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POTENTIAL EFFECT OF *SKIMMIA LAREOULA* LEAVES EXTRACTED DIFFERENT FRACTIONS AND NANOPARTICLES RESCUES YOUNG RATS AGAINST ETHANOL INDUCED NEUROTOXICITY

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Abstract
The study explored the efficacy of *Skimmia lareoula* leaf extracts in ameliorating ethanol-induced neurotoxicity in postnatal day 7 rat pups. Utilizing various fractions and nanoparticles derived from the leaves, including oil, crude extract, chloroform, methyl acetate, silver nitrate, and copper sulphate, researchers administered ethanol intraperitoneally at a dose of 5 g/kg. Following a four-hour period, the animals were euthanized, and their brain tissues were subjected to western blotting analysis. Significantly, all four fractions and both nanoparticles exhibited pronounced reductions in neurotoxicity, as indicated by lowered levels of neuroinflammatory markers like NF-kB, along with decreased expression of neurodegenerative markers such as Caspase-3 and PARP-1. These results underscore the promising therapeutic potential of *Skimmia lareoula* leaf fractions and nanoparticles in mitigating ethanol-induced neurotoxicity. The findings suggest a multifaceted neuroprotective role for *Skimmia lareoula*, offering insights into its potential application as a therapeutic agent against ethanol-induced neuronal damage. By targeting pivotal pathways implicated in neuroinflammation and neurodegeneration, these extracts and nanoparticles provide a promising avenue for the development of innovative interventions aimed at mitigating ethanol-induced neurotoxicity. Further exploration of the underlying mechanisms governing the neuroprotective effects of *Skimmia lareoula* constituents could potentially lead to the development of effective therapeutic strategies for ethanol-induced neurotoxicity and associated disorders.

Key Words: *Skimmia lareoula*, Caspase-3, PARP-1, NF-kB, neurotoxicity

1. INTRODUCTION

Ethanol-induced neurotoxicity is a significant concern, particularly in young populations, as it can lead to long-lasting cognitive deficits and behavioral abnormalities [1]. Despite the widespread consumption of ethanol, effective therapeutic interventions to mitigate its neurotoxic effects are limited [2]. However, recent research has shown promise in the utilization of natural compounds and nanoparticles derived from plant sources for neuroprotection [3]. *Skimmia lareoula*, a plant species rich in bioactive compounds, has garnered attention for its potential therapeutic properties [4]. Extracts from *Skimmia lareoula* leaves have demonstrated various pharmacological activities, including antioxidant, anti-inflammatory, and neuroprotective effects. Recent studies have investigated the potential of *Skimmia lareoula* leaf extracts and their fractions, as well as nanoparticles derived from these extracts, in mitigating ethanol-induced neurotoxicity in young rats [5]. Ethanol exposure during early developmental stages can disrupt normal brain development and lead to neuronal damage, oxidative stress, and inflammation [6]. Consequently, this can result in cognitive impairments, learning deficits, and behavioral abnormalities that persist into adulthood. Therefore, identifying interventions capable of mitigating ethanol-induced neurotoxicity is crucial for minimizing its detrimental effects on brain health [7]. Natural compounds derived from plants have emerged as promising candidates for neuroprotection due to their antioxidant and anti-inflammatory properties. *Skimmia lareoula* leaf extracts contain a plethora of bioactive compounds, including flavonoids, alkaloids, and terpenoids, which have been implicated in various neuroprotective mechanisms [4]. These compounds have the potential to scavenge free radicals, reduce oxidative stress, modulate inflammatory responses, and promote neuronal survival and regeneration [8]. Fractionation of *Skimmia lareoula* leaf extracts allows for the isolation of specific bioactive components, potentially enhancing their therapeutic efficacy. By separating the crude extract into different fractions based on polarity or molecular weight, researchers can identify fractions enriched with compounds that exhibit potent neuroprotective properties [9]. These fractions can then be evaluated for their ability to rescue neuronal function and ameliorate ethanol-induced neurotoxicity. Furthermore, recent advancements in nanotechnology have paved the way for the development of nanoparticles loaded with bioactive compounds from plant extracts. Nanoparticles offer several advantages for drug delivery, including increased stability, improved bioavailability, and targeted delivery to specific tissues or cells [10, 11]. By encapsulating *Skimmia lareoula* leaf extract-derived compounds into nanoparticles, researchers can enhance their pharmacokinetic properties and optimize their therapeutic potential for neuroprotection. Ethanol-induced neurotoxicity poses a significant health risk, particularly in young individuals, and effective therapeutic interventions are urgently needed [12]. *Skimmia lareoula* leaf extracts and their fractions, as well as nanoparticles derived from these extracts, hold promise as potential neuroprotective agents. By elucidating their mechanisms of action and evaluating their efficacy in preclinical models, we can pave the way for the development of novel treatments to safeguard against the detrimental effects of ethanol on the developing brain [13, 14]. In light of these considerations, the present study aims to investigate the neuroprotective effects of *Skimmia lareoula* leaf extract fractions and nanoparticles in young rats exposed to ethanol-induced neurotoxicity. By assessing various parameters such as neuronal morphology, oxidative stress markers, inflammatory cytokine levels, and behavioral outcomes, we seek to elucidate the mechanisms underlying the protective effects of these interventions. Ultimately, our findings may contribute to the development of novel therapeutic strategies for mitigating ethanol-induced neurotoxicity and preserving brain health in vulnerable populations.

2. Material and Methods:

2.1. Mice and Their Grouping

In this experimental study, conducted on 7-day-old albino mouse pups, researchers aimed to assess the efficacy of various treatments in mitigating ethanol-induced neurotoxicity. The pups were strategically divided into groups, with Group A receiving ethanol (5g/kg) alone or combined with different substances: oil (30 mg/kg), crude extract (30 mg/kg), chloroform fraction (30 mg/kg), or

methyl acetate fraction (30 mg/kg). Group B received ethanol (5g/kg) in combination with specific metal salts: silver nitrate (30 mg/kg) or copper sulfate (30 mg/kg). After a 4-hour interval, brain samples were collected for analysis via western blotting. Ethical clearance from the NMMRC committee ensured adherence to rigorous ethical standards throughout the study.

2.2. Plant collection Drying and Grinding

Following the collection of fresh *Skimmia laureola* leaves from the regions of Swat and Buner within the Malakand division of Khyber Pakhtunkhwa, Pakistan, meticulous care was taken to preserve their integrity. These botanical specimens were sheltered in shaded environments to facilitate gradual dehydration over a period spanning two to three weeks. Subsequently, the desiccated leaves underwent pulverization using an electric grinder, yielding a finely powdered form. This methodical process ensured the maintenance of botanical authenticity and consistency, laying a robust foundation for subsequent scientific inquiry and experimentation.

2.3. Oil Extraction Process by Soxhlet Extraction Method (SE)

Initiating the extraction process, 100 grams of *Skimmia laureola* leaves were meticulously positioned within a thimble holder before integration into the Soxhlet apparatus. This crucial step preceded the careful dispensation of 500 milliliters of petroleum ether into a conical-bottomed flask, marking the inception of the extraction procedure. Operating within a controlled temperature range of 30 to 60 degrees Celsius, the extraction ensued for a duration of six hours. The cessation of the process was discerned upon the attainment of a clear solution within the thimble, indicating the comprehensive extraction of oil from the leaves. Subsequently, the extracted oil, distinguished by its heightened viscosity and characteristic greenish hue, underwent solvent removal through immersion in a water bath, adhering to established methodologies (18).

2.4. Gas Chromatography- Mass Spectrometery (GC-MS) of essential oil

The essential oil from the leaves of *Skimmia loureola* was analyzed by GC-MS Model QP 2010 plus ((Shimadzu) operating in EI mode at 70 ev equipped with a split-splitless injector (Split ratio, 1:50 was used). Helium was used as carrier gas with flow rate of 1ml/min. A capillary column (Length: 30 m, id: 0.25 mm, thickness: 0.25µm, DB-5MS Agilent technologies, USA) treated with 95 % dimethyle and 5 % diphenyle poly silphenylene. The identification of the constituents was based on comparison of their retention times (RT) and mass spectra of the samples with those obtained from standards used. Relative percentage of compounds was calculated from the total chromatogram by computer.

2.5. Crude Extract Preparation

Utilizing 50 grams of finely ground *Skimmia laureola* leaves, we initiated the extraction process by immersing them in a solution comprising 3 parts methanol to 1 part water, housed within a shaker. Following a 30-minute incubation period, the resulting mixture underwent filtration, with the filtrates collected subsequent to passage through filter paper. To enhance concentration, a combination of a rotary vacuum evaporator and a water bath was employed, allowing for the gradual removal of solvent from the filtrate. This step aimed to yield a more concentrated extract, rich in bioactive compounds present in *Skimmia laureola* leaves.

2.6. Chloroform and Methyl acetate Fraction Preparation

Commencing the extraction process, 10 grams of dry *S. laureola* powder were meticulously added to 300 milliliters of distilled water. This solution was then combined with chloroform and methyl acetate within a separating funnel, where vigorous agitation ensued. Following separation, the chloroform/methyl acetate layer was carefully isolated and transferred to a water bath for concentration, facilitating the extraction of bioactive compounds from *S. laureola* leaves.

2.7. Green synthesis of nanoparticles

2.7.1. Preparation of plant extract

To initiate the synthesis of nanoparticles (NPs), 30 grams of fresh leaves were subjected to boiling in 200 milliliters of distilled water on a hot plate until the water color transitioned to a dark yellow hue. Subsequently, the resulting filtrate served as both the reducing and stabilizing agent in the NP synthesis process.

2.7.2. Preparation of $AgNO_3$ Nanoparticle

To prepare the solution, 0.017g of silver nitrate was dissolved in 100ml of distilled water, yielding a 1mM AgNO3 concentration. This solution was then heated on a hot plate at 80℃ for 10 minutes. Subsequently, 10-20ml of plant extract was gradually added until the solution turned brown. After centrifuging twice at 4,500 rpm for 30 and 5 minutes respectively, the resulting pellet was treated with methanol and dried in a water bath [14, 15].

2.7.3. Preparation of $CuSO_4$ Nanoparticle

To prepare a 10mM copper sulfate (CuSO4) solution, 0.16g of copper sulfate was dissolved in 100ml of distilled water. The solution was heated on a hot plate at 80°C for 10 minutes. Gradually, 10-20ml of plant extract was added stepwise until the solution changed color to green. Afterward, the solution was centrifuged twice at 4,500 rpm for 30 minutes and 5 minutes respectively. A small quantity of methanol was added to the resulting pellet, followed by drying in a water bath until complete desiccation.

2.8. Western Blotting Analysis

At the conclusion of the treatment and behavioral studies, all animals were euthanized following previously established methods. Mice were decapitated, and the entire brain was swiftly extracted and carefully transferred to RNA later solution and PBS (1:1) on ice. The whole brain was homogenized in total protein extraction (T-PER) solution by Thermo Scientific, and the tissue supernatant was collected and stored at -20℃ for future analysis. Protein concentration was determined using the Bio-Rad protein estimation assay, with absorbance measured at 595nm. Sample proteins were normalized to 30µg per group, and gel electrophoresis was conducted using SDS-PAGE 12-15%. Running conditions were maintained at 50 amps for the first 50 minutes, followed by a switch to 120 volts for approximately 3-4 hours until the run was complete. Proteins from the gel were then trans-blotted to a PVDF membrane (Santa Cruz Biotechnology, USA) using the semi-dry transblot technique by Bio-Rad. Primary antibodies derived from mice, including anti-NF-kB, anti-Caspase-3, anti-PARP-1, and anti-monoclonal antibodies (Santa Cruz, CA, USA), were employed, followed by anti-mouse HRP-conjugated secondary antibody (Santa Cruz, CA, USA). The results were visualized using X-ray films (Shah et al., 2017).

2.9. Statistical Analysis

All the X-rays of results were scanned and compiled and their statistical analysis was done through specified computer based software's. It include image J, Prism 5 graph Pad, Adobe Photoshop etc (Shah et al., 2015).. The density of proteins is expressed in arbitrary units (A.U.s) as the mean ± S.E.M. $^{\#}$significantly different from normal saline treated and *significantly different from scopolamine treated mice, respectively; $^{*\#}P < 0.05$, $^{**\#\#}$ $P < 0.01$, $^{***\#\#\#}$ $P < 0.001$.

3. RESULTS

3.1. GC-MS Analysis of essential oil of *Skimmia laureola* leaves

The essential oils from the leaves of Skimmia laureola were extracted through hydro distillation and analyzed for chemical composition through GC-MS (Fig. 3.7). Various identified components with their respective chemical nature, percent concentration, retention time and charge to mass ratio are given in Table 3.7. A total 31 different components were identified including Bergamot mint oil

(53.41%), hydrocarbon monoterpenes (11.87 %), alcoholic monoterpenes (22.06 %), acetate monoterpenes (12.12 %), aldehyde monoterpenes (0.13 %), oxygenated monoterpenes (0.07 %) and sesquiterpenes (0.42 %). Bergamot mint oil is a mixture of acetate and alcoholic monoterpenes (Linalool and linalyl acetate) was the major constituents (53.41%). Other components detected in the *S.lareoula* were geraniol acetate (9.16 %) and pmeth-1-en-8-ol (6.67 %). beta- Myrcene (3.69 %), Trans-beta-Ocimene (2.48 %) and alpha- pinene (2.42 %) are the major monoterpenes of hydrocarbon nature. Minor components detected were Nerol acetate (1.82 %), cis-beta-Ocimene (1.69 %), Sabinene (1.06 %), p-meth-1-en-8-ol, acetate (1.05 %) and cis-Geraniol (1.03 %). Very small amount of oxygenated monoterpenes were present as compared to other constituents. Gamma elemine (0.21 %) was the major sesquiterpenes, although total amount of sesquiterpenes were found to be very low.

Table 1: GC-MS profile of the *Skimmia laureola* leaf essential oil

S. No	Name	Compound type	% composition	r/time	m/z
1	alpha- pinene	Monoterpene hydrocarbon	2.42	8.889	93
2	Camphene	Monoterpene hydrocarbon	0.01	9.575	93
3	Sabinene	Monoterpene hydrocarbon	1.06	10.594	93
4	Beta – Pinene	Monoterpene hydrocarbon	0.08	10.779	93
5	beta- Myrcene	Monoterpene hydrocarbon	3.69	2.21	93
6	beta – phellandrene	Monoterpene hydrocarbon	0.34	13.258	40
7	Cineole	Monoterpene hydrocarbon	0.14	13.323	43
8	Trans-beta-Ocimene	Monoterpene hydrocarbon	2.48	13.587	93
9	cis-beta-Ocimene	Monoterpene hydrocarbon	1.69	14.079	93
10	3-Carene	Monoterpene hydrocarbon	0.01	14.59	93
11	gamma turpentine	Monoterpene hydrocarbon	0.01	14.59	93
12	Terpinolene	Monoterpene hydrocarbon	0.12	15.893	93
13	.alpha.-Methyl-.alpha.- [4-methyl-3-pentenyl] oxiranemethanol	Monoterpene alcohol	0.04	15.997	59
14	beta- Linalool	Monoterpene alcohol	14.19	16.733	71
15	1-Terpinene-4-ol	Monoterpene alcohol	0.13	19.548	71
16	p-meth-1-en-8-ol	Monoterpene alcohol	6.67	19.974	59
17	n-octyle acetate	Monoterpene acetate	0.06	20.384	43
18	cis-Geraniol	Monoterpene alcohol	1.03	20.705	41
19	Brgamot mint oil	Mixture of acetate and alcohol monoterpenes	53.41	21.327	93
20	Alpha-Citral	Monoterpene aldehyde	0.13	21.686	69
21	Bornyl acetate	Monoterpene acetate	0.03	22.059	95
22	cis-Limonene oxide	Monoterpene oxygenated	0.03	22.941	43
23	3-Nonanol,1,2;6,7dipoxy-3,7-dimethyle acetate	Sesquetepene	0.06	23.152	43
24	p-meth-1-en-8-ol, acetate	Monoterpene acetate	1.05	23.227	121
25	Nerol acetate	Monoterpene acetate	1.82	23.401	69
26	Geraniol acetate	Monoterpene acetate	9.16	23.739	69
27	Caryophyllene	Sesquiterpene	0.04	24.543	41
28	alpha-Limonene diepoxide	Monoterpene oxygenated	0.04	25.567	43
29	gamma-Elemene	Sesquiterpene	0.21	25.701	161
30	trans-Nerolidol	Sesquiterpene	0.08	25.506	69
31	Caryophyllene oxide	Sesquiterpene	0.03	27.22	41

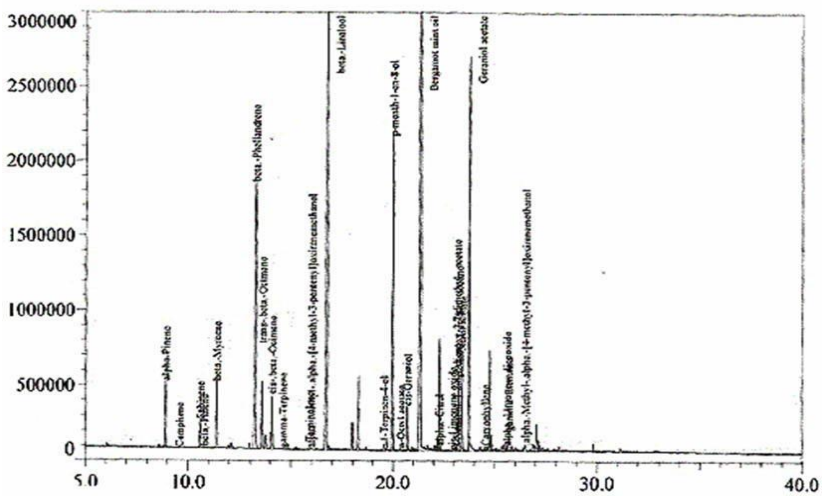

Fig.1a: Typical GC-MS chromatogram of *Skimmia laureola* leaf essential oil (SVO) showing the separation of chemical components

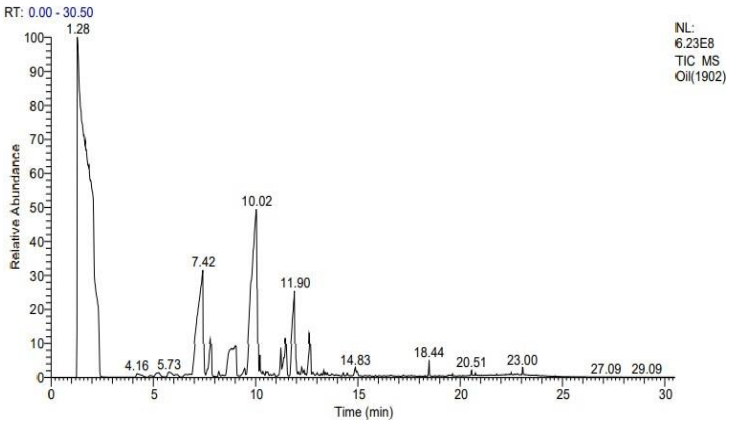

Fig.1b: GC-MS Chromatogram of *Skimmia laureola* Leaf Essential Oil

3.2. *Skimmia lareoula* fractions reverse ethanol-induced

Caspase-3 downregulation in PND-7 mice Ethanol exposure during early developmental stages is recognized for its capacity to induce oxidative stress, resulting in neuronal cell death and subsequent neurological disorders, as established in prior research. Our study, as depicted in Fig.1A, reaffirms the deleterious impact of ethanol, demonstrating its ability to trigger apoptotic cell death in the developing rat brain, ultimately culminating in neuroinflammation and neurodegeneration. In response to these findings, we aimed to explore the therapeutic potential of various fractions of *Skimmia lareoula* leaves in counteracting ethanol-induced neuronal damage. To investigate this, we administered a single subcutaneous dose of ethanol (5g/kg) to postnatal day 7 (PND7) rats, which led to a significant increase in reactive oxygen species (ROS) production and activation of microglia and astrocytes, along with induction of the apoptotic marker Caspase-3. However, treatment with *Skimmia lareoula* leaf fractions, encompassing Oil, crude, chloroform, and methyl acetate, exhibited promising protective effects. These interventions effectively mitigated ROS production, suppressed the activation of microglia and astrocytes, and reversed other ethanol-induced changes, including elevated levels of Caspase-3, NF-kB, and PARP- 1 ratios observed in the developing rat brain (Fig.1B-D). Overall, our findings underscore the potential therapeutic benefits of *Skimmia lareoula* leaf fractions in mitigating ethanol-induced neuroinflammation and neurodegeneration during critical developmental stages. Further exploration of these fractions may offer novel insights into their neuroprotective mechanisms and pave the way for the development of targeted therapeutic interventions for mitigating ethanol-induced neurotoxicity.

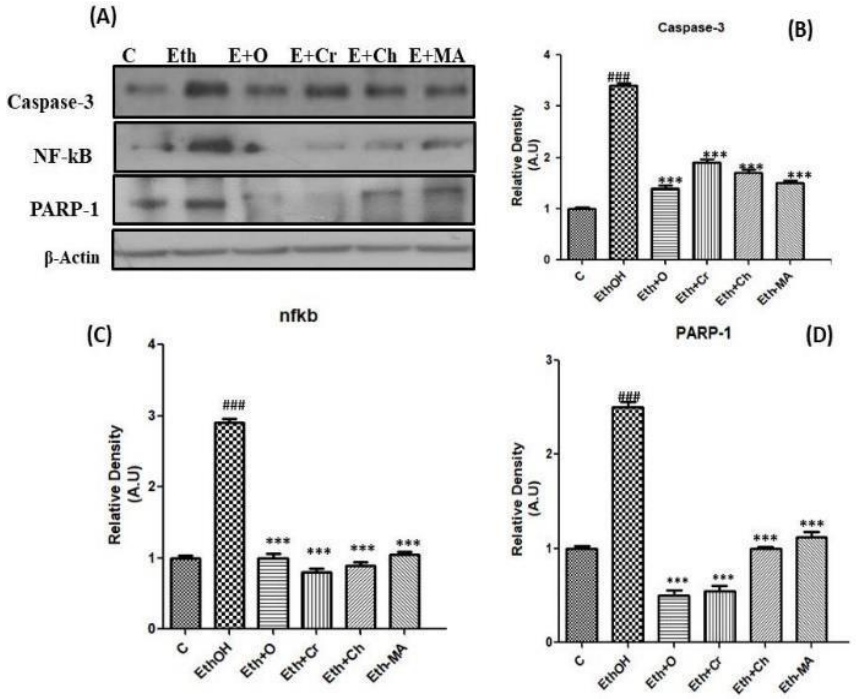

Fig.2: Different fractions of *Skimmia lareoula* leaves reversed ethanol induced apoptosis by downregulation of Caspase-3 in PND-7 mice. The administration of four frictions extracted from Skimmia leaves significantly downregulate Caspase-3, NFKB and PARP-1 protein expression in the brain homogenates of mice. (A) The results of a Western blot for apoptotic and neuro-inflammatory markers are shown. The histograms of (B) Caspase-3, (C) NFKB and (D) PARP-1. The materials and procedures sections already covered the treatment. The output from Image J was provided in arbitrary units (A.U). The mean is shown as a histogram in A.U. SEM. The sign # denotes the comparability of the control and Ethanol, whereas the symbol * denotes the comparability of Ethanol with oil, crude, chloroform and methyl acetate frictions. Significance: $^{**, ##}p\leq0.01$ and $^{***, ###}p\leq0.001$.

3.3 *Skimmia lareoula* leaf nanoparticles reversed ethanol-induced neuroinflammatory complex content

Caspase-3 plays a pivotal role in orchestrating cellular apoptosis, a process implicated in various neuroinflammatory and neurodegenerative conditions. Elevated levels of Caspase-3 have been associated with increased neuronal cell death and the progression of neurological disorders. Similarly, the activation of NFKB (Nuclear Factor Kappa B) and PARP-1 (Poly (ADP-ribose) polymerase 1) further exacerbates neuroinflammation and neuronal damage, contributing to the pathogenesis of neurological diseases. In our study, western blot analysis provided insights into the expression levels of these key proteins in the brain homogenates of postnatal day 7 (PND-7) albino mice. The results depicted a significant upregulation of Caspase-3, NFKB, and PARP-1 proteins following ethanol administration, indicating the induction of apoptotic and inflammatory pathways. However, treatment with Copper sulfate and Silver Nitrate Nanoparticles isolated from *Skimmia lareoula* leaves exerted a noticeable inhibitory effect on the expression of these proteins. Notably, this suppression occurred even when nanoparticles were administered four hours after ethanol exposure, suggesting their potential therapeutic efficacy in mitigating ethanol-induced neuroinflammation and neurodegeneration. Overall, our findings highlight the promising neuroprotective properties of *Skimmia lareoula* leaf nanoparticles, offering a potential avenue for the development of novel therapeutic interventions targeting neuroinflammatory and neurodegenerative conditions associated with ethanol exposure.

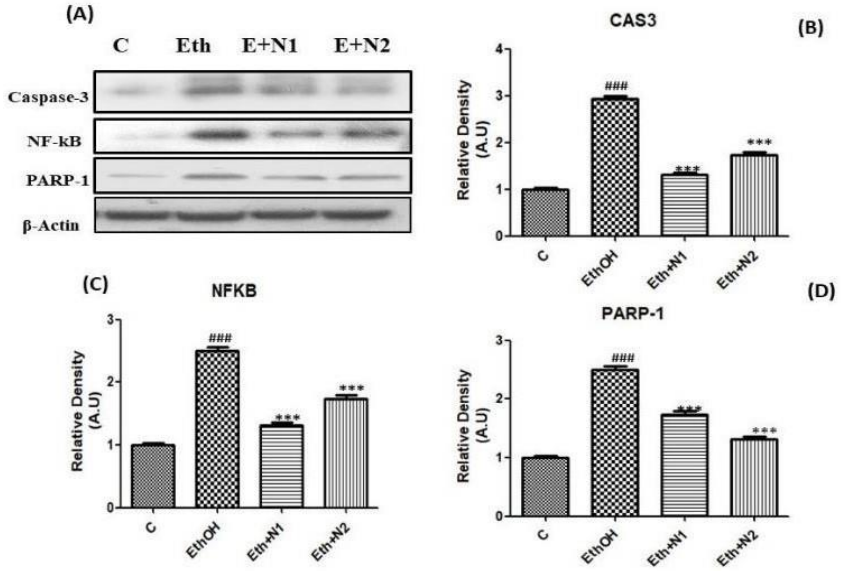

Figure.3: Nanoparticles isolated from the leaves of *Skimmia lareoula* reversed ethanol induced neuroinflammatory complex content in PND-7 albino mice. The administration of nanoparticles isolated from Skimmia leaves significantly downregulate Caspase-3, NFKB and PARP-1 protein expression in the brain homogenates of mice. The administration of nanoparticles isolated from Skimmia leaves significantly downregulate Caspase-3, NFKB and PARP-1 protein expression in the brain homogenates of mice. (A) The results of a Western blot for *Skimmia laureola* and apoptotic and neuro-inflammatory markers are shown. (A-D) are the histograms of (A) blots, (B) Caspase-3 (C) NFKB and (D) PARP-1. The materials and procedures sections already covered the treatment. The output from Image J was provided in arbitrary units (A.U). The mean is shown as a histogram in A.U. SEM. The sign # denotes the comparability of the control and Ethanol, whereas the symbol * denotes the comparability of Ethanol with copper sulphate and silver nitrate nanoparticles. Significance: $^{**,\ ##}p\leq0.01$ and $^{***,\ ###}p\leq0.001$.

4. DISCUSSION

This study delved into the therapeutic potential of different fractions and nanoparticles extracted from *Skimmia lareoula* leaves against ethanol-induced neurotoxicity in postnatal day 7 (PND-7) rat pups. Utilizing the PND-7 rat model was strategic due to its established efficacy as a rapid and reliable method for studying ethanol intoxication effects [16]. Ethanol exposure during early developmental stages poses a significant risk, as it can induce neuroinflammation and neuronal death, leading to long-term neurological impairments. Numerous studies have underscored the neuroprotective properties of herbal medicines against ethanol-induced toxicity [17-19]. These studies have highlighted the potential of herbal remedies to mitigate ethanol-induced brain damage and apoptosis [20, 21]. In the present investigation, four fractions and two nanoparticles isolated from Skimmia lareoula leaves were evaluated for their neuroprotective effects using western blot techniques [22]. Our findings revealed that treatment with *Skimmia lareoula* leaf fractions and nanoparticles significantly mitigated ethanol-induced neuroinflammation and attenuated neuronal apoptosis in the brains of PND-7 male rat pups. Specifically, these treatments were effective in suppressing the activation of inflammatory pathways mediated by caspase-3 and PARP-1/NF-κB signaling. The selection of the PND-7 developmental stage was deliberate, as this period corresponds to a critical phase of brain growth spurt in rodents, during which they exhibit heightened vulnerability to ethanol-induced apoptotic neurodegeneration [23]. Male rats were chosen to ensure consistency and avoid potential confounding effects of sex-dependent differences. Ethanol is a well-established neurotoxin capable of inducing inflammation in the central nervous system (CNS) [24, 25]. Exposure to ethanol triggers neuroinflammation, ultimately leading to neuronal death, particularly in vulnerable brain regions such as the hippocampus and cerebellum, which exhibit lower levels of antioxidant enzymes

and vitamin E [26-28]. Our findings suggest that *Skimmia lareoula* fractions and nanoparticles effectively mitigate ethanol-induced neurotoxicity by modulating the NF-κB and PARP-1 pathways and reducing caspase-3 activation in postnatal rat pup brains. Previous research has demonstrated that ethanol administration activates inflammatory pathways such as the JNK pathway and upregulates inflammatory markers like PARP-1 and NF-κB [29]. However, co-treatment with herbal medicines has been shown to reverse these effects, highlighting their potential as therapeutic agents in mitigating ethanol-induced neurodegeneration. Overall, this study presents a novel approach using plant fractions and nanoparticles to combat ethanol-induced neurotoxicity in an animal model of neurodegeneration, offering promising insights into potential therapeutic interventions for alleviating alcohol-induced brain damage.

5. CONCLUSION

Medicinal plants serve as valuable reservoirs of bioactive compounds, offering potential solutions for combating various chronic complications associated with neurodegenerative disorders. *Skimmia lareola* stands out as one such medicinal plant with promising therapeutic properties against neurotoxicity and associated neuronal damage and oxidative stress. This research primarily focuses on investigating the ameliorative effects of *Skimmia lareola* in mitigating neurotoxicity. Among the various fractions studied, the crude extract of *Skimmia lareola* emerges as particularly effective against alcohol-induced toxicity. Additionally, different nanoparticles derived from *Skimmia lareola*, such as silver nitrate and copper sulphate, exhibit notable efficacy in reducing neurotoxicity. The extract of *Skimmia lareola* is notably rich in antioxidants and neuroprotective compounds, which operate on multiple levels including neuroendocrine-immune modulation. These bioactive constituents work synergistically to enhance brain function, mitigate neurodegeneration, and reduce neurotoxicity.

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