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Evaluation of the Efficacy of *Carpesium Cernuum* leaves nanoparticles on Apoptotic Pathway in MNU-induced Brain injury in Male Albino Rats

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Abstract

Background: N-methyl-N-nitrosourea (MNU) is an alkylating reagent that causes oxidative stress, inflammation and disruption of apoptotic signaling leading to cell injury. *Carpesium cernuum* possesses bioactive phytochemical constituents that may possess antioxidant and anti-inflammatory activities. The present study aims, to investigate the protective effects of nano-formulated *Carpesium cernuum* leaf extract on 30 mg/Kg b.w. of MNU-induced brain injury in male albino rats affecting apoptotic, inflammatory and oxidative stress parameters and histological changes. **Methodology:** The objective of this experiment was to test the effectiveness of Nano-*Carpesium cernuum* leaf extract on regulating the apoptotic process in the brain tissues of male albino rats induced with N-methyl-N-nitrosourea (MNU). Forty male albino rats were arbitrarily assigned into four groups: n = 10/group) , one group being the control group, another MNU-induced group, and the last Nano-*Carpesium cernuum* leaf extract with MNU group. “At the end of the experimental period, blood samples were collected to determine the levels of BCL-2, Caspase-3, Caspase-8, CEA, TNF-α, IL-6, IL-1β, COX, COX-2, and the antioxidant enzyme SOD. Brain tissues were also examined grossly and histopathologically. **Results:** The results demonstrated that MNU exposure significantly decreased BCL-2 and SOD levels, while significantly increasing Caspase-8, CEA, TNF-α, IL-6, IL-1β, and COX-2,

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indicating activation of apoptosis, oxidative stress, and inflammatory responses". Histopathological examination revealed inflammatory cell infiltration, spongiform changes, and liquefactive necrosis in brain tissue. In contrast, treatment with Nano-*Carpesium cernuum* Leaf Extract + MNU significantly improved apoptotic, inflammatory, and oxidative stress biomarkers and markedly reduced histopathological brain damage. **Conclusions:** These findings suggest that the nano formulation of *Carpesium cernuum* leaf extract support the potential neuroprotective activity, antioxidant, anti-inflammatory, and anti-apoptotic properties against MNU-induced brain injury in male albino rats.

Keywords: *Carpesium cernuum*, nanoparticles, N-Methyl-N-nitrosourea (MNU), apoptosis, oxidative stress, inflammation, neuroprotection, male albino rats.

Introduction

Carpesium cernuum L. is a medicinal plant belonging to the family Asteraceae and the genus *Carpesium*, which comprises several species distributed throughout Europe and East Asia, particularly in China and Japan. Different parts of this plant have long been used in traditional medicine because they contain a variety of bioactive compounds, including sesquiterpene lactones, phenolic compounds, and flavonoids. These phytochemicals possess potent antioxidant and anti-inflammatory properties and have been reported to protect cells against oxidative damage and toxic insults "making *Carpesium cernuum* a promising source for the development of novel therapeutic agents" [1] [2].

N-Methyl-N-nitrosourea (MNU) is a highly potent chemical toxicant and alkylating agent capable of inducing severe DNA damage. Exposure to MNU promotes oxidative stress and inflammatory responses within brain tissue, resulting in neuronal injury and impaired brain function. Owing to its neurotoxic effects, MNU is widely used in experimental models to investigate the molecular mechanisms underlying neuronal damage and neurodegenerationn [3], Apoptosis is a highly regulated physiological process that maintains tissue homeostasis by eliminating damaged or abnormal cells without provoking excessive inflammation. Nevertheless, exposure to toxic substances like MNU disturbs this equilibrium by activation of intrinsic and extrinsic apoptotic mechanisms. This phenomenon is linked to changes in the apoptotic biomarkers that include BCL-2, Caspase-3, and Caspase-8 along with the increase in the levels of

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inflammatory cytokines and oxidative stress, thereby resulting in the damage of neurons and brain tissue [4].

With recent advances in the field of nanotechnology, the scope for using medicinal plants in drug development has been significantly improved. For this reason, the current study was undertaken to assess the effectiveness of the nano formulation of leaf extract of *Carpesium cernuum* in modulating the apoptotic pathway in the brain tissues of male albino rats exposed to MNU. The study was performed to assess its efficacy on apoptosis biomarkers, inflammatory mediators, oxidative stress markers, and histopathological changes in brain tissues to establish the neuroprotective role of *C. cernuum* leaf extract against MNU induced brain injuries.

Materials and Methods

Forty albino rats of *Rattus norvegicus* and weighing 200-240 g, were randomly divided into four groups, (n = 10 per group): Control Group, MNU Group (50 mg/kg), Nano-*Carpesium cernuum* leaf extract Group (11 mg/kg), and Nano-*Carpesium cernuum* leaf extract + MNU Group. At the end of experiment, blood samples and brain tissues samples were collected for further analysis. Serum levels of BCL-2, Caspase-3, Caspase-8, CEA, TNF-α, IL-6, IL-1β, COX-1, COX-2, and SOD were quantitatively determined using commercial ELISA kits according to the manufacturers' instructions. "Brain tissues were fixed in 10% neutral-buffered formalin, routinely processed, sectioned, and stained with hematoxylin and eosin (H&E) for histopathological examination. Analysis of data was performed using SPSS and LSD test was used for comparisons among groups at $P \leq 0.05$.

Results and Discussion

Table (1) demonstrates significant differences among the experimental groups in the investigated molecular biomarkers, including BCL-2, Caspase-3, Caspase-8, and CEA, indicating that the different treatments exerted marked effects on the regulation of cell survival and apoptotic pathways [5], as well as on the level of the tumor marker. Statistical analysis was performed using the Least Significant Difference (LSD) test at a significance level of $P \leq 0.05$ to compare the group means.

Means sharing identical or similar superscript letters were considered not significantly different, whereas means with different letters indicated statistically significant differences.

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Table (1). Effect of Different Treatments on the Molecular Biomarkers BCL-2, Caspase-3, Caspase-8, and CEA in the Studied Experimental Groups

Group	Group | Mean ± Standard Error (SE)			
	CEA (ng/mL)	Caspase- 8 (ng/mL)	Caspase-3 (ng/mL)	BCL-2 (pg/mL)
Control	0.24 ± 1.241C	0.66 ± 44.200C	1.12 ± 55.911A	0.64 ± 84.391B
MNU	0.90 ± 16.531A	1.75 ± 84.700A	1.22 ± 46.69B	0.70 ± 53.026D
Nano-Carpesium cernuum Leaf Extract	0.64 ± 5.140B	1.20 ± 63.800B	0.63 ± 25.574D	0.79 ± 77.723B
Extract + MNU	0.79±77.723 B	1.20±63.3800 B	0.63±25.574 D	0.79±77.723 B
L.S.D 0.05	**1.7031**	**3.5043**	**3.1244**	**2.3604**

*** Means which have similar superscript letter(s) do not differ significantly (P ≤ 0.05).**

The result present in Table (1) showed significant differences among the experimental groups in the investigated molecular biomarkers. The MNU-treated group exhibited a significant decrease in BCL-2 and significant increases in Caspase-8 and CEA compared with the control group, indicating activation of the apoptotic pathway. This aligns with [6]. In contrast, treatment with the nano-*Carpesium cernuum* leaf extract, either alone or in combination with MNU, improved these biomarkers toward normal levels, demonstrating its neuroprotective and anti-apoptotic effects. Caspase-3 levels also differed significantly among the experimental groups Expand after verification of Caspase-3 raw data [7],

Effect of Different Treatments on Immune and Inflammatory Biomarkers

Table (2) shows significant differences among the experimental groups in the investigated immune and inflammatory biomarkers, "including TNF-α, COX-1, IL-6, COX-2, and IL-1β, indicating that the different treatments exerted marked effects on regulating the immune and inflammatory responses associated with the apoptotic pathway in brain tissue. Statistical comparisons were performed using the Least Significant Difference (LSD) test at a significance level of P ≤ 0.05". Means sharing the same or similar superscript letters were considered not significantly different [8], these findings demonstrate that treatment with the nanoparticles of *Carpesium cernuum*

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leaf extract significantly modulated the levels of inflammatory cytokines and mediators compared with the MNU-treated group, supporting its protective role in attenuating the inflammatory response associated with activation of apoptotic pathways in the brains of male albino rats. These results are consistent with the primary objective of the present study, which was to evaluate the efficacy of these nanoparticles in reducing MNU-induced neurotoxicity.

Table (2). Effect of Different Treatments on the Immune and Inflammatory Biomarkers TNF-α, COX-1, IL-6, COX-2, and IL-1β in the Studied Experimental Groups.

Mean ± Standard Error (SE)					Group
IL1B (pg/mL)	COX-2 (pg/mL)	IL6 (pg/mL)	COX-1 (pg/mL)	TNF (pg/mL)	
0.32 ± 12.66B	0.51 ± 11.300C	0.37 ± 11.900C	1.77 ± 21.500A	0.19 ± 8.056C	Control
7.47 ± 43.580A	1.18 ± 68.200A	1.22 ± 80.800A	0.84 ± 6.730B	4.25 ± 54.250A	MNU
0.37± 21.421B	0.92± 23400B	0.93 ± 23.400 B	0.43 ± 3.342 C	0.62 ± 27.320B	Nano-Carpesium cernuum Leaf Extract
0.37 ±21.421B	0.93 ±23.400B	0.92 ±32.100B	0.43 ±3.342C	0.62 ±27.320C	Extract + MNU
10.754	**2.4571**	**3.3794**	**2.9109**	**6.2071**	**L.S.D 0.05**

*** Groups that have identical or similar superscripts are not statistically different from one another (P ≤ 0.05).**

In Table (2), there are differences among the experimental groups in the inflammatory and immune biomarkers studied (TNF-α, COX-1, IL-6, COX-2, and IL-1β). The group that was treated with MNU has shown the highest level of TNF-α, IL-6, COX-2, and IL-1β than the control group, and this implies that the MNU treatment had an important effect on inflammatory conditions. On the other hand, the levels of TNF-α, IL-6, COX-2, and IL-1β were significantly lower in the Nano-*Carpesium cernuum* Leaf Extract + MNU group than in the MNU group, and they were closer to the control group. These findings suggest that nano-*Carpesium cernuum* leaf extract attenuated the MNU-associated inflammatory response.

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Effect of Different Treatments on the Antioxidant Enzyme SOD

Table (3) shows significant differences between the experimental groups ($P \leq 0.05$) with regard to the level of the antioxidant enzyme superoxide dismutase (SOD), which suggests that there were significant impacts of the different treatment methods on the performance of the antioxidant defense mechanism. Differences between the means of the various groups were statistically analyzed through the LSD test with a significance level of $P \leq 0.05$. Different superscripts indicate significantly different means, while the same superscript indicates non-significantly different means.

Table (3): Effect of Different Treatments on the Level of the Antioxidant Enzyme Superoxide Dismutase (SOD) in the Studied Experimental Groups**.**

Mean ± Standard Error (SE) SOD	Group
0.94 ± 28.830C	**Control**
0.79 ± 23.780D	**MNU**
1.59 ±33.356 B	**Nano-*Carpesium cernuum* Leaf Extract**
1.59 ± 33.356B	**Extract + MNU**
4.3619	**L.S.D 0.05**

*** Values that have the same or similar superscripts are not significantly different ($P \leq 0.05$).**

Table (3) demonstrates significant differences among the experimental groups in the antioxidant enzyme SOD, this study agreement with [9]. The MNU-treated group exhibited the lowest SOD level, indicating that exposure to MNU induced oxidative stress and impaired the antioxidant defense system. In contrast, the Nano-*Carpesium cernuum* Leaf Extract + MNU group showed a significantly higher SOD level than the MNU-treated group, indicating that the nano formulation effectively enhanced antioxidant activity and reduced oxidative stress. These findings suggest that the nanoformulation of *Carpesium cernuum* leaf extract exerts a protective antioxidant effect, contributing to the preservation of brain tissue and attenuation of MNU-induced neurotoxicity

The results demonstrated that MNU induced significant activation of the apoptotic pathway, as evidenced by decreased BCL-2 levels and increased Caspase-8 and CEA levels, indicating enhanced neuronal cell damage and apoptosis. These findings are

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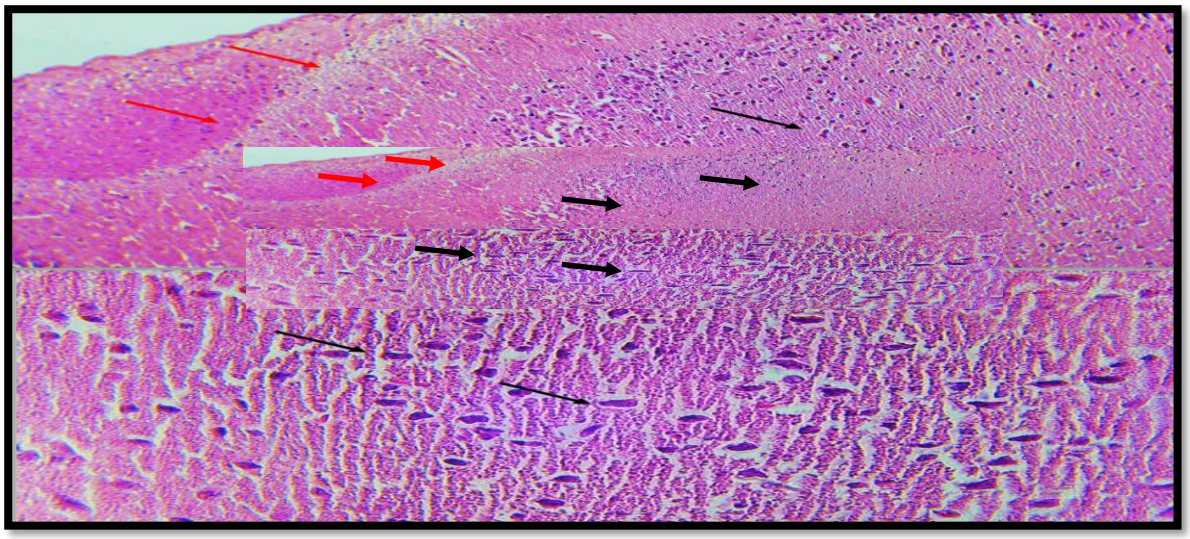
consistent with previous studies reporting that MNU causes DNA damage, oxidative stress, and activation of apoptosis-related signaling pathways [10]. In contrast, treatment with Nano-*Carpesium cernuum* Leaf Extract + MNU significantly improved these biomarkers by increasing BCL-2 expression and reducing Caspase-8 and CEA levels, suggesting a protective effect against MNU-induced neurotoxicity، [11]. This neuroprotective activity may be attributed to the antioxidant and anti-inflammatory bioactive compounds present in *Carpesium cernuum*, which regulate apoptotic signaling pathways and promote neuronal cell survival [9].

Overall, these findings indicate that the nano formulation of *Carpesium cernuum* leaf extract effectively attenuates neuronal apoptosis and protects brain tissue against MNU-induced damage in male albino rats.

The histopathological change of brain tissues

Histopathological examination of the brain revealed a normal cerebral cortex with well-organized neuronal layers. The neurons exhibited round to oval vesicular nuclei, prominent nucleoli, and abundant eosinophilic cytoplasm. No histopathological alterations were observed in the cerebral cortex (black arrow), hippocampus (red arrow), midbrain, or cerebellum, all of which displayed normal histological architecture. [12]. Picture. 1.



Picture (1): Histopathological Description (Magnification ×400):

The following figure clearly shows perivascular cuffing which is the result of an inflammation process in the brain. Perivascular cuffing includes the aggregation of

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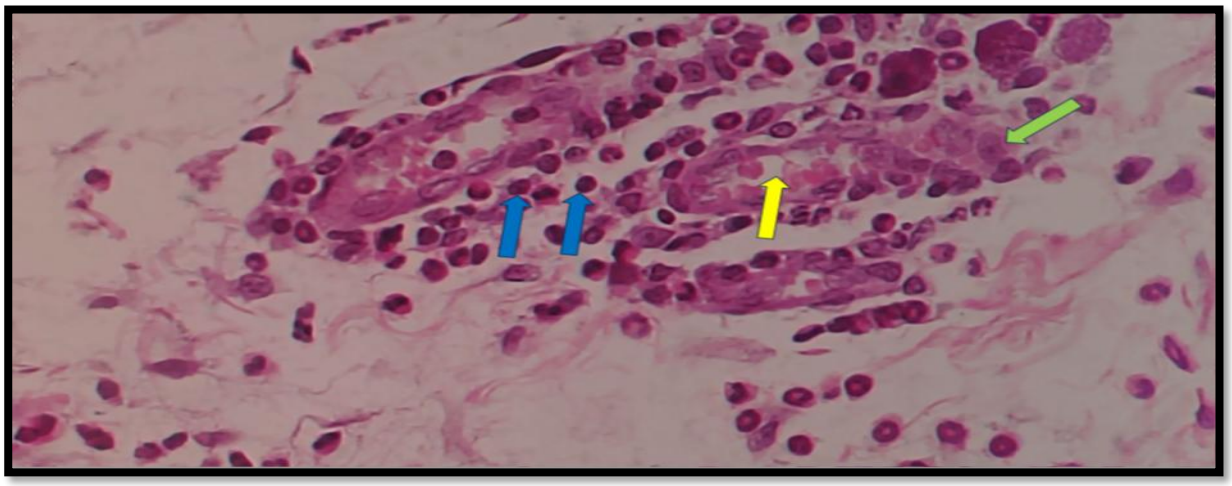
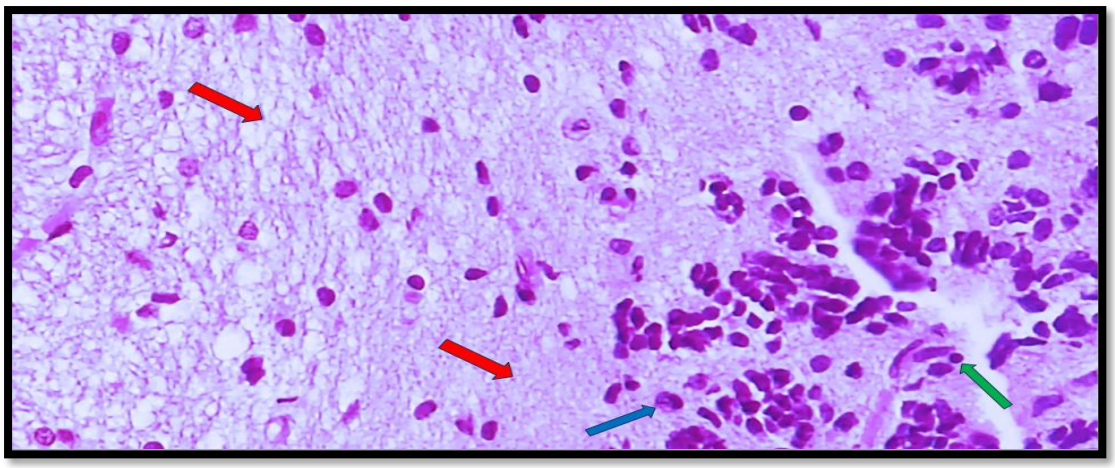
lymphocytes (blue arrows) and monocytes (yellow arrow) around a blood vessel (yellow arrow). Picture 2.



Picture (2): Histopathological Description (Magnification ×400):

In this section, there is evidence of very pronounced liquefactive necrosis with severe spongiosis and destruction of the brain parenchyma. An intense cellular inflammation, predominantly neutrophilic in nature with lymphocytes present in smaller numbers, can be seen here. Name of the picture: cerebl4_10x_1bundle nerves muscle connect tissue (Magnification ×100):

This section demonstrates intact bundles of nerve fibres located in connective tissue along with muscle fibres. Picture 3.



Picture (3): Cerebl3_10x_1zfocal necrosis inflame

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Histopathological Description (Magnification ×100):

The lesion characterized by liquefaction necrosis with abundant vacuolization was seen in the cerebellum, along with the focal presence of inflammatory cells such as microglia, lymphocytes, and macrophages, suggesting acute inflammatory and toxic tissue injury. Picture 4

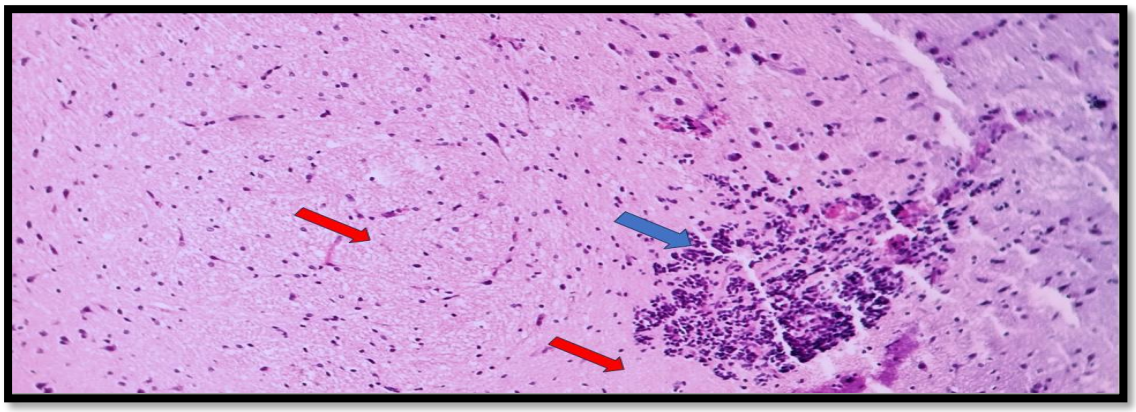

Figure 4. Liquefactive Necrosis and Inflammatory Cell Infiltration in the Cerebellum (H&E, ×100).

Conclusions

In this study, it was observed that MNU induced apoptosis via the reduction of BCL-2, upregulation of Caspase-8 and CEA, along with increased levels of inflammatory biomarkers and oxidative stress. Nano formulation of leaf extract of *Carpesium cernuum* was found to be effective in the improvement of biomarkers via BCL-2 and SOD upregulation and Caspase-8, CEA, TNF-α, IL-6, COX-2, and IL-1β downregulation. Collectively, these findings suggest a potential protective effect of nano-formulated *C. cernuum* leaf extract against MNU-induced brain injury through modulation of apoptosis-associated, inflammatory, and antioxidant responses.

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