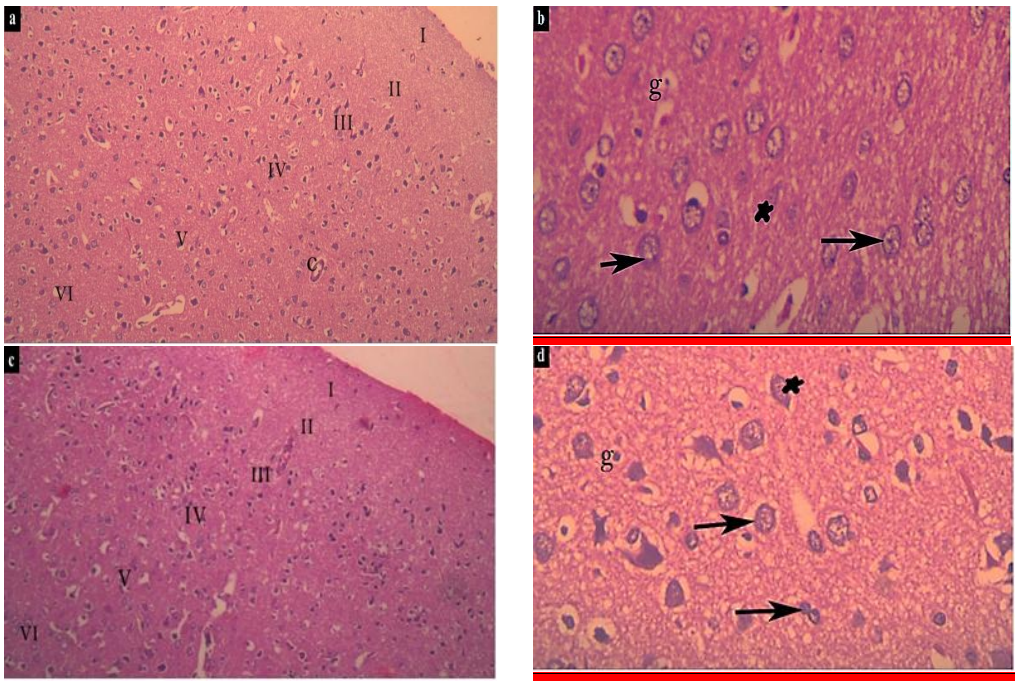


Fig (2): **(a)** & **(c)** photomicrographs of coronal section in cerebral cortex of the control group & Resveratrol treated group respectively showing, normal neuronal cells in layers I, II, III, IV, V, VI and normal blood vessel (C). (H& E X 100). **(b)** & **(d)** photomicrographs of coronal section in cerebral cortex of the control group & Resveratrol treated group respectively showing layer III contained pyramidal cell with basophilic cytoplasm, large vesicular nucleus and long apical dendrite (star), granular cell (arrow) and glial cell with small dense nuclei (g). (H& E X 400)

Immunohistochemical study:

Resveratrol dropped Caspase3 in tramadol intoxicated group

Caspase3positive staining becomes visible as brown cytoplasm indicating the degree of nuclear apoptosis. Control and resveratrol groups seemed normal with negative reaction of rats' brain to caspase3**, (Fig.6& 7).** The tramadol group represented a lot of neurons with strong positive Caspase3 reaction, **(Fig.8).** The tramadol+resveratrol group demonstrated a few neurons with mild positive Caspase3 expression **(Fig.9).**

Histomorphometry: Tramadol administration resulted in a statistically significant increase in the mean area percentage of caspase3 immunoexpression in comparison with control and resveratrol groups. Whereas coadministration of resveratrol with tramadol exerted a decline of caspase**3** mean area percentage with statistically significant variance versus tramadol intoxicated group. **(Table 5) (Fig.10)**

Caspase3 immunostaining

Group (I) Control: it demonstrated Negative Caspase3 immunostaining

Group (II) Resveratrol: it demonstrated Negative Caspase3 immunostaining

Group (III) Tramadol: it demonstrated Strong positive Caspase3 immunostaining

Group (IV) Tramadol and resveratrol: it demonstrated Slightly positive Caspase3 immunostaining

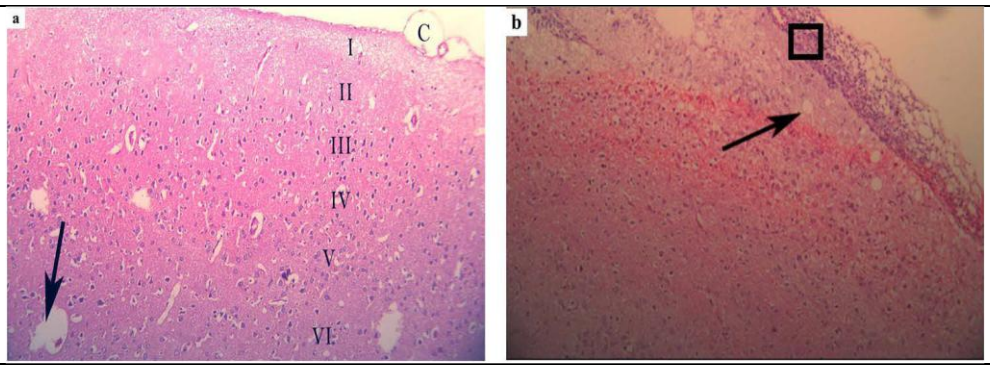


Fig (3): (a) A photomicrograph of a coronal section in cerebral cortex of tramadol treated rats with showing neuronal cells in layers I, II, III, IV, V, VI with neural disorganization and dilated congested blood vessel (C). Separation between the layers was clearly noticeable (arrow). (H& E X 100) **(b)** A photomicrograph of a coronal section in cerebral cortex of tramadol treated rats with showing inflammatory cells (square) and degenerative vacuolization (arrow) with in the neural cells. (H&E×100).

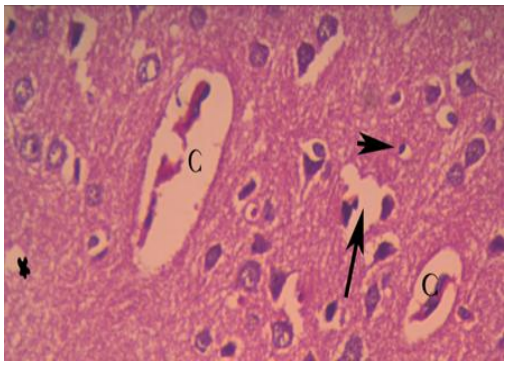


Fig (4): A photomicrograph of a coronal section of cerebral cortex of tramadol treated group showing, apoptotic cells characterized by neuronal shrinkage and chromatin condensation (arrowhead), degenerative vacuolization (star), wide intercellular space (arrow) and dilated congested blood vessels (C). (H& E X 400).

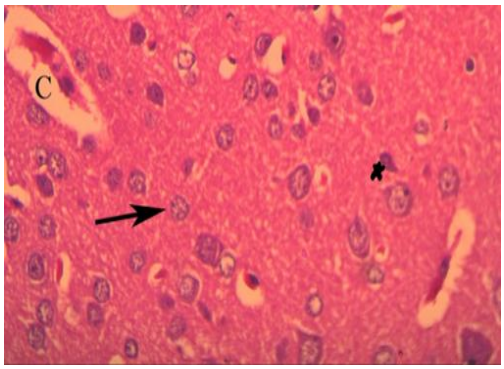


Fig (5): A photomicrograph of a coronal section of cerebral cortex of tramadol and resveratrol treated group showing, normal cells with vesicular nuclei (arrow), normal pyramidal cells (star), and dilated congested blood vessels(C). (H&E X 400)

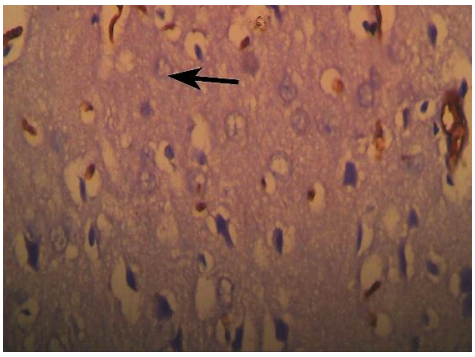


Fig (6): A photomicrograph of a coronal section in cerebral cortex of control group, showing the normal negative reaction of brain tissue to the Caspase–3 antibody (arrow) (Caspase immunostaining × 400)

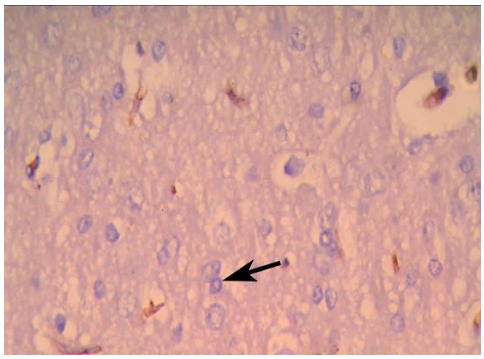


Fig (7): A photomicrograph of a coronal section in cerebral cortex of resveratrol group, showing the normal negative reaction of brain tissue to the Caspase–3 antibody (arrow) (Caspase immunostaining × 400)

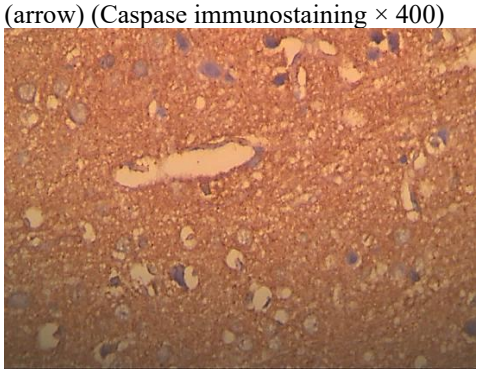


Fig (8): A photomicrograph of a coronal section of cerebral cortex of tramadol treated group showing many neurons with strong positive reaction of brain tissue to the Caspase–3 antibody (Caspase immunostaining × 400)

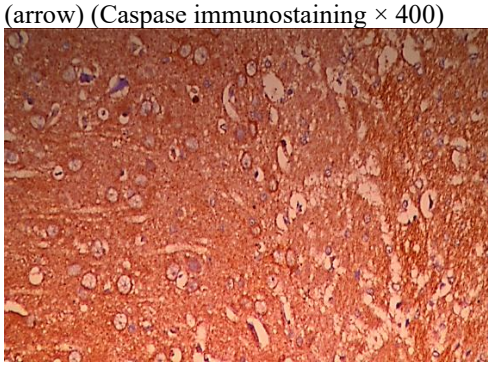


Fig (9): A photomicrograph of a coronal section of cerebral cortex of tramadol and resveratrol treated group showing, many neurons with mild positive reaction of brain tissue to the Caspase–3 antibody (Caspase immunostaining × 400)

DISCUSSION

Tramadol used as an analgesic whereas may be abused leading to various psychological and physical problems such as CNS, and GIT troubles. The CNS is more prone to tramadol toxicity-provoked injury ***(Mraisel et al., 2021).***

A natural resveratrol is a phenolic substance presents in red grapes and red wine. Previous investigations had demonstrated that Resveratrol owns antioxidative, anti-inflammatory and antitumor effects. Although there is a link between resveratrol and cognitive function, few researches used it as neurodefensive agent **(Zhang et al., 2019**).

The mechanism of Resveratrol neuroprotective properties was that resveratrol recovers the oxidative injury and dysfunction of mitochondria. Resveratrol also clears amyloid β (Aβ). Additionally, Resveratrol downregulates inflammatory markers (IL-β and IL-6), therefore relieving neuroinflammation (**Abozaid, et al., 2022)**

This study demonstrated that rats exposed to tramadol had a statistically highly significant decline in rats' weight and RBW percentage in comparison with Control group, Resveratrol administration with Tramadol documented non-significant elevation of body weight compared to intake of tramadol alone.

Our findings align with ***Mraisel et al. (2021)*** who showed that tramadol lowered relative brain weight significantly as compared with control. ***Elsukary et al. (2022)*** reported that misuse of tramadol diminished the body weight.

Meghawry et al., (2024) assured that chronic use of tramadol decreases the physical parameters (body weight & RBW%)

GIT side effects like nausea, vomiting and constipation resulted from tramadol abuse, leading to decline of appetite, and therefore weight loss ***(FitzHenry et al., 2020)***.

Parallel to our results, ***(Balogun et al.,*2020)** recorded that opiate abuse causes fatigue which alters food consumption, and metabolic rate triggered lowering body weight,

Reda et al., (2022) reported that resveratrol could not reduce the body weight gain in the metabolic syndrome but, resveratrol (400 mg/kg body weight/day) for long time, could decrease the body weight. so that prolonged resveratrol intake may lead to weight loss.

In present study, regarding cognitive skills, administration of tramadol to rats resulted in prolonged time to locate the platform during initial acquisition latency (IAL) of MWM test, while concomitant administration of resveratrol with tramadol significantly decreases IAL to arrive the hidden platform. Following training, tramadol-treated animals showed elevation in both 1stRL(day21) and 2ndRL(day28) latencies suggesting significant cognitive impairment effect of tramadol. However, administration of resveratrol with tramadol showed a significant decrease in retention latencies.

Mowaad et al. (2023) observed that tramadol intake was associated with neurological impairment using Open Field Test. In addition, ***Elsukary et al. (2022*)** documented that tramadol declines Open Field test parameters.

Ekpo et al. (2024) reported memory impairment after administration of 50 mg/kg tramadol to rats because of their inability to locate the escape platform early in Morris Water Maze test.

Ability of tramadol to impair memory may be through its inhibitory effect on serotonin, and norepinephrine reuptake, also, tramadol stimulates the mu-opioid receptors causing cognitive impairment, including memory loss **(Ekpo *et al*. 2024)**

In contrast with our findings, ***Ibrahim and Salah-Eldin (2019)*** found that tramadol intoxicated animals have hyperactivity comparing with control animals having a normal behavior.

Wang et al., (2021) reported that administration of resveratrol can recover memory, learning skills and cognitive deficits.

Tramadol provoked oxidative damage was well recognized as diminished memory, it compromises the antioxidant cascade, which tangles up redox reactions in the cells. It alters its biochemistry (↑TBARS and ↓SOD content), which provokes cerebral oxidative damage **(Hindawy et al., 2024).**

Our experimental study demonstrated that tramadol produced a significant lowering in the brain CAT, and GPx which elevated significantly after co-administration of resveratrol with tramadol. Along with a significant raise of brain MDA after tramadol intake, which diminished in tramadol + resveratrol group.

Our work agreed with **Meghawry et al., (2024)** who observed that tramadol mediated neurotoxicity in male and female Wister rats

increases the MDA level and decreases SOD and GSH levels in brain tissue.

Mraisel et al. (202I) documented that rats taking tramadol for 8 weeks showed significantly increase of MDA level in cerebral tissue. Also, ***Xia et al. (2020)*** revealed that oxidative stress is a main mechanism in tramadol neuronal injury due to significant elevation of malondialdehyde.

Olaniyi et al., (2025) evaluated that oxidative/antioxidant biomarkers revealed a rise in MDA, and a reduction of GSH & SOD in tramadol administrated Wistar rats.

Previous studies reported that administration of resveratrol elevates glutathione (GSH) levels and decline superoxide radical production in models exposed to arsenic and manganese which support antioxidant activity of resveratrol **(Cong et aI., 202I) (Taheri et aI., 202I) (Yingrak et aI., 224**),

Yu-Te Lin et al., (2018) demonstrated that administration of resveratrol improves cognitive function by decreasing oxidative stress and preserving SOD function

Hui Ye et al., (2025) reported that supplementation of resveratrol for short time at 1000–2000 mg/kg upregulates GP_X, SOD, CAT and decrease level of MDA in dexamethasone -induced oxidative stress of yellow-feathered broilers.

Former studies found that decrease of antioxidant markers (SOD, GP_X, CAT), elevation of oxidative stress indicators and a rise of inflammatory biomarkers were accompanied with neurdegeneration. Additionally, enhancement of apoptotic pathways and caspases promote cellular disabilities **(Hindawy et aI., 224).**

Our study noticed significant rise in caspase3, and TNF-α, as well as significant decrease in IL10 and FST of tramadol group. The administration of Resveratrol with tramadol improved significantly changes of these parameters.

Our results agreed with **Meghawry et al., (2024)** who evaluated increase casepase3 expression in chronic tramadol administration in adult albino rats for 6 months which ameliorated by melatonin administration.

Also, ***Awadalla and Salah-Eldin (2015) and 1brahim & Salah-E1din (20I9) mentioned*** marked increase in caspase3 after tramadol administration in comparison with control.

Li et al., (2026) showed that reduction of infarct size, lowering in serum ROS and increasing IL-10 levels in group receiving resveratrol after transient middIe cerebraI artery occIusion/reperfusion (MCA0/R).

Runge et al., (2022) observed that brain IL-10 has a neuroprotective effect. **Bachis et al., (2001)** mentioned that IL-10 obstructs cell demise by inhabitation of caspase3.

Wang et al., (2021) reported that resveratrol treatment attenuate effect of lead exposure on caspase3 as apoptotic marker.

Liu et al., (2023) demonstrated that resveratrol reduced neuronal apoptosis which manifested as down-regulation of TNF-α and caspase 3 in paclitaxel (PTX)-induced cognitive disabilities.

Abozaid et al., (2022) reported that formulated resveratrol-Selenium nanoparticles decrease interleukin-1β (IL-1β) level, which indicated attenuation of neuroinflammation in Alzheimer's disease.

Szkudelska et al., (2020) revealed that resveratrol treatment can alleviate inflammatory and oxidative stress by decreasing MDA, IL-1β and TNF-α levels in skeletal muscle and liver of type 2 diabetic rats.

Follistatin (FST) is an extracellular glycoprotein. FST modifies early inflammatory steps, so stimulates tissue repair, which indicated its usage as a novel therapy **(Nissine et al., 202I) (Yaden et al., 2014).**

Administration of resveratrol reversed the increase of brain serotonin & NOR after exposure to tramadol, in our experiment.

Our results were in line with **Meghawry et aI., (2024)** who mentioned that tramadol increase levels of brain tissue serotonin and noradrenaline.

Arakawa et al. (2019) mentioned that tramadol can increase 5-HT and NOR levels. ***Hussein and Abdel Aal (2017)*** documented that analgesic effect of tramadol is due to at least two mechanisms: activation of opioid receptor, and inhibition of serotonin and noradrenaline neuronal reuptake.

Ahmed et al., (2014) found that oxidative stress may aggravate brain dopamine levels, they observed that resveratrol as antioxidant (80 mg/kg) markedly declines 5-HT, dopamine and NOR levels in the brain.

In our study, histopathological sections of cerebral cortex of group treated by tramadol alone demonstrated disruption and separation of cerebral cortex layers, apoptotic cells, vacuolation and vascular congestion. After administration of resveratrol+tramadol, normal cerebral cortex was noticed.

Our results were like that of **Olaniyi et aI., (2025)** who found neuronal damage vacuolation, and pyknosis in rats' cerebral cortex after tramadol administration.

Ekpo *et al*. (2024) and Meghawry et al., (2024) observed neuronal changes (loss organization of layers of cerebral cortex, vascular congestion, and apoptotic cells) in group treated by tramadol alone.

Elsukary et al. (2022) reported that shrinkage of pyramidal cells with darkly stained nuclei, reduction in size of granular cells, also vascular congestion and apoptotic cells found in the tramadol treated group.

Li et al., (2026) noted that resveratrol group restored normal brain appearance compared with the MCAO/R group, in the form of reduction in number of damaged neurons, which indicates the neuroprotective effects of resveratrol.

The results of the current study as regards caspase3 immuno-histochemical reaction demonstrated that brain of tramadol treated group showed a lot of cells with strong +ve Caspase3 reactions, while tramadol + resveratrol treated rats showed little neurons with mild +ve Caspase3.

Meghawry et al., (2024) revealed that brain immunohistological examination after chronic tramadol administration increases casepase3 expression, which could be diminished by melatonin.

Awad-Alla & SaIah-EIdin (2015) mentioned that rats receiving opioids illustrating apoptotic cells and damaged neurons with severe rise of caspase3 (proapoptotic marker) with moderate decline in Bcl-2 (antiapoptotic marker). Opioids exert neuropathological and neurochemical changes, which cause marked cerebral demise which impairs memory and attention.

Abd-Elhafiz and Issa (2021) documented that resveratrol significantly diminishes hepatic caspase3 immunopositively versus cisplatin treated rats.

In accordance with our results, resveratrol possesses neurodefensive impact by its antioxidant, anti-inflammatory, anti-apoptotic effects which ameliorate tramadol induced behavioral, biochemical, and histopathological disturbances. **Nasralla et al., (2021)** noted that resveratrol decreased

Bcl-2-associated X (proapoptotic marker) immunohistochemical reaction in acrylamide mediated nephrotoxicity.

SUMMARY AND CONCLUSION

Our outcomes reported that resveratrol alleviates tramadol -induced neurotoxicity. Anti-oxidant, anti-apoptotic, and antinflammatory characteristics of resveratrol made it neuroprotector.

RECOMMENDATIONS

Further studies are needed to investigate neurotoxicity of tramadol and neurodefensive mechanisms of resveratrol after tramadol administration in animals and humans.

CONFLCT OF INTEREST

The authors decIare that there is no conflict of interest.

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الملخص العربى

التأثير الوقائى للريسفيراترول على التسمم العصبي للترامادول على الفئران البيضاء البالغة

هدف البحث: يهدف هذا البحث التجريبي إلى تقييم التأثيرات السامة للترامادول على أدمغة الفئران البيضاء البالغة من خلال المعايير البيوكيميائية والنسيجية المرضية ، وإلى دراسة التأثيرات التحسينية المحتملة للريسفيراترول.

خطوات اجراء الدراسة: أُجريت هذه الدراسة على 50 فأراً أبيض بالغاً يتراوح وزنها بين 180 و200 غرام. قُسّمت الفئران عشوائياً إلى اربع مجموعات،:
مجموعات ضابطة مجموعة سلبية، ومجموعة ضابطة بالمذيب
ومجموعة مُعالَجة بالريسفيراترول (20 ملغم/كغم/يوم)]. حُضّر الريسفيراترول في محلول ملحي عادي، وأُعطي للفئران بالفم , مجموعة مُعالَجة بالترامادول (خمسون ملغم/كغم)عن طريق الفم، ومجموعة مُعالَجة بالترامادول والريسفيراترول. عُولجت الفئران مرة واحدة يومياً لمدة 28 يوماً
سيتم عمل الاتى تم عمل قياس وزن الفئران و كذلك مخ الفئران فى اليوم الثامن وعشرون

-التحاليل البيوكيميائية: 1. تحليل أنسجة لمؤشرات الإجهاد التأكسدي(MDA, GPx, CAT)

2. مستويات البروتين في الدماغ (المضاد للالتهابات (IL-10)، المحفز للالتهابات (TNF)، وعلامة تكوين الأوعية الدموية؛ الفوليستاتين (FST).)و كاسبيس3

3. تقييم النواقل العصبية في أنسجة الدماغ:. استُخدمت مجموعات ELISA الخاصة بالفئران لقياس مستويات السيروتونين والنورأدرينالين.

-الدراسة النسيجية: ثُبّتت عينات من أنسجة القشرة الدماغية لمدة 24 ساعة في محلول فورمالين متعادل مخفف بنسبة 10%.

- لتحليل الإحصائي:تم تحليل البيانات باستخدام (ANOVA)، ثم مقارنة بين المجموعات، باستخدام برنامج التحليل الإحصائي IBM SPSS Statistics

النتائج: قد نتج عن هذه الدراسة زيادة MDA او نقص GPx, CAT ذو دلالة احصائية,انترليوكن , معامل تكسير الاورام وكاسبيس 3 فى مجموعة الترامادول مقارنة بالمجموعة الضابطة. ونقص ذو دلالة احصائية فى مستوى انترليوكن10 , زيادة معامل تكسير الاورام وكاسبيس 3 وزيادة ذو دلالة احصائية فى مسنوى السيروتونين والنورأدرينالين فى مجموعة الترامادول مقارنة بمجموعة الترامادول و الريسفيرترول.وتتوافق هذه التغيرات البيوكميائية مع التغيرات الباثولوجية فى انسجة المخ للمجموعات

الخلاصة: اثبتت التأثيرات السامة للترامادول على أدمغة الفئران البيضاء البالغة من خلال المعايير البيوكيميائية والنسيجية المرضية ، يوجد اتأثيرات تحسينية للريسفيراترول.