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Ameliorative effect of Mg-*Salvia Officinalis* Nanoparticles against Aluminum Chloride induced oxidative stress in rat model of Alzheimer's Disease

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ABSTRACT

Alzheimer's disease (AD) which is usually referred to as Alzheimer's is one of the popular reasons for dementia. Mg-*Salvia Officinalis* NPs has been known as an interesting compound with antioxidant, anti-inflammatory and cognitive properties. The possible therapeutic effect of *Mg-Salvia Officinalis NPs* was evaluated in Aluminum Chloride ($ALCL_3$) induced AD. AD was induced by intraperitoneal injection of $ALCL_3$ at a dose (100 mg/kg b. wt.) for 2 weeks. Fifty male rats were equally divided into 5 groups. Group I (Normal control): Rats received no drugs, Group II ($ALCL_3$- induced AD): Rats injected with $ALCL_3$ (100mg/kg b. wt./I. P) for two weeks, Group III (*Mg-Salvia Officinalis NPs* treated): After 14 days of $ALCL_3$ injection, rats treated with *Mg-Salvia Officinalis NPs* (5mg/kg b. wt./day, orally) for four weeks. Group IV (Donepezil treated): Rats injected with $ALCL_3$ as group II and treated with Donepezil (1mg/kg b. wt./day, orally) for four weeks. Group V (*Mg-Salvia Officinalis NPs* and Donepezil treated): Rats injected with $ALCL_3$ and treated daily with *Mg-Salvia Officinalis NPs* and Donepezil for four weeks. The results revealed that $ALCL_3$-induced AD rats causing significant alterations in Tau protein and acetylcholine levels in brain tissue in addition to marked elevation of serum ALT, AST activities, urea, creatinine concentrations and oxidative stress biomarkers. Treatment with *Mg-Salvia Officinalis NPs* to AD rats caused marked improvement effect in all previous parameters. Conclusively, *Salvia Officinalis NPs* treatment ameliorates oxidative stress induced by $ALCL_3$ in rat's model of AD and enhances antioxidant defense system and prevent the lipid peroxidation.

1. INTRODUCTION

Alzheimer's is the number one cause for dementia and is considered a permanent neurodegenerative syndrome defined by an advanced injury of memory and deficiency of cognitive capacities. Alzheimer's became a global cognitive decline that involves memory, perception, and reasoning which eventually affects the daily activities of human beings (Tanzi and Bertram, 2005).

Alzheimer's is multi-factorial, including the lack of acetylcholine which acts as an important neurotransmitter in brain cells, and destruction of cholinergic capacity in CNS extracellular accumulation of amyloid-beta peptides (Aβ), and intracellular neurofibrillary tangles (Bachurin et al., 2017). There are other mechanisms linked with AD include oxidative stress and inflammation (Zhang et al., 2013).

Aluminum can simply penetrate the blood-brain barrier (BBB) because of its high similarity to the receptors of transferrin in the brain(Liaquat et al., 2019). Animals exposed to Aluminum have suffered from the formation of neurofibrillary tangles, cholinergic neuronal terminal loss in the hippocampus and cortex, aggregation of amyloid protein (Aβ), development of oxidative stress, and neuronal apoptosis in the hippocampus which is a site for memory formation and synaptic plasticity occurs during learning (Kumar et al., 2019).

Until now, we have no cure for Alzheimer's and all we have are some drugs that can only reduce the symptoms for a short time(Tariot and Federoff, 2003). Donepezil is known as an inhibitor for the acetylcholinesterase enzyme, which decreases the hydrolysis of acetylcholine to elevate the endogenous level of acetylcholine in the brain and to boost cholinergic neurotransmission (Abozaid et al., 2017b). This drug increases the concentration of acetylcholine in the brain (ACh) (Rogers et al., 2000)and has a protecting effect on the neuroinflammation that occurred in the brains of Alzheimer's patients(Herrmann et al., 2011).

The Lamiaceae family, is a big plant family, consists of group of genus from which *Salvia Officinalis* genus(sage) that includes over 900 species spread throughout the world.The name of the plant came from the Latin word "*salvare*" which means "To heal" and it's commonly used in medicinal studies and researches (Lopresti, 2017). *Salvia Officinalis* L. has a lot of polyphenolic compounds such as Caffeic acid, rosmarinic acid and because of this, it's known to have antioxidant properties. (Lu and Foo, 2002) and studies have shown that phenolic components increase neuronal development and protection (Sul et al., 2009).Accordingly, this work intended to study the possible effect of *Mg-Salvia Officinalis* NPs on the improvement of protein tau, hepato-renal function and antioxidants

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alterations in $AlCl_3$-induced Alzheimer's Disease model in rats.

2. MATERIAL AND METHODS

2.1 Chemicals and Drugs:

Aluminum chloride was obtained from (Sigma-Aldrich Co., USA), it came as a bottle containing a powder of half kilo with molecular weight 133.34 g. mol. Aluminum chloride was dissolved in distilled water, freshly prepared and administered I/P and daily at a dose level of 100 mg/kg. body weight for group II for 2 weeks (Zhao et al., 2020).

Donepezil (ARICEPT®) : chemical drug of Pfizer Canada Inc., it came as film coated tablets each one contains 10mg Donepezilhydrochloride which dissolved in distilled water, freshly prepared and administered orally and daily at a dose level of 1 mg/kg. body weight for group IV and V for 4 weeks (Chiroma et al., 2019).

2.2 Salvia Officinalis:

Urine samples were collected by using urethral catheterization (Kelly, 1984) and analysis was made by using commercial urine strips (COMBI-9 strips Produced by Pasteur Lab, Egypt).

2.2.1-Preparation of extract of Salvia Officinalis and its nanoparticles:

Preparation of extract of *Salvia Officinalis* and Synthesis of Mg-*Salvia Officinalis NPs* according to methods of Essien et al. (2020). Diseased rats were treated daily with Mg-*Salvia Officinalis NPs* at a dose of (5mg/kg b. wt.) orally for 4 weeks according to LD50.

2.2.2-Induction of Alzheimer's disease:

Alzheimer's disease was induced by intraperitoneal injection of $ALCL_3$ at a dose of (100mg/kg b. wt.) for a duration of 2 weeks (Zhao et al. 2020). The LD50 of the prepared Mg-*Salvia Officinalis NPs* concentration was found to be 5 mg/kg body weight for the oral treatment according to Bab et al. (1982).

2.3 Experimental Animals:

Fifty male Wister albino rats of 3 months old age and average body weight between 250- 350g. The rats were obtained from the Laboratory Animals Research Center, Faculty of Veterinary Medicine, Moshtohor, Benha University. Animals were housed in metal cages under good environmental and nutritional conditions during the experiment. Rats were left for a duration of 2 weeks for acclimatization before the start of experiment.

2.4 Design of the experimental work:

The rats were randomly divided into five groups of ten rats each, placed in separated cages, and classified as following:

Group I (Normal control): Rats received no drugs.

Group II ($ALCL_3$- induced AD): Rats injected with $ALCL_3$ (100mg/kg b. wt./I. P) for two weeks.

Group III (AD + Mg-*Salvia Officinalis NPs*treated): After 14 days of $ALCL_3$ injection, rats treated with Mg-*Salvia Officinalis NPs* (5mg/kg b. wt./day, orally) for four weeks.

Group IV (AD + Donepezil treated): Rats injected with $ALCL_3$ as group II and treated with Donepezil (1mg/kg b. wt./day, orally) for four weeks.

Group V (AD + Mg-*Salvia Officinalis NPs* and Donepezil treated): Rats injected with $ALCL_3$ and treated daily with Mg-*Salvia Officinalis* NPs and Donepezil for four weeks.

2.5 Sampling:

Blood Samples: Blood samples were collected after overnight fasting from retro-orbital plexus of rats at the end of experiment (after 45 days). Blood samples were collected in dry, clean plain test tubes and incubated for 1/2 h at room temperature to allow clotting for serum separation. Clear sera were separated by centrifugation at 3500 r.p.m. for 15 min, then serum collected in Eppendorf's tubes using automatic micropipettes and kept in a deep freeze at _20℃ until used for estimation of biochemical parameters.

Brain tissue homogenate: Briefly, brain tissues were cut and minced into small pieces, homogenized with a glass homogenizer in 9 volumes of ice cold 0.05 mM potassium phosphate buffer (pH 7.4) to make 10 % homogenates. The homogenates were centrifuged at 6000 r.p.m for 15 minutes at 4°C then the resultant supernatant was used for estimation of ACH level and Tau protein concentration.

2.6 Analysis of the biochemical parameters:

Serum ALT and AST activities, Urea, and Creatinine concentrations were determined according to the methods described by (Reitman and Frankel, 1957; Mariami and Yoshikawa, 1979; Brzezinski, 1987 and Skov, 1970), respectively. However, serum MDA, SOD and GSH were determined using the methods of (Yoshioka et al., 1979 and Ellman et al., 1961), respectively. Additionally, brain tissue Tau protein concentration was evaluated using Sandwich ELISA technique (Herrmann et al., 1999), while Acetylcholine was measured and presented as µmol/g of tissue using the method of Liaquat et al. (2018).

2.7 Statistical analysis:

We used ANOVA which is a one-way analysis of variance to calculate the differences in means of variables between the groups. The obtained results were displayed as the mean±SE and were examined by a software tool named Statistical Package for Social Science (SPSS) V20, at $P<$ 0.05 where this probability is considered as significant.

3. RESULTS

3.1 Characterization of Mg-Salvia Officinalis NPs:

Nanoparticles of Mg-SONPs were characterized by using Fourier transform infrared (FT-IR) spectroscopy, transmission electron microscopy (TEM), and dynamic light scattering (DLS), according to Abozaid et al. (2017a).

3.2- Determination of the biochemical parameters

Data presented in (Tables 1) revealed that serum ALT and AST activities, urea and creatinine concentrations were significantly increased in AD induced rats when compared with a control normal group. Administration of Mg-*Salvia Officinalis* NPs and/or Donepezil to Diseased rats exhibited a significant decrease in all previous serum parameters as compared with AD non treated group.

The obtained results in (Tables 2) exhibit a significant increase in serum MDA concentration, while serum SOD activity and GSH level were significantly decreased in AD induced rats when compared with a control normal group. However, treatment with Mg-SONPs and/or Donepezil exhibited a significant decrease in serum MDA concentration with marked increase in serum SOD activity and GSH contents when compared with a diseased non treated group.

Table 1 Effect of Mg-SONPs and/or Donepezil treatment on serum ALT, AST activities, urea and creatinine concentrations in Aluminum Chloride induced - Alzheimer's Disease.

Animal groups	Parameters			
	ALT (U/L)	AST (U/L)	Urea (mg/dl)	Creatinine (mg/dl)
GI: Control normal	13.14 ± 0.74f	13.16 ± 0.56f	29.63 ± 1.71f	0.70 ± 0.02f
GII: $ALCL_3$	56.45 ± 2.04a	52.93 ± 1.92a	72.33 ± 2.62a	1.64 ± 0.06a
GIII: $ALCL_3$ + Mg-*Salvia Officinalis* NPs	42.34 ± 1.54c	39.69 ± 1.44c	54.25 ± 1.97c	1.23 ± 0.04c
GIV: $ALCL_3$ + Donepezil	33.87 ± 1.23d	31.76 ± 1.15d	43.40 ± 1.58d	0.98 ± 0.03d
GV: $ALCL_3$ + Mg-Mg-Salvia Officinalis NPs+ Donepezil	25.40 ± 0.92e	23.81 ± 0.86e	34.72 ± 1.26e	0.79 ± 0.03e

Data are presented as Mean ± S.E. S.E = Standard error. Mean values with different superscript letters in the same column are significantly different at P<0.05.

Table 2 Effect of Mg-SONPs and/or Donepezil treatment on serum SOD activity, MDA and GSH concentrations in Aluminum Chloride induced - Alzheimer's Disease.

Animal groups	Parameters		
	MDA (µmol/m1)	SOD (U/ml)	GSH (mg/dl)
GI: Control normal	8.55 ± 0.32f	62.81 ± 2.87f	5.31 ± 0.19f
GII: $ALCL_3$	50.86 ± 1.84a	16.47 ± 0.60a	1.44 ± 0.05a
GIII: $ALCL_3$ + Mg-*Salvia Officinalis* NPs	38.15 ± 1.38c	20.59 ± 0.75c	1.79 ± 0.07c
GIV: $ALCL_3$ + Donepezil	30.52 ± 1.11d	24.71 ± 0.90d	2.15 ± 0.08d
GV: $ALCL_3$ + *Mg - Salvia Officinalis NPs*+ Donepezil	24.42 ± 0.88e	29.65 ± 1.08e	2.58 ± 0.10e

Data are presented as Mean ± S.E. S.E = Standard error. Mean values with different superscript letters in the same column are significantly different at P < 0.05.

The presented data in (Tables 3) indicated that rats injected with $ALCL_3$, showed a significant increase in brain tissue Tau protein concentration, while brain acetylcholine (ACH) concentration was significantly decreased when compared with a control group. Diseased rats, treated with Mg-SONPs and/or Donepezil exhibited a significant decrease in brain Tau protein concentration with marked increase in brain acetylcholine (ACH) concentration when compared with AD induced non treated group.

4. DISCUSSION

This study presents a new perspective on the beneficial effects of *salvia officinalis* for Alzheimer's treatment in comparison with Aricept® (Donepezil) as cholinesterase inhibitors drug.

A significant increase in serum ALT and AST activities, urea and creatinine concentrations were observed in AD induced rats. These results are agreed with the recorded data of Al Dera (2016) who reported $ALCL_3$ administration caused increase levels of urea and Creatinine significantly by158.4. % and 258.5. The marked increase in serum ALT and AST activities and renal function tests in AD rats may be due to that $ALCL_3$ exposure induce hepatotoxicity with the increased level of oxidative stress in the cells and tissues refers to enhance the creation of ROS and/or exhaustion of antioxidant defense system (Abozaid et al., 2017c), leading to destruction of hepatic cells and leakage of the liver enzyme into the blood circulation (Cheraghi and Roshanaei, 2019). Renal toxicity because that the kidneys are responsible for $ALCL_3$ excretion, and it causes marked degeneration of tubules due to its critical accumulation in the kidneys, eventually resulting in renal failure as reported by Belaïd-Nouira et al. (2013).

Table 3 Effect of Mg-SONPs and/or Donepezil treatment on brain Tau protein and Acetylcholine concentrations in Aluminum Chloride induced-Alzheimer's Disease.

Animal groups	Parameters	
	Tau protein (pg/mg. Tissue)	Acetylcholine (pg/mg. Tissue)
GI: Control normal	1.59 ± 0.15f	133.22 ± 5.88f
GII: $ALCL_3$	13.44 ± 0.83a	57.76 ± 2.75a
GIII: $ALCL_3$ + Mg-*Salvia Officinalis* NPs	5.06 ± 0.10c	102.02 ± 2.31c
GIV: $ALCL_3$ + Donepezil	3.24 ± 0.30d	108.70 ± 2.16d
GV: $ALCL_3$ + Mg-Salvia Officinalis NPs+ Donepezil	2.48 ± 0.18e	121.12 ± 2.80e

Data are presented as Mean ± S.E. S.E = Standard error. Mean values with different superscript letters in the same column are significantly different at P < 0.05.

Administration of Mg-*Salvia Officinalis* NPs and/or Donepezil to Diseased rats exhibited a significant decrease in all previous serum parameters (ALT, AST, urea and creatinine) as compared with AD group, subsequently Mg-*Salvia Officinalis* NPs decreases the susceptibility of rat hepatocytes to oxidative stress (Liaquat et al., 2018). Aqueous extract protects hepatocytes against di-methoxy naphthoquinone and H2O2 induced DNA damage through elevation of glutathione peroxidase activity (Kozics et al., 2013). The carnosol and rosmarinic acid are the main antioxidant constituents of *S. Officinalis* (Cuvelier, 2002).

The existing results showed a significant increase in serum MDA concentration, while serum SOD activity and GSH level were significantly decreased in AD induced rats when compared with a normal group. These are nearly similar with those reported by Benyettouet al. (2017) who found that MDA was significantly increased in the $ALCL_3$-treated rats, while reduced GSH and SOD were significantly compromised in the model samples. This result may be due to that $ALCL_3$ causes neuronal damage through extensive oxidative damage by increasing levels of reactive oxygen species and reduction in the mitochondrial membrane potential which further activates the mitochondrial apoptosis pathways (Guner et al., 2009).

Administration of Mg-*Salvia Officinalis* NPs and/or Donepezil to Diseased rats significantly adverse the previous results because Mg-Salvia Officinalis NPs has antioxidant effects through its phenolic compounds as reported by Ren et al. (2003). Also, Rosmarinic acid is the main phenolic compound in Mg-*Salvia Officinalis* NPs and its effects was attributed to the compound's antioxidant properties acting as scavenger of ROS (Osman and Abd El–Azime, 2013).

Rats injected with $ALCL_3$, showed a significant increase in Tau protein concentration, neurofibrillary tangles are aggregates made up of hyper-phosphorylated microtubule-associated protein tau in brain tissue homogenates. These results are nearly similar with those of Zaher et al. (2019) who found that $ALCL_3$ caused significantly increase in mean value of tau protein in the brain tissue by 11.48±2.01 ng/mg when compared with the normal value. Aluminum Chloride inhibits the PI3K/AKT pathway and the inhibition of the PI3K/AKT pathway induces GSK-3ß activity that increases tau phosphorylation through PI3K/AKT/ GSK-3ß pathway (Yu and Koh, 2017). PI3K-Akt Pathway is an intracellular signal transduction pathway that promotes metabolism, proliferation, cell growth and angiogenesis in response to extracellular signals. This is mediated through serine and/or threonine phosphorylation of a range of downstream substrates. Administration of Mg-*Salvia Officinalis* NPs enhance Tau protein level by decrease hyperphosphorylation through inhibition of PI3K/AKT/ GSK-3ß pathway.

On the other hand, the obtained results showed that injection of $ALCL_3$ to normal rats exhibited a significant decrease in brain acetylcholine concentration when compared with a control group, that agrees with Zaher et al. (2019) who showed that rats obtained $ALCL_3$ developed a significant damage of cholinergic terminals. These results may be due to that $AlCl_3$ caused increase of AChE activity, which contributes to decreased and loss of cholinergic terminals (Thomas et al., 2004).

Diseased rats, treated with Mg-SONPs and/or Donepezil (Aricept®) exhibited a significant decrease in brain Tau protein level with marked increase in brain acetylcholine (ACH) concentration as compared with AD rats, that due to that the components of Salvia specially rosmarinic acid and come phenolic compounds can cross the blood– brain barrier and increase cholinergic transmission via cholinesterase inhibition so improves mental functions including memory (Wake et al., 2000).

5. CONCLUSION

From the existing study it could be concluded that administration of Mg-*Salvia Officinalis* NPs and/or Donepezil (Aricept®) cholinesterase inhibitors drug to $ALCL_3$-induced Alzheimer's disease provided an effective treatment against oxidative stress, abnormality of brain tissue, Tau protein and acetylcholine neurotransmitter; since these natural compounds are able to improve the related neurochemical changes of brain and may be provide a unique role for treating Alzheimer's disease. So, we recommend administration of Mg-*Salvia Officinalis* NPs for treatment of Alzheimer's.

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