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The possible ameliorating effect of date (*Phoenix Dactelifera* L) against brain toxicity induced by $AlCl_3$ in male rats

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Abstract: The study aims to see if date palm fruits (*Phoenix Dactelifera* L) could protect male albino rats from neurological abnormalities and oxidative stress caused by $ALCL_3$ exposure. In rats given $AlCl_3$ at a dose of 100 mg/kg, levels of brain neurotransmitters, DA , 5-HT, AChE, Na-K ATPase activity, and ATP value are significantly decline, whereas the level of NE was elevated. Furthermore, $AlCl_3$ intoxicated rats had a large rise in the end product of the brain lipid peroxidation, MDA and in NO, H_2O_2 levels, and the activity of xanthine oxidase (XO), as well as a pronounced drop in the antioxidant parameters, TAC, SOD, and GSH. As well as the apoptotic marker, Caspas3, Caspase9, Bax, and Bax/Bcl 2 showed a significant increase, while a decrease in anti-apoptotic marker ,Bcl2 was recorded. As well as, the serum levels of Na, and Ca were significantly decreased, while the level of K was elevated. Serum TC, TG, LDLC, and VLDL levels, as well as the TC/HDL and LDL/HDL ratios, all increased significantly, while serum HDLC levels declined dramatically. Supplementation with date palm fruit (1.6 g/kg bw) demonstrated a substantial neuroprotective effect against the metabolic abnormalities and oxidative stress generated by $AlCl_3$ exposure. As a result, it can be inferred that date fruit consumption may be beneficial in the treatment of neurological illnesses and oxidative stress. At the same time $ALCL_3$ exposure was linked to apoptotic and anti-apoptotic indicators, indicating free radical scavenging and significant antioxidant action. as well as the apoptotic and anti-apoptotic markers associated with $AlCl_3$ exposure revealing its powerful antioxidant and free radical scavenging properties.

keywords: Date palm fruits (*Phoenix Dactelifera* L), Aluminum chloride, Neurotransmitters, Oxidative stress, Antioxidants, Apoptotic and anti-apoptotic markers.

1. I ntroduction

1.1 General background

Human aluminum exposure comes primarily from diet, it is a ubiquitous element found in every food product. Food interaction with Al during preparation and storage can raise Al levels, with acidity, prolonged contact, high salt content, and uncoated Al cookware amplifying the effect. Purification is also achieved by adding it to drinking water. (74). It is used in ceramics; and in some products as antacids, food additives, and antiperspirants.The Al complex is neurotoxic, causing cytoskeletal abnormalities and cell death (10). The Al complex causes apoptosis in N2a neuroblastoma cells by upregulating the transcription factor p53, increasing the expression of the pro-apoptotic protein Bax, and decreasing the expression of the anti-apoptotic protein Bcl-2 through the intrinsic pathway (Johnson et al. 2005).

Alzheimer's disease (AD) is a chronic neurological illness that causes memory loss, as well as mental and abnormalities in seniors (36).

Aluminum's catalytic activity appears to be the production of free radicals. Furthermore, the accumulation of beta-amyloid protein in Alzheimer's patients' brains causes the formation of free radicals (102). Aluminum (Al) can induce oxidative damage through multiple mechanisms although it is a relatively low redox element. It has the ability to connect to negatively charged phospholipids in the brain, particularly polyunsaturated fatty acids, making it vulnerable to reactive oxygen species (ROS) such as H_2O_2, O_2, and OH (94). Because of the high oxygen consumption rate (20%), the abundance of polyunsaturated fatty acids in cell membranes, the high concentration of iron (Fe), and low anti-oxidative enzyme activity, brain tissues are extremely sensitive to oxidative damage (Youdim 2002). Natural antioxidants present in vegetables and fruits have been demonstrated to lower the risk of age-related neurological diseases like dementia and macular degeneration (99).

Phytochemicals derived from herbs and plants have been demonstrated to aid in the treatment of a variety of ailments. Various medicinal herbs and plants were advised by the Prophet Mohammed (peace be upon him) for the treatment of various maladies (60).

Phoenix dactylifera (date palm) fruits , the most important medicinal and nutritional plants was recommended by Prophet Mohammed to protect against various agents-induced body organs toxicity. Prophet Muhammad say according to a "Hadith." (7) who eats seven date fruits every morning would not be afflicted by poison or sorcery on the day he eats them,"

Carotenoids, polyphenols (e.g., phenolic acids, isoflavones, lignans, and flavonoids), tannins, and sterols are abundant in date fruit (Martín-Sánchez *et al*., 2014). P. dactylifera has multiple medicinal properties, including antioxidant, anticancer, antidiabetic, anti-hyperlipidemic, and antibacterial properties, as well as the ability to protect various cells from various environmental hazardous substances. P. dactylifera's phenolics, flavonoids, and small molecules like vitamin C and vitamin E have antioxidant properties (Naskar *et al*., 2010). These antioxidant constituents of P. dactylifera may either directly react with ROS to destroy them by accepting or donating electrons to eliminate the unpaired state of ROS, or they may indirectly reduce cellular free radicals by increasing the activities and expressions of antioxidant enzymes, resulting in the prevention of LPO, DNA damage, and protein modification (Vayalil, 2012).

Up-regulation of anti-apoptotic molecules like Bcl-2-associated X protein (Bax) or down-regulation of apoptotic molecules like B cell lymphoma-2 have both been employed as strong anticancer treatments in *Phoenix Dactelifera L* plants (Bcl-2) (Rodríguez *et al*., 2013)**.** Environmental neurotoxicity and environmental neurotoxicants such as aluminium are linked to both peripheral and central neurotoxicity. The methanolic extract of P. dactylifera protected against oxidative stress and brain damage in a dose-dependent manner (Zangiabadi *et al*., 2011).

In light of the foregoing, the current study was carried out in male albino rats to investigate the beneficial effects of date palm fruits, , as well as the possible protective effect of date fruits as a natural antioxidant against their adverse effects.

2. Materials and methods

2.1 Chemicals

Aluminium chloride was obtained from Sigma Chemical Company (St. Louis, USA). Fresh date fruits (*Phoenix Dactelifera L*) were purchased from local market (Mansoura, Egypt). The tested doses of aluminum chloride ($AlCl_3$; 100mg/kg bw) also, the date palm fruits (1.6 g/kg bw) was selected bases on the earlier studies (60). All of the kits used in the experiment were purchased from Egypt's Biodiagnostic Company. All of the chemicals and reagents utilised were of analytical grade.

Experimental animals

Forty-eight adult male Wister albino rats' weight 100-120g, were obtained from the holding company for biological product & vaccines (VACSERA), Cairo, Egypt. Rats were received human care, good ventilation; adequate stable diet and water were allowed *ad libitum*. Throughout the experimental period, rats were maintained on normal light/dark cycle and accommodated to the laboratory conditions for two weeks before being experimented.

Experimental design

After the acclimation period, rats were classified into four groups comprising of twelve rats in each. Group 1: the untreated control rats group (C). Group 2: date palm fruits (Phoenix dactelifera L) treated group; rats given date mixed with diet at dose of 1.6g/kg bw. Group 3: (AlCl3): rats were given AlCl3 mixed with the diet at a dose of 100mg/kg bw. Group 4: (date +AlCl3) rats were given date and AlCl3 at the same mentioned dose. Rats were given their respective doses daily for three months.

Blood collection and tissue homogenate

Blood samples were taken from the retro-orbital venous plexus of overnight fasting rats after three months (Schermer 1967), centrifuged at 860 Xg for 20 minutes in clean tubes. For biochemical investigation, the isolated sera were kept at -20^{o}C. Each group's rats were decapitated, and brain specimens were carefully removed, weighed, and homogenised using phosphate buffer solution (10 percent w/v) for biochemical analysis.

Biochemical analysis

Using the equation of Pagel and Youdim (2002), neurotransmitters (DA, 5-HT and NE) levels were estimated in the brain tissues. The acetyl choline esterase (AChE) activity in the brain tissues was determined according to the method of Ellman *et al*., (1961). Na,K ATPase activity was assayed according to Taussky and Shorr (1953) and ATP content was calculated according to (57). Malondialdehyde was measured according to the method of Ohkawa *et al*., (1982). NO production was measured according the method of Montgomery and Dymock (1961), H2O2 level was assayed as described by Aebi (1984). According to the method described by Litwack *et al*., (1953) brain Xanthine oxidase was calculated. According to (51), SOD activity according to Nishikimi and Yogi (1972) and GSH content was assayed according to (15).

Using a colorimetric caspase-3 assay kit and Bcl-2 protein and Bax protein levels were measured in brain tissue by an ELISA kit.

Serum concentration of sodium Na and potassium K were evaluated according Henry (1974), while Calcium Ca according to Gindler (1972)

Total cholesterol (TC) level was estimated according Young (1995). Triglycerides (TG) according to Fossati and Prencipe (1982). HDLC , LDLC and VLDLC levels calculated as described by Friedewald *et al*., (1972).

Statistical analysis:

By One Way ANOVA, the obtained results were evaluated, as described by Snedecor and Cochran (1982).

3. Results

As observed in the data below, and as recorded in Tables 1, 2, 3 and 4 the administration of date fruits alone for three months results in a non-significant change in the parameters estimated when compared to the control rats' group.

The obtained results in Table 1 revealed that the administration of aluminum chloride showed significant decline in the DA, 5-HT, AChE, Na-K ATPase and ATP but a significant elevation, NE in the brain as compared with control rats group. While, administration of the AlCl3 intoxicated rats with date resulted in a significant amelioration in these parameters when compared to the intoxicated rat groups without date treatment.

As seen in Table 2, a significant increase in MDA and H2O2 levels, as well as, an increase in NO level and XO activity were observed in AlCl3 treated rats' group. On the other hands a significant decrease in TAC, SOD and GSH in the AlCl3 intoxicated rats' group. Meanwhile, an improvement in these parameters is recorded when the rats co-administrated with date.

Also, the obtained results in Table 3 declared a significant decrease in serum Na, and Ca levels, but an increase in K level was recorded in the AlCl3 treated rats. As well as a significant increase in Apoptosis markers, Caspase 3, Caspase 9, Bax levels and Bax/Bcl2 ratio, while a decrease in the anti-apoptotic marker Bcl2 in aluminum chloride rat group compared with that of the control rat group. Meanwhile the administration with date caused significant improvement in these parameters in comparison with intoxicated rats group.

However, as shown in Table 4, a marked increase in serum TC, TG, LDL-C, VLDL-C levels as well as TC/HDL-C and LDL-C/HDL-C ratio while, a significant decrease in serum

HDLC level was observed in aluminum chloride rats' group. But when the AlCl3 intoxicated rats received date an improvement in these parameters were recorded.

Table (1): Brain neurotransmitters (DA, 5-HT and NE), enzymes activity (AChE and Na-K ATPase) and ATP value in different rat groups**.**

Animal groupsParameters	Control	Date	$AlCl_3$	Date+ $AlCl_3$
Dopamine (DA)(µg/g)	0. 39±0.03	0.41±0.02	0.11^{a}±0.01	$0.29^{a,b}$±0.02
Serotonin (5-HT) (µg/g)	0.30±0.02	0.32±0.01	0.13^{a}±0.02	0.24^{ab}±0.01
Norepinephrine(NE) (µg/g)	0.24±0.03	0.23±0.03	0.64^{a}±0.03	$0.50^{a,b}$±0.04
AChE(µmolSH./mg/min)	0.51±0.08	0.52±0.09	0.24^{a}±0.087	$0.35^{a,b}$±0.066
Na-K ATPase(µmolpi./min/mg)	0.48±0.07	0.51±0.06	0.17^{a}±0.08	$0.26^{a,b}$±0.05
ATP(ng/g)	0.39±0.04	0.40±0.03	0.08^{a}±0.02	0.19^{ab}±0.01

Results are presented as mean ±S.E of six rats. Significant change at ($P < 0.05$)
a: compared to control untreated group , b: compared to $AlCl_3$ group

Table (2): Brain (MDA, H_2O_2 , NO , XO) and (TAC, SOD and GSH) in different rat groups.

Animal groupsParameters	Control	Date	$AlCl_3$	Date+ $AlCl_3$
MDA(nM/g)	0.078±0.008	0.075±0.007	0.248^{a}±0.036	0.164^{ab}±0.013
H2O2(mM/g)	0.069±0.008	0.068±0.02	0.246a±0.02	0.17ab±0.01
NO(µM/g)	0.109±0.007	0.096±0.007	0.587a±0.08	0.303ab±0.04
XO(µM/h/g)	0.165±0.008	0.15±0.009	0.26a±0.018	0.19 ab±0.011
TAC(mM/g)	0.51±0.08	0.54±0.07	0.14a±0.01	0.27ab±0.03
SOD(U/g)	0.255±0.04	0.261±0.04	0.079 a±0.01	0.128 ab±0.01
GSH(mg/g)	0.54±0.03	0.55±0.04	0.18 a±0.03	0.31a,b±0.02

Results are presented as mean ±S.E of six rats. Significant change at ($P < 0.05$)
a: compared to control untreated group , b: compared to AlCl3 group

Table (3): Apoptotic and anti-apoptotic markers and serum electrolytes concentration in different rat groups.

Animal groupsParameters	Control	Date	$AlCl_3$	date+ $AlCl_3$
Caspase 3	0.197±0.02	0.182±0.02	0.345^{a}±0.03	$0.280^{a,b}$±0.03
Caspase 9	0.159±0.09	0.143±0.04	0.307^{a}±0.08	$0.259^{a,b}$±0.07
Bax	0.111±0.015	0.110±0.015	0.414^{a}±0.009	$0.222^{a,b}$±0.019
Bcl2	0.263±0.016	0.264±0.018	$0.156^{a,}$±0.009	$0.19^{a,b}$ 1±0.024
Bax/Bcl2Ratio	0.42±0.05	0.44±0.08	2.70^{a}±0.11	$1.25^{a,b}$±0.17
Na(mEq/L)	102.9±5.7	103.4±5.7	64.5^{a}±4.6	$81.4^{a,b}$±4.4
K(mEq/L)	1.39±0.07	1.38±0.05	2.05^{a}±0.02	$1.9^{a,b}$±0.07
Ca(mg/dl)	3.23±0.24	3.67±0.21	1.83^{a}±0.13	$2.38^{a,b}$±0.12

Results are presented as mean ±S.E of six rats. Significant change at ($P < 0.05$)
a: compared to control untreated group , b: compared to AlCl3 group

Table (4) : Serum lipid profile level in different rat groups**.**

Animal groupsParameters		Control	Date	$AlCl_3$	Date+ $AlCl_3$
mg /dl	**TC**	126.8±4.4	124.7±4.2	222.2^{a}±8.1	$148.8^{a\,b}$±6.7
	TG	131.2±3.3	130.3±3.3	199.2^{a}±9.0	$152.8^{a\,b}$±6.1
	LDLC	59.5±03. 3	52.8^{a}±5.1	159.3^{a}±9.8	$85.3^{a,b}$±3.7
	VLDLC	26.3±0.3	26±0.5	38.9^{a}±0.8	30.5^{b}±0.9
	HDL	45.4±3.5	49.5±4.2	25.5^{a}±2.4	$32.7^{a\,b}$±2.4
	TC/HDL ratio	2.89±0.25	2.61±0.23	9.24^{a}±0.74	$4.74^{a\,b}$±0.59
	LDL/HDLratio	1.36±0.15	1.23±0.19	6.45^{a}±0.53	$2.68^{a\,b}$±0.21

Results are presented as mean ±S.E of six rats. Significant change at ($P < 0.05$)
a: compared to control untreated group , b: compared to AlCl3 group

4- Discussion

Toxic metal exposure is linked to a variety of negative physiological outcomes, including neuropathological alterations. Aluminum is one of the most widely dispersed hazardous metals in the environment, and it is frequently used, making it easier for humans to be exposed to it. According to reports, aluminium forms a compound in the brain, which is likely to produce long-term neurotoxicity, leading to neurodegeneration and synapse loss (17).

Al reaches and accumulates in practically most organ of the human body, but the central nervous system is especially vulnerable to the most harmful consequences and

neurodegenerative disorders (80). Aluminum neurotoxicity is caused by a synergistic effect between aluminium and iron. Aluminum binding to the neuronal membrane accelerates attacks by iron-induced free radicals, and membrane oxidation enhances aluminium coordination over time, increasing the ability of iron ions to produce oxidative damage to neurons (14).

The results of the current investigation revealed that rats exposed to $AlCl_3$ had significantly lower levels of dopamine and serotonin in the brain, but significantly higher levels of noradrenalin. Observations similar to this were also recorded by (53) and Bahalla *et al*., (2010), they revealed region-specific changes in serotonin levels, with the lowest being in the brain, while the decrease in serotonin was only visible after 60 days of Al exposure in the cortex, hippocampus, and cerebellum. This could be owing to the adult rats' sluggish accumulation and efficient clearance of Al. Further, (77) found a decrease in serotonin levels in cortex, septum, striatum and hippocampus of rats intoxicated with Al. Similarly, Al-induced neurochemical alterations in regions having the highest neurofibrillary degeneration in rabbits exposed to Al, according to (13). Serotonin levels were found to be significantly lower. Decreased release of serotonin suggested deactivation of the serotoninergic system. The serotonin reuptake transporter is targeted in depression therapy (SERT). Serotonin is allowed to work in the synapse for a longer duration when SERT is inhibited, which is the desired outcome in depression treatment. (79).

As an alternative, Al has been identified as a cholinotoxin. Long-term Al exposure is thought to have an inhibitory effect on the 5-HT system due to the withdrawal of cholinergic input, resulting in lower levels of 5-HT in various areas. (53).

In toxic modes, serotonin neurons' principal purpose is to facilitate gross motor output. Within the deman of continuing motor outputs, the serotonin system inhibits sensory information processing and coordinates autonomic and neuroendocrine activities. However, in cases of senile dementia, lower levels of 5-HT and its metabolites have been found (40). Neurons in the upper brain stem, particularly those of the serotoninergic group, play a key role in the formation of neurofibrillary tangles in Alzheimer's disease. Increased brain Al levels have been linked to poor visuo-motor coordination, poor long-term memory, and intellectual disability (19), implying a neurochemical imbalance. Regarding, inhibition of dopamine level seen due to Al exposure, it may be explained partly due to the effect of Al on the sensitivity status of 5-HT_{2C} receptors, The reactivity of the 5-HT_{2C} receptor may be a compensatory response for the damage to the central dopaminergic system reflecting on the dopamine level (20). The inhibitory impact of Al on the activity of dopamine-beta-hydroxylase was also attributed to the potential implications of Al in the etiopathogenesis of neurological illnesses (62). Surprisingly, dopaminergic transmission inhibition in the CNS may play a key role in Al-induced neurotoxicity (104). In the current study, the increase in cerebral noradrenalin (NE) seen in rats exposed to Al could be due to the metal's oxidative stress, which accelerates its synthesis pathway, notably the phase that includes the conversion of dopamine to NE via hydroxylation (37), which could also explain the decrease in dopamine levels.

However, the inhibition of dihydropteridine reductase, an enzyme essential for the synthesis of tyrosine, L-dihydroxy phenylalanine, and 5-hydroxytryptophan, by Al has been hypothesised as a probable source of clinical indications of intoxication in the brain. When the enzyme dihydropteridine reductase is blocked, phenylalanine builds up in the brain and basal ganglia, causing seizures, rigidity, chorea, spasms, and muscle hypotonia. Al can also interact with the catalytic core of tyrosine hydroxylase (39). Furthermore, considerable changes in brain catecholamine neurotransmitters (DA, 5-HT, and NE) in rats intoxicated with aluminium chloride could be due to an increased rate of synthesis of O2-, H2O2, which could be linked to neurodegenerative disorders. Monoamine oxidase oxidises (DA, 5-HT, and NE) to create hazardous catecholamine metabolites as catecholamine aldehydes (a precursor of oxidative stress) (21) It could also be because of increased neural activity, which promotes

catechol-O-methyl release, or because enzymes involved in their synthesis, such as dopamine-hydroxylase, DOPA decarboxylase, and tyrosine hydroxylase, are underactive (32). A drop in serotonin, a monoamine neurotransmitter, was also seen. Serotonin converts to melatonin via activating N-acetyltransferase, a rate-limiting enzyme, via a cyclic adenosine monophosphate route (41). Melatonin is a powerful antioxidant, and its levels were found to be high throughout the body, particularly in the mitochondria and cell nucleus (78). The drop in serotonin could be the result of serotonin being converted to melatonin (a potent antioxidant) to battle the oxidative stress generated by AlCl3. Serotonin and melatonin are inversely proportional; when melatonin levels rise, serotonin levels fall. It's also been proposed that withdrawal of cholinergic input has an inhibitory effect on the 5-HT system, resulting in lower levels of 5-HT in different locations after exposure (53). Changes in the activity of biosynthetic and degradative enzymes, as well as the availability of their precursor amino acid tyrosine, could explain the lower levels of dopamine found after exposure (42). Concerning the increase in brain norepinephrine seen in this study, it could be a result of the metal's oxidative stress, which activates its synthesis pathway, notably the step involving the conversion of dopamine to norepinephrine via hydroxylation (George and Siegel 2000).

Slow accumulation of aluminium in the brain and creation of an aluminium complex with a high affinity for binding to this enzyme's active site, resulting in oxidative stress, and, as a result, lowering AChE activity in all parts of the brain, could explain the observed reduction in brain AChE activity. As a result, acetylcholine (ACh) is not digested and builds up in cholinergic sites, causing the nervous system's normal function to be disrupted (65).

The buildup of ACh due to AChE inhibition may promote lymphocyte stimulation, enhanced lymphocyte motility, and cytotoxicity. Because AChE is a membrane-bound enzyme and ACh can be released from its interaction with AChE, resulting in lower AChE activity, the effect on synaptic transmission could be explained by AChE inhibition in the brain (54). Acetylcholine deficiency has a significant influence on short-term memory and learning. Some symptoms of AD might be alleviated by impeding the enzyme for the breakdown of acetylcholine (Namm *et al*., 2020).

The present study showed that brain Na-K ATPase and ATP decreased significantly in intoxicated rat groups. These results were previously reported (63), (59). The activity of the Na+-K+ ATPase is critical for maintaining Na+ and K+ electrochemical gradients across the membrane. As a result, variations in this enzyme's activity could be linked to problems with neural action potential firing (103). This membrane-bound enzyme is especially vulnerable to oxidative stress since phospholipid is required for its action. As a result, the inhibitory mechanism involves either direct protein damage from reactive oxygen species and other lipid peroxidation products, or change of the enzyme's phospholipid microenvironment (24). Inhibition of the Na+-K+ ATPase may result in partial membrane depolarization, enabling enough Ca2+ to enter neurons, causing toxic effects similar to excitotoxicity and contributing to physiological and pathological abnormalities (47).

In the mammalian brain, ATP depletion induces rapid membrane depolarization, yet the enzyme is plentiful in the brain and muscles (86). Fluoride toxicity appears to begin with enzyme inhibition and ATP depletion, followed by the buildup of reactive oxygen species and impaired antioxidant defences (3).

Aluminum-induced mitochondrial dysfunctions, such as respiratory chain dysfunction, also resulted in lower adenosine triphosphate generation and higher oxygen free radical production (83). Furthermore, because glycolysis is a major source of ATP in mammalian cells, inhibiting it at the enolase stage could disrupt essential cellular activities such protein phosphorylation and ion transport through the membrane, disrupt membrane potential, and disrupt membrane cytoskeleton connections. Surely, inactivation of Na+–K+ and Ca2+ pumps due to ATP depletion can explain Na+ and Ca2+ buildup in rat cells, as well as Ca2+-dependent K+ loss and cell injury (3).

Furthermore, an imbalance in pro-oxidant generation and neutralisation causes oxidative stress. Various diseases throw off this balance by increasing the production of free radicals in comparison to the antioxidants present. Increased levels of MDA, H2O2, NO, and XO in the brains of AlCl3-exposed rats indicate oxidative stress and free radical generation, as well as reduced antioxidant levels of TAC, SOD, and GSH, limiting their ability to scavenge high amounts of ROS generated in the brain. Overproduction of reactive oxygen species (ROS) is known to cause macromolecule oxidation, resulting in free radical attacks on membrane phospholipids, resulting in membrane damage, mitochondrial membrane depolarization, and death by lipid peroxidation induction (11). Increased free radicals and oxidative stress would assault the membrane, resulting in polypeptide changes as well as a decrease in Ca2+ uptake, transport, and permeability.

As well as, revealed by (1), membrane lipid peroxidation can damage the membrane's anatomical integrity and lower its fluidity, inhibiting a variety of membrane-bound enzymes, including the Na+-K+ ATPase.

Under normal condition xanthine oxidoreductase remains in dehydrogenase form and consumes NAD+ and there is hypo production of superoxide anions. Under NAD+ deficient condition, there is subsequent membrane Ca2+ gradient loss due to ATP depletion leading to increase calcium levels that is cause selective proteolysis by activation of Ca2+ dependent proteases of the dehydrogenases to change over it into xanthine oxidase which acts on both xanthine and hypoxanthine and consumes molecular oxygen to produce superoxide ions (Ates *et al*., 2007). The effects of elevated nitric oxide (NO) are mediated by hazardous metabolites such as peroxynitrite (OONO-), which impede the activities of mitochondria's oxidative phosphorylation complexes, producing metabolic abnormalities and ATP depletion, resulting in damaging conditions for brain tissues (45). The synthesis of NO may be encouraged by uncontrolled histamine production in the body, resulting in increased permeability of the blood-brain barrier (61).

A shortage in serum Zn and Cu could be linked to a decrease in SOD activity in the brains of AlCl3-exposed rats, resulting in mitochondrial impairments and dysfunction, explaining the large alterations in total SOD (Radjicic *et al.,* 1999). Also, disturbances in serum electrolytes have been observed as a result of Al ion binding to various brain cells, including astrocytes, neural cells, and synaptosomes, but it interacts more with the former, causing inhibition of membrane bound Na+, K+, Ca2+ ATPase activity, altering the electrolytes level and possibly leading to death (Rao 1992). Additionally, because of the disruption of Ca2+ metabolism in neural cells, aluminium accumulation in the brain can inhibit the development of long-term potentiation by influencing multiple signalling pathways, including the Ca2+ calmodulin kinase II - dependent protein kinase signal transduction system and the protein kinase C (PKC) signalling pathway, causing rats' memory to deteriorate (Wang *et al.*, 2010).

Increased oxidative stress in rats following Al treatment was shown to be accompanied by a significant inhibition of the antioxidant defence system in the current investigation. Increased scavenging of reactive substances produced as a result of toxin metabolism, which could be associated with inflammatory reactions and possibly decreased antioxidant production, could explain the observed decrease in GSH level and activities of antioxidant enzymes such as TAC, SOD, and GST in the brains of AlCl3 intoxicated rats (Lii *et al.,* 1998).

This led to loss of balance between ROS production and antioxidant defense with subsequent production of oxidative stress and tissue damage. It has been reported that, GSH is a highly effective cellular non enzymatic antioxidants in the body. It contributes to two main defense processes in the cell: i- it offers a reducing agent for oxidant molecules (such as, H_2O_2 and hydroperoxides) as it donates hydrogen and converted to the oxidized form (GSSG), and this process is catalyzed mainly by GPx enzyme; ii- GSH is used in detoxification of xenobiotics since it used as a substrate for conjugation reactions in phase II metabolism which are catalyzed by GST enzyme.

Consumption and consequently depletion of cellular GSH under the conditions of oxidative stress is therefore expected. So, for restoration of the normal concentration of GSH inside the cell, an important antioxidant enzyme called glutathione reductase (GR) is necessary for this biochemical process since it catalyzes the reduction of GSSG (Masella *et al.*, 2005). It is clearly appeared that, GSH and its related enzymes (GPx, GR and GST) represent a large part of antioxidant defense system and can catalyze the reduction of oxidized reactive molecules into non-toxic products and then can end the chain reaction of LPO process (Chao *et al*., 2013). The decline in activities of the GSH-related enzymes together with the level of GSH itself is therefore used as a marker for oxidative stress and cytotoxicity. Beside GSH and its related enzymes, the antioxidant enzyme SOD was found to exert an effective role in the defense mechanism against reactive molecules-induced cell injury. They represent the first line used for cytoprotection against ROS. Considering SOD, it plays a major role in catalyzing the dismutation of superoxide radicals ($O2^{\cdot-}$) into H2O2 (Valko *et al*., 2006).

The associated drop in SOD activity could be due to its role in removing free radicals from the cells, indicating a high degree of free radical production and LPO. When compared to the control group, the increase in LPx was followed by a decrease in the activity of some antioxidant enzymes involved in the detoxification of ROS, such as SOD, as well as the amount of GSH in brain tissues, demonstrating that Al has a prooxidant effect.

These findings backed up previous work by (81), (71), and Johnson *et al.* (2005), who found that Al exposure increased neuronal lipid peroxidative damage with concomitant changes in enzymatic antioxidant defence status, posing a serious threat to the central nervous system's functional and structural development (Dua and Gill, 2001). Similar research found a decrease in antioxidant activity in the brains of Al-exposed rabbits (Yousef, 2004), rats (23), and humans (9). (Dua and Gill, 2001). Furthermore, the findings are consistent with those of Nehru and Anand (2005), who found a substantial decrease in SOD and CAT activity in the brain after Al therapy. SOD activity in the hippocampus was lowered by 50% and in the cerebral cortex by 30% after intragastrical exposure to Al (54-55). Reduced enzyme protein synthesis as a result of greater intracellular Al concentrations could explain both the decrease in enzyme activity and the decrease in enzyme activity (67).

Because of a variety of factors, including its high lipid content, fast oxygen turnover, low mitotic rate, and low antioxidant concentration, the brain is particularly prone to peroxide damage. These considerations may explain why Al exposure affects the brain more than any other organ. SOD is an endogenous enzymatic scavenger that helps to counteract the oxidative destruction of free radicals by destroying H2O2 produced by oxidase enzymes within peroxisomes (Fridovich, 1989; Ryan *et al*, 2008).

According to our findings, $AlCl_3$ activated caspase-3, caspase-9, and Bax in the brains of rats, causing apoptosis. The mitochondrial permeability transition pore controls the permeability of the mitochondrial membrane, which causes the release of cytochrome c from the mitochondria into the cytoplasm, resulting in mitochondrial failure (Yang *et al.,* 2014). When cytochrome c is released, it binds to Apaf-1 in the cytoplasm, forming a complex that can activate caspase-9, which subsequently activates caspase-3, which causes death (16). The Bcl-2 family of proteins regulates the mitochondria-mediated intrinsic pathway. Anti-apoptotic proteins like Bcl-2 and Bcl-XL are categorised as anti-apoptotic proteins, while pro-apoptotic proteins like Bax and Bak are classified as pro-apoptotic proteins. The balance of pro- and anti-apoptotic proteins in the Bcl-2 family is crucial for cell survival or death. Bcl-2 may protect against injury by reducing lipid peroxidation, which is triggered by cytotoxic stimuli such as reactive oxygen species (ROS), which cause inflammation (6). Bcl-2 has also been shown to stop cytochrome c from being released. In contrast, Bax regulates apoptosis through regulating cytochrome c release and subsequent caspase-3 activation, as well as dimerizing with anti-apoptotic Bcl-2 proteins (22).

Aluminum-exposed animals' serum and brain lipid components have previously been found to be disrupted. (44 & 87). This finding

could be due to the fact that aluminium toxicity can alter the lipid profile of brain myelin as a result of the metal's pro-oxidant activity, as evidenced by increased lipid peroxidation, which interferes with the lipids of cellular membranes and thus influences their functional ability and integrity. As a result, aluminium toxicity has been found to have a significant effect on the various membrane bound enzymes (Pandya *et al.*, 2004). Hyperlipidemia can also be caused by an increase in fatty acid synthesis in the liver or by lipid metabolism disturbances caused by aluminium accumulation in the liver (101). Aluminum may inhibit enzymes such as unspecific esterase, triglyceride lipase, and pyrophosphates, which appear to be one of the most prominent causes of an increase in blood triglycerides and cholesterol (84). A decrease in the clearance of low-density lipoprotein (LDL) and very low-density lipoprotein (VLDL) from the circulation, as well as unregulated VLDL generation by the liver, could induce anomalies in the lipoprotein profile (92). Given the inhibitory action of date on AlCl3-induced oxidative stress, earlier investigations shown that pretreatment with Siwa DPFE dramatically reduced MDA levels and increased GSH levels in the liver tissue of rabbits intoxicated with CCl4 (El-Gazzar *et al.*, 2009). Furthermore, date extract considerably reduced hepatic oxidative stress parameters in CCl4-intoxicated rats, as seen by significantly lower levels of MDA and significantly higher levels of GSH, GPx, and SOD (Al-Rasheed *et al*., 2015). In rats, concomitant administration of ajwa date extract and CCl4 resulted in a considerable decrease in MDA content, and restoration in GSH level and SOD activity in the hepatic tissue (27). Date fruits are widely known for their excellent antioxidant activity due to their high polyphenol content. Furthermore, they have become increasingly recognized as having significant potential for human health, and have been shown in animal studies to improve short-term memory performance (85). Date phytochemicals are degraded and undergo several changes in the gastrointestinal tract after intake. Pheonix spp. were once thought to be key sources of neuroprotective metabolites. By activating adaptive cellular stress response pathways such GSH regulation, reduction of ROS production, and caspase activation, digested metabolites from these dates can protect brain cells from oxidative damage, one of the most critical mechanisms of neurodegeneration (91).

In comparison to intoxicated AlCl3 group, treatment with either CCl4 or DENA, administration with date improved the lipid profile in the serum of rats by lowering the amounts of TC, TG, and LDL-C and raising the concentration of HDL-C. According to published studies, DPFE's lipid-lowering effects have been confirmed. After 14 days of therapy with date fruit 90 suspensions (300 mg/kg), TC, TGs, LDL-C, and VLDL-C, as well as HDL-C and the LDL-C/HDL-C ratio, were considerably reduced in hyperlipidemia-induced albino rats, according to (5). (27) found that Ajwa date extract resulted in significant reductions in serum levels of TC, TG, and LDL-C, as well as a significant increase in HDL-C.

In rats treated with date extract (1g/kg) was observed to alter serum lipid parameters (30). Free radicals have been shown to affect liver functioning and can cause a disruption in the perfect harmony of hepatic metabolic reactions, which can lead to hyperlipidemia through a variety of mechanisms, including increased synthesis of TC, TG, and LDL-C. (8). As a result, it's possible that free radicals and oxidative stress caused by DENA or CCl4 poisoning are the primary risk factor for altered hepatic lipid metabolism in the treated rats (30-31).

Date fruits have been discovered to be a neuroprotective mediator against aluminum-induced oxidative stress and neuronal degeneration. The majority of the markers evaluated, such as oxidative stress, endogenous antioxidant system, neurotransmitters, and electrolytes levels, improved, corroborating this conclusion. Flavonoids (anthocyanin), vitamins (A, B complex, E, and C), minerals (Na, K, Ca, Zn, Se, and P), phenolic polymers (ellagic acids), and phenolic acids (ferulic, p-coumaric, caffeic, and galic), phenolic polymers (ellagic acids), and phenolic acids (ferulic, p-coumaric, caffeic, and galic), phenol (ferulic) (29).

P.dactylifera includes a range of phytonutrients, including carotenoids, sterols, tannins, and polyphenols, including flavonoids,

according to (89). Dates are strong in phenolic compounds, which have been proved to be good antioxidants and free radical scavengers. (25). These activities have been linked to P. dactylifera's neuroprotective characteristics (Essa *et al.,* 2019). Dates defend against oxidants in a variety of ways. By battling the harmful hydroxyl radical-generating mechanism, which is a primary generator of oxidants in the body, it can protect lipids in cellular membranes, membrane related enzymes, and amino acids like tyrosine from peroxynitrite, a highly reactive oxidant (92). Furthermore, anthocyanins and non-anthocyanin phenolics in date may operate as anti-oxidants and/or pro-oxidants in different biological situations, resulting in the reported favourable changes (68). The preventive effects of dates can also be linked to the synergistic antioxidant activity of vitamins E and C in reducing oxidative stress and cell degeneration. When vitamin E combines with free radicals, a vitamin E radical is formed, which works as a chelating agent before being transformed back to an antioxidant with vitamin C. Vitamins E and C's usefulness in avoiding aluminium toxicity demonstrates their detoxifying, scavenging, and curative abilities (43). Dates also have a hypolipidemic impact and protect against AlCl3-induced toxicity by restoring the altered lipid level, as well as drastically lowering the activity and mRNA levels of different enzymes involved in hepatic fatty acid production. The mechanism underlying date's hypolipidemic impact has been suggested to be a reduction in hepatic lipogenesis (48).

5. Conclusion

The use of date with aluminum exposure may be recommended for reversing oxidative damage in the brain and neurological problems. Intracellular pathways are involved, with improvements in neurotransmitter changes as well as other elements of brain dysfunction, as well as the suppression of oxidative stress, the scavenging of free radicals, and the modification of antioxidant defence mechanisms. This impact is likely owing, at least in part, to the synergistic anti-oxidant activity of its numerous nutritional ingredients. As a result, supplementing with edible dates may aid in the safe application of toxins, as well as a variety of other facets of modern life. However, future research will focus on the separation and bioavailability of the major components of dates that are responsible for antioxidant activity

6. References

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