Skimmia lareoula fractions via TLR-4 /COX2 / IL-1β rescues adult mice against Alloxan induced hyperglycemia induced synaptic and memory dysfunction

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Abstract-In the current study, we have evaluated the therapeutic efficacy of two Nanoparticles i.e. copper sulphate ($CuSO_4$) and silver Nitrate ($AgNO_3$) isolated from *Skimmia lareoula* against Alloxan induced hyperglycemia and oxidative stress mediated neuroinflammation and memory dysfunction in male adult albino mice brains. The results indicate that both nanoparticles significantly reduced Alloxan-induced hyperglycemia oxidative stress, neuroinflammation neurodegeneration accompanied by the improvement in memory and behavior via TLR-4 /COX2 / IL-1β signaling pathway. Copper sulphate show more significant result comparative to silver nitrate. In summary, these results indicate that these two nanoparticles are natural, potent, safe and easily available therapeutic agents and acts as a drug candidate to treat diabetes mediated AD pathology in adult mice.

Index Terms- Copper sulphate, hyperglycemia oxidative stress, silver nitrate, *Skimmia lareoula* and signaling pathway.

I. INTRODUCTION

Type 1 diabetes mellitus (T1DM) is characterized by the death and malfunctioning of pancreatic beta-cells (β-cells), resulting in abnormal blood glucose levels (1). Around 411 million individuals worldwide are affected by diabetes mellitus (DM). T1DM accounts for around 10% of all diabetic patients (2,3). Diabetes has an impact on metabolism in a variety of tissues, including the liver, which serves as a metabolic hub and plays a key role in metabolic processes. (4). In other investigations, oxidative stress and inflammation have also been linked to insulin dysfunction (5,6). In order to develop T1DM in animal models, Alloxan, a pancreatic-cell toxin, is generally utilized. Hyperglycemia and free fatty acids, as well as lower serum insulin levels, influence pancreatic β-cells in a alloxan-induced diabetes model (7,8). There is strong evidence that diabetes mellitus (DM) can harm the central nervous system, with cognitive impairment being the most common symptom (9,10**)**. Cognitive deterioration in patients with type 1 diabetes mellitus (T1DM) is caused by hyperglycemia-related microvascular alterations in the brain; intense insulin therapy for T1DM treatment improves glycemic control. Insulin therapy also seeks to improve spatial learning and memory deficiencies (11,12). However, C-peptide (an insulin manufacturing byproduct) supplementation from the outset of diabetes almost completely prevents cognitive losses, but C-peptide has little effect on blood glucose levels (13,14). As a result, other biomedical factors that are insidiously linked to diabetes can influence diabetes-related cognitive deterioration. The presence of amyloid plaques and neurofibrillary tangles in the hippocampus at autopsy has been linked to a decline in cognitive abilities in diabetics (15,16,17,18). Amyloid-β (Aβ) buildup was also seen in the brains of diabetic mice, either naturally (19) or as a result of Alloxan, a diabetogenic drug toxic to pancreatic β island cells (20,21,22). Furthermore, diabetes-accelerated memory dysfunction was due to cerebrovascular inflammation and Aβ deposition in an Alzheimer mouse model with diabetes. These findings suggested that Aβ accumulation in the brain in diabetic condition may be one of the important reasons for diabetes-associated cognition impairment.

Pakistan's flora are abundant with bioactive compounds (23,24,25). Some medicinal plants, are commonly employed in life science as a source of bioactive chemicals with a wide range of chemical structures and biological activity (26,27). *Skimmia lareoula* are being investigated as a potential source of bioactive compounds. It contain a variety of inorganic and organic compounds that can benefit

human health (28). *S. lareoula* have been found to contain chemical compounds that have antioxidant, antiviral, antifungal, and antibacterial properties (29,30). The compounds isolated from some plants has been reported to act as a neuroprotective agent in neurodegenerative diseases (31). Recent studies have revealed their benefits to the brain. Furthermore, the investigated plant leaves fractions are the subject of numerous studies because they are a major source of natural bioactive compounds with specific biological functions, as well as because they exhibit a wide range of health benefits due to their antioxidant, anticoagulant, anti-inflammatory, and antiviral properties (32). Antioxidants have also been demonstrated to help prevent oxidative stress-induced neuronal damage in Alzheimer's disease (33). Research into the biological activities of many medicinal plants and their applications in biomedical and pharmaceutical industries have attracted much interest recently. In the current study we have analyzed for the first time to our knowledge the antihyperglycemic effect of two nanoparticles extracted from *Skimmia lareoula* leaves. Alloxan was used to induce hyperglycemia accompanied by the AD neuropathology in adult mice. These two nanoparticles significantly restored the memory and synapse and reduced neuroinflammatory and neuroapoptotic markers in the brains of adult albino mice. Additionally, two nanoparticles inhibited TLR-4 /COX2 / IL-1β (signaling pathways to reduce alloxan induced memory dysfunction.

II. Materials and Methods

2.1 Mice and Their Grouping

During this study adult male albino mice (7-8 weeks old) were randomly divided into five groups (n=5) as given below. These mice were purchased from NIH (National Institute of health), Islamabad, Pakistan and brought to Neuro Molecular Medicines Research Center (NMMRC, Peshawar). Then the mice were allowed to acclimatize to the environment. Mice were separately placed in their respective cages (Biobase China). Their grouping were,

1. Normal mice
2. Alloxan treated mice (30 mg/kg)
3. Alloxan treated mice (30 mg/kg) + (N1) nanoparticle $CuSo_4$ (30 mg/kg)
4. Alloxan treated mice (30 mg/kg) + (N2) nanoparticle $AgNO_3$ (30 mg/kg)

The male mice (30–32 g average body weight) were place in the breeding room with a 12 /12-h light/dark cycle at 25 ±°C temperature, provided with water and food *ad libitum*. All the animals were treated with great care as per the recommendations of the local animal ethics committee of the NMMRC, Peshawar.

2.2 Plant collection Drying and Grinding

After the fresh *Skimmia laureola* leaves had been obtained from the districts of Swat and Buner in the Malakand division of Khyber Pakhtunkhwa, Pakistan, they were stored in the shade until they had completely dried out for two to three weeks, and then they were ground into powder using an electric grinder.

2.3 Green synthesis of nanoparticles

2.3.1 Preparation of plant extract

For the synthesis of NPs 30 gm of fresh leaves 30gram were boiled in 200 ml of distilled water on a hot plate until water color changed to dark yellow. The filtrate obtained was further used as reducing and stabilizing agent.

2.3.2 Preparation of $AgNO_3$ Nanoparticle

0.017g silver nitrate was dissolved in 100ml distilled water to prepare 1mM $AgNO_3$. Solution was kept for 10 min on hot plate at 80°C and 10-20 ml of plant extract was added step wise until color of solution changed to brown. Then solution was centrifuge twice at 4,500 rpm for 30 min and 5min respectively. A small amount of methanol was added in the pellet and kept in water bath until the pellet was fully dry.

2.3.4 Preparation of $CuSO_4$ Nanoparticle

0.16g copper sulphate was dissolved in 100ml distilled water to prepare 10mM $AgNO_3$. Solution was kept for 10 min on hot plate at 80°C and 10-20 ml and plant extract was added step wise until color of solution changed to green. Then solution was centrifuge twice at 4,500 rpm for 30 min and 5 min respectively. A small amount of methanol was added in the pellet and kept in water bath until the pellet was fully dry.

2.4 Chemicals

Alloxan, PBS (Phosphate Buffer Saline) tablets [Cat. No; P4417-50TAB], sodium dodecyl sulphate (SDS), ammonium per sulphate (APS), acrylamide, bis-acrylamide, ,trizma base, sodium chloride (NaCl), potassium chloride (KCl), were procured from Sigma Aldrich Chemical Co. (St. Louis, MO, USA) &Daejung Chemicals & Metals Co. Ltd (Gyeonggi-do, Shiheung, South Korea) respectively. The antibodies used in the Western blot and immunofluorescence studies were anti-COX-II (sc-376861, anti-tumor necrosis factor-α (TNF-α) (sc-52,746), and anti-β- actin (sc-47,778) (Santa Cruz Biotechnology, Dallas, TX, USA). For β-actin, the antibody was diluted in TBST (1:10000) (Santa Cruz Biotechnology, Dallas, TX, USA). Other primary

antibodies were diluted in 1x TBST (1:1000), and Secondary anti-mouse HRP (Horseradish peroxidase) conjugated were diluted 1:10,000 in 1× TBST and were purchased from Promega, (Fitchburg, WI, USA).

2.5 Behavioral Tests

To demonstrate the therapeutic effects of nanoparticle isolated from *Skimmia* plant on Alloxan induced memory impairment, two behavioral tests were executed. Alloxan was administered interaperitonealy (i.p.) to the mice of group III and IV with Nanoparticle 1 and 2 respectively. Later all the research animals were tagged and randomized into four (04) groups, while the behavioral studies were carried out as single blind trial. The researcher carrying out the behavioral tests was kept blind of the tags and mice treatment group.

2.6 Morris Water Maze Test

To probe the hippocampal-dependent long-time spatial learning abilities of mice, the Morris water maze (MWM) test was carried out. The design & dimensions of MWM test apparatus are specified in detail reported recently (34). Before the actual commencement of the tests, the mice were trained for swimming twice a day to acclimatize with water tank and platform for 03 days. Later for each mouse the escape latency was calculated for a period of 60 sec to find the hidden/submerged platform and this practice continued for 05 days. The mice were manually directed and placed on platform for 10 sec if it failed to find platform in given time (60 sec). Escape latency time (Sec) for each day was recorded and maintained. Mice were placed on rest for period of 02 days before final probe testing i.e. platform was hidden and the mean time spent by each mice in target quadrant was calculated.

2.7 Y-Maze

The Y-maze behavioral test was carried out as reported earlier (35). Y-maze apparatus consists of 3 arms with dimensions of 50x10x20 cm^3 (LxWxH) connected at an angle of 120^0 to each other. Each time mice were allowed to adjust to this new environment for 10 minutes. Afterwards the mice were placed in maze center, and allowed to move freely in 3 arms for 08 minutes. Total arm entries of each mice and successive triplets were computed by software and the percentage of alternations was calculated by the formula as follows:

$$\text{Percentage alternations} = \frac{\text{Successive triplet sets}}{\text{Total number of arm entries} - 2} \times 100$$

The spatial memory function of mice was positively correlated with the percentage of alternations.

2.8 Western Blotting Analysis

All the animals were killed at the end of treatment and behavioral studies as per method reported previously. Decapitated the mice and extracted the whole brain carefully and quickly transferred in RNA later solution and PBS (1:1) on ice. Whole brain was homogenized in total protein extraction (T-PER solution by Thermo Scientific), tissue supernatant was collected and stored at -20ºC for future analysis. The protein concentration was quantified using Bio-Rad protein estimation assay and absorbance was taken at 595nm. All the sample proteins were normalized to 30µg/group and gel electrophoresis was performed using SDS-PAGE 12-15%. Running conditions were maintained at 50amp for first 50 minutes then switched to 120volts for almost 3-4 hours till the run was complete. Proteins from gel were trans-blotted to PVDF membrane (Santa Cruz Biotechnology, USA) using semi-dry transblott technique (Bio Rad). Different mouse derived primary antibodies such as anti-Iba, anti-COX-II, anti-actin, anti-Aβ, anti- monoclonal antibodies from (Santa Cruz, CA, USA) followed by anti-mouse HRP conjugated (Santa Cruz, CA, USA) secondary antibody were employed. The results were developed on the X-rays films (34).

2.9 Biochemical analysis of plasma

Once the drug treatment is finished the animals were killed and blood collected was used for biochemical analysis i.e LDL, HDL, Triglycride and Total cholesterol, random blood glucose and GTT etc.

2.10 Antioxidant analysis of brain homogenates

2.10.1 Catalase assay (CAT)

Catalase activity (CAT) was estimated by method developed earlier with few modifications. The 3 mL of reaction mixture contained 2500 µL of phosphate buffer (50 mM) at pH 5.0, 400 µL of H_2O_2 (5.9 mM) and 100 µL of brain supernatant. Change in absorbance of the reaction mixture was measured at interval of one minute at 240 nm. Change in absorbance of 0.01units/minute was considered as one unit of activity (36).

2.10.2 Peroxidase assay (POD)

Chance and Maehly (1955) method with slight modifications was employed to measure the peroxidase activity. Reaction mixture for peroxidase assay consists of 2500 µL of phosphate buffer (50 mM, pH 5.0), 300 µl of H_2O_2 (40 mM), 100 µL of guaiacol (20 mM) and 1000 µL brain homogenate

supernatant. Change in absorbance of the reaction mixture was measured at one minute interval at 470nm. One unit of peroxidase (POD) activity was regarded as change in absorbance of 0.01units/minute (22).

2.10. 3 Superoxide dismutase assay (SOD)

To estimate the superoxide dismutase (SOD)activity reaction mixture contained 100 phenazine methosulphate (186 µM), 1200 µL sodium pyrophosphate buffer (0.052 mM; pH 7.0) and 300 µL of brain homogenate supernatant. To initiate enzymatic reaction 200 µL of NADH (780 µM) was added to reaction mixture and after 1 minute 1000 µL of glacial acetic acid was added as the stopping agent. The amount of chromogen formed was determined by taking the absorbance of reaction mixture at 560nm, results were expressed as units per mg of protein (23).

2.10.4 Catalase assay (CAT)

Methods previously established for estimating catalase activity (CAT) was slightly modified for this study (22). The reaction mixture at pH 5.0 which took up 3 mL, consisted of 100 µL of brain supernatant, 400 µL of H_2O_2 (5.9 mM), and 2500 µL of phosphate buffer (50 mM). The reaction mixture's absorbance's were monitored every minute as it changed at a wavelength of 240 nm. At the rate of 0.01 units per minute in absorbance change, one unit of activity was defined.

2.10.5 Estimation of lipid peroxidation (TBARS)

Lipid peroxidation (TBARS) assay was carried out by slight modification in method developed earlier (25). Reaction mixture volume of 1000 µL for said assay comprised of 580 µL of 0.1 M phosphate buffer (pH 7.4), 200 µL of 100 mM ascorbic acid, 200 µL of brain homogenate supernatant, and 20 µL of 100 mM ferric chloride. The reaction mixture was incubated for 1 h in shaking water bath maintained at 37 ºC. To stop the reaction 1000 µL of trichloroacetic acid (10%) solution was added. Later 1000 µL of 0.67% thiobarbituric acid was added to the tubes and tubes were placed in hot water bath (95 ºC) for 20 minutes, then rapidly shifted to the crushed ice bath and centrifuged for 10 minutes at 2500*g. The amount of lipid peroxidation (TBARS) formed in every samples was quantified by measuring the absorbance of supernatant at 535 nm on spectrophotometer. The results were expressed as nM TBARS/min/mg of tissue at 37 ºC (TBARS molar extinction coefficient is 1.56×10^5 $M^{-1}cm^{-1}$). 25. Aguilar Diaz De Leon J, Borges CR. Evaluation of oxidative stress in biological samples using the thiobarbituric acid reactive substances assay. J Vis Exp [Internet]. 2020 May 1 [cited 2021 Mar 14];2020(159). Available from: https://pubmed.ncbi.nlm.nih.gov/32478759/.

2.11 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. 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; $^{*\#}P < 0.05$, $^{**\#\#}$ $P < 0.01$, $^{***\#\#\#}$ $P < 0.001$.

III. Results

3.1 *Skimmia lareoula* Isolated Nanoparticles reduced hypercholesterolemia and hyperglycemia induced by Alloxan in mice

The blood of all the experimental animals was tested to know whether Alloxan administration for one time can cause hyperglycemia and hypercholesterolemia in mice. The results shown here depicts that Alloxan significantly increased the random blood glucose and also these animals could not tolerate the oral glucose (Fig. 1a, b) administered to them in oral glucose tolerance test (OGGT). All these results suggest that Alloxan can induce diabetes in the mice. It is accompanied by the hypercholesterolemia i.e. higher levels of total cholesterol (Fig. 1c), triglycerides (Fig. 1d), LDL (Fig. 1e) and less HDL (Fig. 1f) in the blood of mice. On the other hand the two nanoparticles isolated from *Skimmia* leaves significantly reduced both random blood sugar as well as hyperglycemia due to OGTT and hypercholesterolemia in mice.

(a) **(b)**

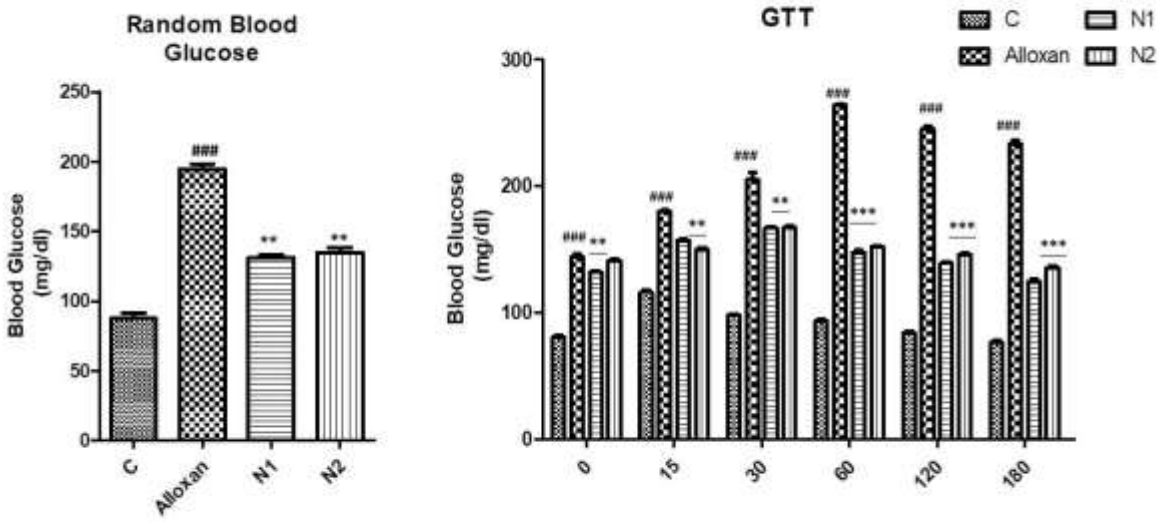


Fig.1a, b: Alloxan significantly increased the random blood glucose and also these animals could not tolerate the oral glucose administered to them in oral glucose tolerance test (OGGT).

(c) **(d)**

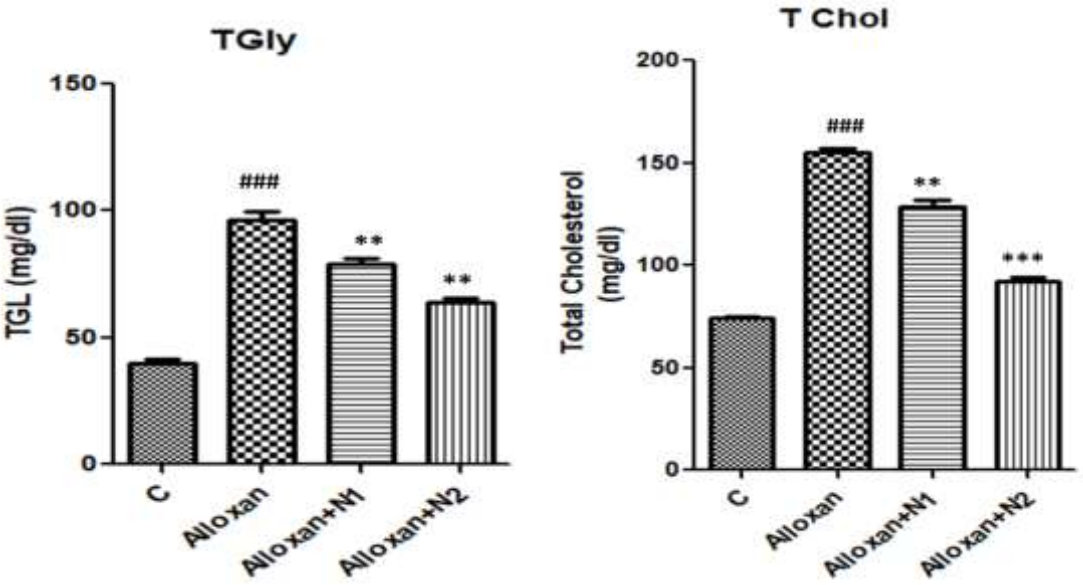


Fig.1 c, d: Alloxan significantly increased the hypercholesterolemia i.e. higher levels of total cholesterol, triglycerides (TGL) in the blood of mice.

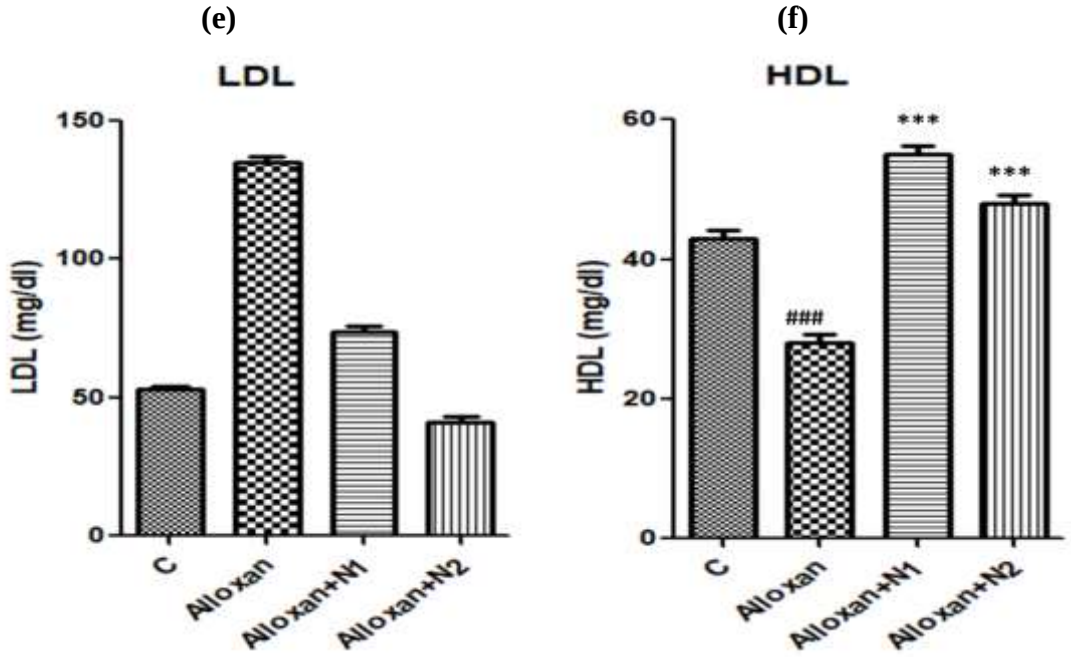


Fig.1 e, f: Alloxan significantly increased the hypercholesterolemia i.e. LDL and less HDL in the blood of mice. Shown are the different blood biochemical assays (a) RBG, (b) OGTT (c) Triglyceride (d) Total Cholestrol, (e) LDL and (f) HDL performed with brain homogenates of experimental mice treated either Alloxan alone or in combination with two nanoparticles i-e copper sulphate and silver nitrate of *Skimmia laureola* respectively. The treatment details have already been given in material and method. The results are expressed as Mean ± SEM of (n=5) mice each group. Significance of control vs Alloxan is expressed as #, while * denotes Alloxan vs Alloxan + *Skimmia laureola* NPs. Significance: $^{**,\,\#\#}p\leq 0.01$ and $^{***,\,\#\#\#}p\leq 0.001$.

3.2 *Skimmia lareoula* Isolated Nanoparticles reduced Alloxan Induced Oxidative Stress in Mice

Previous literature has reported that Alloxan induces oxidative stress in brain, that's why we have injected Alloxan intraperitonealy once to the adult albino mice. One week later the two nanoparticles i-e copper sulphate and silver nitrate NPs extracted from *Skimmia* leaves were injected intraperitonealy at a dose of 30 mg/kg (after the dose optimization) to adult albino mice. The mice brain (hippocampus) of all the experimental groups were homogenized and different antioxidant assays such as SOD, POD, CAT and lipid peroxidase assays i.e. TBARS were performed according to the protocols to know the antioxidant capability of these two Nanoparticles against Alloxan induced oxidative stress. The results revealed that both nanoparticles have significantly enhanced the activities of antioxidant enzymes such as SOD, POD, and CAT and decreased LPO activities in adult mice brain homogenates as given in the figure 2.

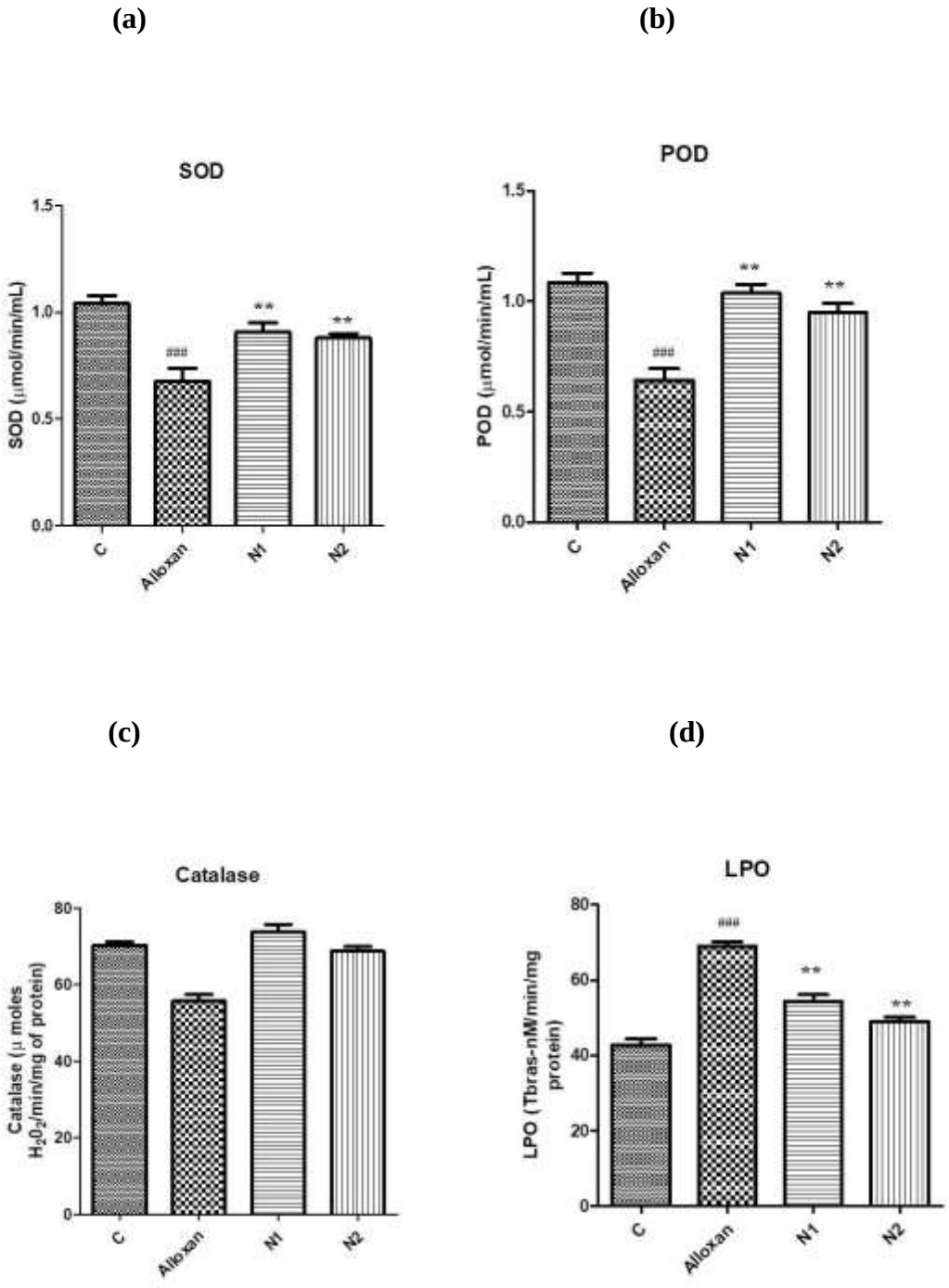


Fig.2 a-d: Three PSs significantly enhanced the activities of antioxidant enzymes such as POD, SOD and CAT and decreased LPO activities in adult mice brain homogenates.
Shown are the different antioxidant enzyme assays (a) SOD, (b) POD (c) CAT (d) LPO Lipid peroxidase (TBARS) performed with brain homogenates of experimental mice treated either scopolamine alone or in combination with two nanoparticles extracted from *Skimmia laureola* respectively. The treatment details have already been given in material and method. The results are expressed as Mean ± SEM of (n=5) mice each group. Significance of control vs

Alloxan is expressed as #, while * denotes Alloxan vs Alloxan + Different *Skimmia laureola* NPs. Significance: **, $^{\#\#}p\leq0.01$ and ***, $^{\#\#\#}p\leq0.001$.

3.3 *Skimmia lareoula* Isolated Nanoparticles reduced Alloxan Induced Neuroinflammation and neurodegeneration in Mice

The brain homogenates of all the experimental animals were subjected to the western blot technique. The immunoblot results reveal that Alloxan significantly induced TLR-4 activation and it is accompanied by the significantly activation of neuroinflammatory and neuro degeneratory markers such as COX-3 and IL-1β. The administration of two nanoparticles not only deactivated TLR-4 but also significantly inhibited COX-3 and IL-1β protein expression in the brain homogenates of mice as given in the figure 3 (a-d).

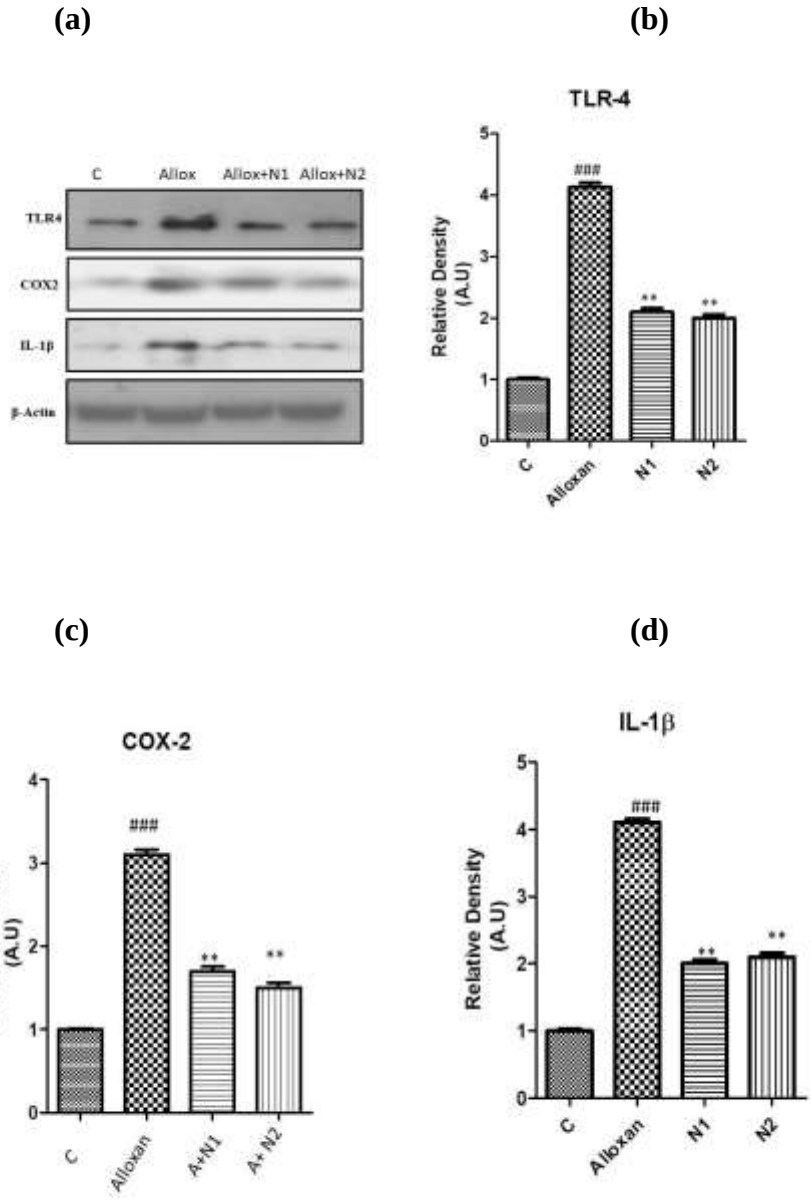


Fig 3 (a-d): The administration of two Nanoparticles extracted from *Skimmia* leaves significantly inhibited TLR-4, COX-3 and IL-1β protein expression in the brain homogenates of mice. Nanoparticles isolated from *Skimmia*

laureola inhibited neuroinflammatory markers and neurodegeneration in mice brain. **(a)** The results of a Western blot for *Skimmia laureola* several neuro-inflammatory markers are shown. (a-d) are the histograms of (a) blots, (b) TRL-4, (c) COX-3 and (d) IL-IBeta. 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 Alloxan, whereas the symbol * denotes the comparability of Alloxan and copper sulphate and silver nitrate nanoparticles. Significance: $^{**, ##}p\leq0.01$ and $^{***, ###}p\leq0.001$.

3.4 Nanoparticles Isolated from *Skimmia lareoula* leaves showed beneficial effect on memory impairment against Alloxan in mice

Previous studies reported that Alloxan impairs memory and behavior in animal model. The administration of Alloxan to adult male mice and Alloxan plus two Nanoparticles and untreated control mice were subjected to two well-known behavioral tasks i.e. Y-maze and Morris water maze (MWM). The mice were trained two times in a day for two days in MWM test. The mice were then given one-day rest and then mean escape latencies data was collected for five days respectively. According to the daily observations the mean escape latencies of the control mice were significantly less from day 1 to day 5 and a constant decrease in the mean escape latencies was noted in those animals as shown in the figure 4a. The mice received Alloxan had higher mean escape latencies from day 1st to day 5th. Although they have shown a little decrease in the mean escape latencies from day 1 to day 5, but still it was on the higher side. Interestingly, the two Nanoparticles treated animals have shown significantly lower mean escape latencies starting from day 1st to day 5th,they have shown a regular decease trend in reaching the platform. Although both the two Nanoparticles have shown less mean escape latencies from day 1st to day 5th and improved behavior but still their escaping time was more than the saline treated animals as shown in the fig. 4a.

(a)

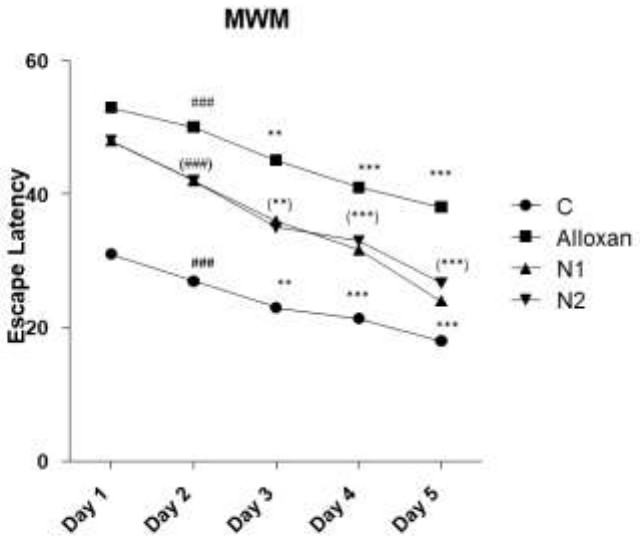
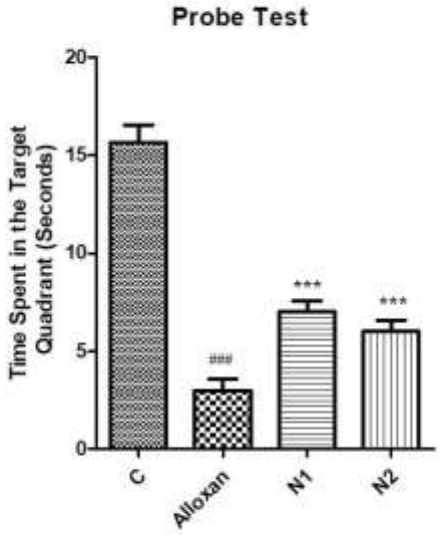


Fig.4a: Constant decrease in the mean escape latencies was noted in those animals. The mice received Alloxan had higher mean escape latencies from day 1st to day 5th. Two Nanoparticles have shown less mean escape latencies from day 1st to day 5th and improved behavior but still their escaping time was more than the untreated animals as shown in the fig above.

After giving on day rest the mice were subjected to probe test in which the platform was hidden. The mice were allowed to find the hidden platform and their time was noted that they have spent in the target quadrant. Here, too the control animals spent more time as compared to the Alloxan

treated animals which spent a very less time in the target quadrantas shown in the fig. 4b. In contrast the two nanoparticles treated animals have spent significantly more time in the target quadrant but overall their spent time was less than the control mice as given in the figure 4b.

(b)



Finally, these animals were subjected to Y-maze test and their % age of spontaneous alternation was noted which believes dependent on spatial memory. Once again in the Y-maze test the control animals shown higher % age of spontaneous alternation, while the Alloxan treated animals have spent significantly less time as given in the figure 4c. The animals received the two Nanoparticles treatments along with Alloxan have shown high % age of spontaneous alternation in the Y-maze task as given in the figure 4c.

(c)

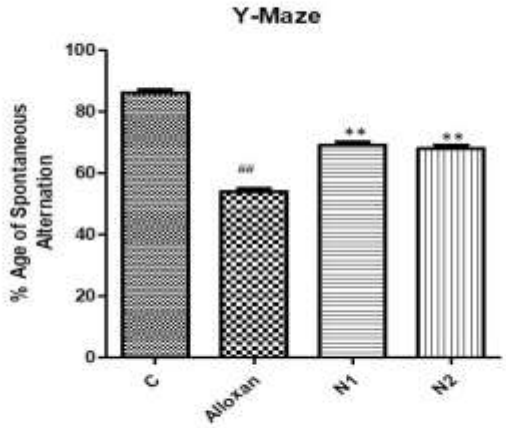


Figure 4a-c. Various Fractions of *Skimmia lareoula* enhanced albino mice's memory impairment produced by scopolamine. (a) Immuno-blot results for MWM and (b) Prob test and (c) Y- maze are shown for control, Alloxan-treated, and Alloxan plus two nanoparticles (copper sulphate and silver nitrate) of *Skimmia lareoula* in the mouse brain. plots of density histograms show how variables vary. The data was processed in Image J and presented in arbitrary units (A.U.). Histogram displaying the mean in A.U ± SEM. Significance: $^{**, ##}p\leq0.01$ and $^{***, ###}p\leq0.001$.

IV. Discussion

This study reported for the first time that the two Nanoparticles isolated from the *Skimmia lareoula* leaves from District Swat, Khyber Pakhtunkhwa Province, Pakistan exhibit neuroprotective capabilities in animal model of Alloxan induced hyperglycemia and oxidative stress mediated Alzheimer disease. During the study we have noticed that two nanoparticles can ameliorates Alloxan induced oxidative stress, neuroinflammation, and improve memory impairment in male adult albino mice. Interestingly, the two nanoparticles in this have inhibited TLR-4/ COX-3/IL-IB to reduce oxidative stress to rescue adult mice brain against Alloxan. This study for the first time evaluated the potential of two Nanoparticles i-e: copper sulphate and silver nitrate extracted from *Skimmia lareoula* leaves. Alloxan induce Alzheimer pathology in adult albino mice interestingly, the two Nanoparticles significantly restored memory as assessed through Y-Maze and MW Maze (MWM) tests. Similarly, some other studies reported the potential of nanoparticles can inhibit the neuropathology of AD in rats/mice model. Here we have reported that Alloxan caused oxidative stress leading to Neuroinflammation, neurodegeneration, synaptic toxicity in mice, it is accompanied by the memory impairment in mice. 0ur this study is of clinical importance as these two Nanoparticles are very important because we have analyzed its neurotherapeutic efficacy induced by Alloxan in mice. There are several studies claiming that Alloxan induces AD neuropathology and memory impairment. The medicinal plant that is *Skimmia lareoula* leaves have been found to exert the neuroprotection in animal model of Neurodegenerative diseases. In short this study reveals that the two Nanoparticles i-e: copper sulphate and silver nitrate extracted from *Skimmia lareoula* leaves are very potent in reducing the AD neuropathology of Alloxan induced in Adult Albino mice. These nanoparticles ameliorated Hyperglycemic and its related complications including AD neuropathology in mice brain. Our study introduce two new nanoparticles with unique anti-diabetic activity, which can be used as a potential dietary supplement or functional food. A more in depth and detail work is warranted to explore the hidden capabilities of these Nanoparticles i-e: copper sulphate and silver nitrate extracted from *Skimmia lareoula* leaves.

V. Conclusion

In summary we can conclude that these two nanoparticles extracted from *Skimmia* leaves are natural, potent and safe therapeutic agent to treat neurodegenerative diseases especially against DB. A more in depth and mechanistic studies are warranted to know in detail the therapeutic efficacy of these two nanoparticles *in vivo* activities.

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