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AMELIORATING ROLE OF *ULVA LACTUCA* EXTRACT ON NEUROBEHAVIORAL AND NEUROCHEMICAL ABNORMALITIES CAUSED BY LITHIUM IN RATS

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ABSTRACT

Lithium is a relevant mood-stabilizing agent that is used in the management of depressive and manic episodes in bipolar, disorder; however, its application may cause diverse side effects including oxidative stress. This work investigated the neuroprotective and anti-stress activity of *Ulva lactuca* extract (MEUL) against lithium induced neuronal toxicity in rats. Lithium (2 g/kg) was administered in diets with or without MEUL (250 mg/kg BW by gavage) for 90 days. Lithium induced oxidative stress lead to an elevation in lipid peroxidation and oxidation protein product levels, a reduced superoxide dismutase, glutathione peroxidase activities, glutathione, and ascorbic acid concentrations in brain. The level of brush border enzymes (Ca^{2+}ATPase, Mg^{2+}ATPase) and acetylcholinesterase activity was reduced after lithium treatment. Histopathological observations confirmed these results. Open field and elevated plus maze behavioral tests showed an impaired recognition memory. Treatment with MEUL alleviated neurobehavioral alterations, attenuated oxidative stress markers and restored these biological parameters to normal standards, as well as a histological improvement. *Ulva lactuca* extract offers neuroprotection, against lithium-induced behavioral and oxidative brain damages.

Keywords: Lithium, *Ulva lactuca*, neurotoxicity, neurobehavioral, oxidative stress.

INTRODUCTION

Lithium (Li) is widely used medication to treat psychiatric illnesses such as bipolar disorder or manic depression and for prevention of their recurrence (Młyniec et al., 2014). The possibility of using lithium in therapy of Alzheimer's and Parkinson's disease has been suggested (Arraf et al., 2012; Hampel et al., 2019). However, neurotoxicity could occur at normal plasma concentrations (Megarbane et al., 2014). Severe side effects such as renal, thyroid, endocrine and metabolic abnormalities, nerves, gastrointestinal tract and dermatological disruptions can be accompany an overflow of the safe threshold (McKnight et al., 2012; Shine et al., 2015; Hanak et al., 2017). Indeed, severe neurotoxicity is the most critical manifestation of lithium poisoning which typically happens when a medicinal dosage is accumulated chronically instead of during an acute overdose. Tremor, delirium, ataxia, drowsiness, myoclonus, dysarthria, confounding, coma, seizures, and death are all neurologically impaired (Lee et al., 2011).

Beneficial studies have suggested that a certain natural products have the potential to biosynthesize phytochemicals that have a range of activities to protect efficiently against oxidative stress and associated diseases. It is well established that secondary metabolites synthesized by green algae (*Ulva lactuca* L.) are effective in terms of antioxidant potentials due to

many bioactive substances (Olasehinde et al., 2019a). *Ulva lactuca,* not only possesses nutritional value, but also have nutraceutical potentials such as hypocholesterolemia hypolipidemic antiinflamatory, antineoplastic, cardioprotective, chemoprotective activities and anti-tumour activities (El-Baky and Baz, 2008; Madhusudan et al., 2011). However, their neuroprotective role has not been fully explored there are no data about in *vivo* effects of *Ulva lactuca* on cerebral oxidative damage induced by lithium toxicity.

Therefore, this research investigated the possible beneficial effects of the *Ulva lactuca* methanolic extract therapy, using rats as an animal model, against lithium-induced neurodegenerative changes. The behavioral status, activity of acetyl cholinesterase (AChE), oxidative stress biomarkers and brain histopathology have been determined.

MATERIALS AND METHODS

Algae Selection and Extraction Procedure

The green algae (*Ulva lactuca*) were collected in April from Al Mahres Beach, Sfax coast during low tides (Longitude: 10.09 E°, latitude: 36.21 N°, altitude: 174 m, rainfall: 483 mm/year).

The alga was well washed, then left to dry under shade. The dried algae were grounded finely, completely flooded with absolute methanol within 24 h with magnetic agitation at room temperature, then filtrated by Whatman N°1 filter paper, the process was repeated three times till exhaustion. The filtrates were collected and evaporated by a rotary evaporator at 50 °C under reduced pressure. The powder has collected and placed at 4 °C in the refrigerator.

i. Determination of Total Phenolic, Flavonoid and Mineral Contents

Total phenolic content was calculated using Folin-Ciocalteu reagent according to Singleton and Rossi (1965), the content of flavonoids was measured using the method of Ordonez et al., (2006). Minerals were estimated in compliance with protocol of Kachiguma et al. (2015). After nitroperchloric mineralization (2:1), the mineral components potassium (K), iron (Fe), magnesium, Zinc, calcium, and sodium found in the date ashes were examined separately by an atomic absorption spectrophotometer (model Thermo-Scientific ICE 3000, Sherwood Scientific Ltd., Cambridge UK).

ii. Antioxidant Testing Assays

The effect of the extract on DPPH radical was calculated using the method reported by Son and Lewis (2002). The ability to scavenge hydrogen peroxide from marine algal extracts was performed according to the procedure of Ruch et al., (1989). Also, the antioxidant capacity of marine algae extracts was done by iron reduction (FRAP assay) according to Bertoncelj et al., (2007).

Animals Housing and Treatments

Studies were performed on adult male albino rats with 160-180 g body weight. Rats were maintained under standard environmental and nutritional conditions for fifteen days to acclimatize to laboratory conditions. The animals were divided into four equal groups that included six mice and were treated as follows: Group I (C): Control rats. Rats in groups II and III were provided with a standard diet supplemented by lithium carbonate in a 2 g/kg diet dose level (Nciri et al., 2012).

Ulva lactuca methanolic extract MEUL was given at 250 mg/Kg b.w via daily oral gavage treatment in groups III and IV. All the treatments continued for three months, mortality, behavioral, neurological and any other abnormalities clinical observations were made and the weight was measured daily. The dose *Ulva lactuca* extract was chosen from our experimental toxicity test.

Toxicity Test of the Methanolic Extract of Ulva Lactuca Crude

The methanolic algae extract toxicity was assessed by oral doses of 100, 200, 250, 300 mg/kg/day for 24 rats divided into four groups. Animals were continuously monitored for changes of autonomic or behavioral responses within 12 and 24 hours and monitored for any mortality.

Preparation of Brain Homogenates

After cervical decapitation, at the end of the experiment, the rats' brains were quickly removed and weighted for biochemical, antioxidant and histological testing. Tissue homogenates were prepared using Ultra-Turrraks homogenizer in Tris NaCl buffer (50 mM Tris, 15 mM NaCl, pH 7.4), then filtered and centrifuged at 4 °C for 15 minute time at 5000 g. Supernatants were collected and frozen in aliquots at - 80°C until analysis.

Behavioral Testing

i. Elevated Plus Maze (EPM) Test

Rats were placed in the middle of the four arms of the labyrinth facing one of the open arms (two open and two closed arms). The number of entries in each arms and the time spent were recorded for 5 minutes. The time spent in the closed arms was used as an index of anxiety. An increase in open arm activity, however, reflects anti-anxiety behavior (Sharma et al., 2013). After each test, the appliance was spiritually cleansed to mask the scent left by the animal in the previous experiment.

ii. Open Field Test (OFT)

The test (OF) is used for the evaluation of the general rat locomotive activity. It was done using the method of Sethi et al., (2009). Each animal was positioned separately in the middle of an open field device divided into equal squares and is let free for 5 min. The entire room, except the OF was kept dark during the experiment. Behavioral tests scored included time of immobility, number of the lines crossed, rearing frequency, and grooming. This test lasts for 15 days.

Oxidative Stress Analysis

Lipid peroxidation in brain tissues homogenate was estimated by measuring Thiobarbituric Acid Reactive Substances (TBARS) according to Fraga et al., (1988). Advanced oxidation protein products levels (AOPP) were determined using the method of Witko-Sarsat et al., (1996). At 340 nm, the absorbance was recorded. Glutathione peroxidase enzymatic activity was determined according to Rotruck et al., (1973). The method used for assessing SOD activity was described in Kakkar et al., (1984).

Reduced glutathione was tested using a spectrophotometric method according to Jollow et al., (1974). Ascorbic acid content was calculated using the method described by Jacques- Silva et al., (2001).

Determination of Acetylcholinesterase (AChE) Activity in Brain

Acetylcholinesterase activity was measured immediately in the brain using acetylthiocholine iodide as a substrate according to Ellman et al., (1961). The hydrolysis rate of acetylthiocholine iodide is measured at 412 nm by releasing the thiol compound producing the DTNB

color forming compound when reacted by DTNB. AChE activities were expressed as micromole of substrate (acetylthicholine) hydrolyzed /min/mg protein.

The Ca^{2+} and Mg^{2+} ATPases Activities

The sediment of the centrifuge was used for estimation of the Ca^{2+}ATPase by Hjerken et al., (1983) and Mg^{2+}ATPase by Ohinishi et al., (1982).

Histological Studies

The brain tissues were fixed in 30% of buffered formalin solution, dehydrated in graduate concentrations of ethanol (50–100%) and embedded in paraffin. For microscopic observations, thin slices (4–5 µm) were cut and stained with Hematoxylin-Eosin (H&E).

Statistical Analysis

The data is expressed as mean ± standard deviation (SD). Differences were analyzed in one way analysis of variance (ANOVA) followed by the Fisher test (Stat View) and P< 0.05 was considered as significant.

RESULTS

Extraction yield and Polyphenol, Flavonoid and Mineral Contents

The mass yields of extractable components are related to the dry algae mass ranged from 12.23% for methanolic extract. The results of the phytochemical analysis showed methanol extract quantitative levels: 76.21 ± 0.28 mg GAE/g E to phenolic compound and 59.32 ± 2.74 mg quercetin/g E to flavonoids.

The macromolecules such as calcium, iron, potassium, sodium, and magnesium are among the minerals which are present in significant amounts in marine algae. The results showed that magnesium (Mg) and calcium (Ca) was the most abundant macro minerals in *Ulva lactuca*, it amounted to 47.23±3.14 and 41.32±4.14 mg/kg respectively, with lower contents of Fe and Zn (Table 1).

Table 1: Minerals composition of methanolic extract of *Ulva lactuca*.

Minerals elements	Amount (mg/g)
Potassium (K)	14.20±0.78
Fer (Fe)	3.81±0.33
Magnesium (Mg)	47.23±3.14
Zinc (Zn)	2.16±0.45
Calcuim (Ca)	41.32±4.14
Sodium (Na)	34.15±2.31

Determination of Antioxidant Activity of Ulva lactuca Extract

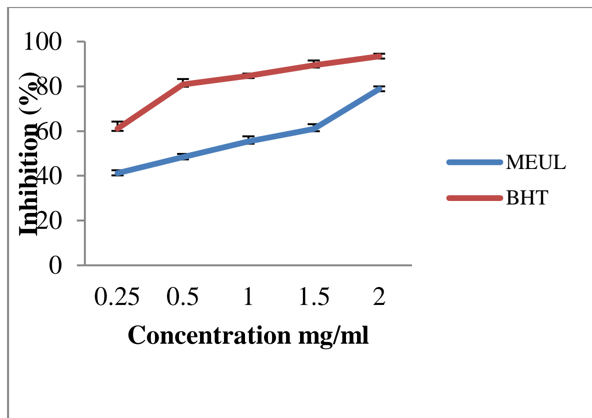
The DPPH inhibition activity presented in Figure 1 showed that 2 mg/ml of methanolic extract was able to inhibit the formation of DPPH by 78.78 ± 1.23% which were compared with standard antioxidant BHT 93.33%.

The percentage of H_2O_2 scavenging activity by same concentration (2 mg/ml) of methanolic extract, and L-ascorbic were found to be 65.46 and 75.16 %, respectively. The methanolic extract had effective H_2O_2 scavenging activity.

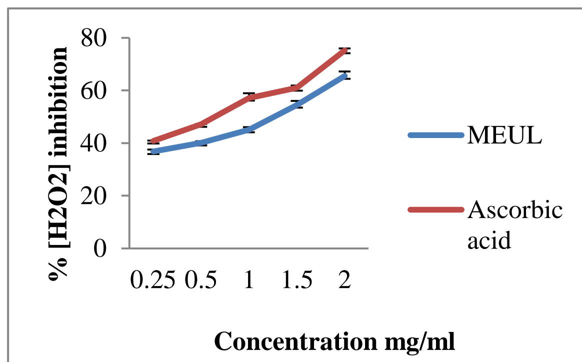
The same results were produced by the ferric reducing power of algae methanolic extract (97.61±2.11 mM Fe^{2+}/ mg sample) compared to ascorbic acid (98.41±1.41 Mm Fe^{2+}/mg sample) at the maximum dosage of 2 mg/ml.

Toxicity Study

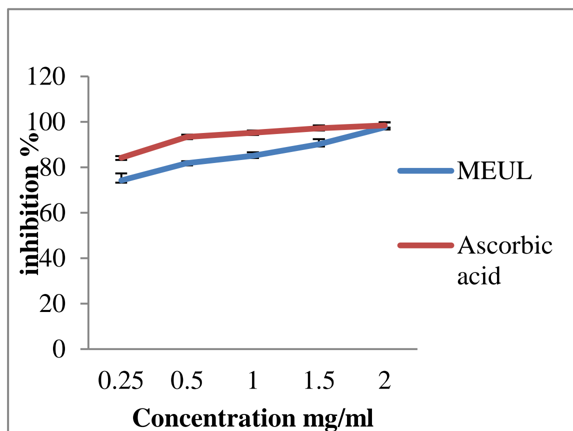
In toxicity test, there was no visible symptom of toxicity or mortality among rats administered with different doses (100, 200, 250, 300 mg/kg) of MEUL. No abnormal behavior was observed in the four-experimental groups of male rats.



A. DPPH radical scavenging activity.



B. H_2O_2 scavenging activity



C. Ferric- reducing antioxidant potential assay

Figure 1: Determination of antioxidant activity of *Ulva lactuca* extract.

Body and Brain Weights

Our results in table 2 exposed a significant decrease ($p < 0.01$) compared to normal rats in the body weight of rats after 3 months of lithium treatment. Co-administration of MEUL substantially reduced body weight loss in rats compared with the Li-treated group. In addition, brain weights in Li-treated animals decreased considerably ($p < 0.05$). MEUL supplementation in Li-treated group (MEUL+Li) induced a significant ($p < 0.05$) increase in brain weights compared to the Li-treated rats.

Effects on the Behavioral Tests

i. Elevated Plus Maze Test

The results in Table 3 showed rats spent more time and more entries in closed arms of the EPM than that of untreated rats. In contrast, in the enclosed arms, the time spent and the numbers of entries have been reduced compared to control animals by extract treatment.

ii. Open Field Test (OFT)

Treatment with Li significantly reduced the amount of ambulation measured by the total distance travelled, rears, and the total number of grooming movements. The immobility time in the central squares was significantly increased relative to the control group. However, in Li+ MEUL-treated rats, these behavioral activities have been significantly restored compared to the Li-treated rats.

Oxidative Stress Analysis

Figures 2 and 3 showed that lipid peroxidation TBARS and protein oxidation AOPP levels in brain of Li group have been significantly increased compared to normal group. The level of TBARS and AOPP products decreased significantly after MEUL co-treatment in combination with Li compared to Li-treated rats.

The results revealed significant decreases in GPx and SOD activities and GSH and ascorbic acid levels in rats treated with lithium compared to controls. However, co-administration of MEUL improved brain antioxidant activity compared to the Li-treated group (Table 5).

Acetylcholinesterase (AChE) Assay

The activity of AChE in the brain of Li-treated group was significantly reduced compared to the normal group. The Li+MEUL-treated rats resulted in a significant increase in brain AChE activity as compared to Li group (Figure 4).

Determination of Ca^{2+} and Mg^{2+} ATPases Activities

In present result data reveal that the level of membrane bound enzymes like Ca^{2+}ATPase and Mg^{2+}ATPase was significantly decreased after Li administration as compared with the control group. However, Ca^{2+}ATPase and the Mg^{2+}ATPase were significantly recovered with dose 250 mg/kg of the extract in the Li +MEUL group (Table 6).

Histological Study of Brain Tissue

Li-treated rat brain tissues displayed irregular cellular morphology in cerebral cortex, followed by extreme vacuolated spaces and neuronal necrosis (Figure 5C). Co-treatment with MEUL, by contrast, has maintained a brain histological architecture and reduces brain injury, with protected cell morphology showing moderate brain improvements in comparison to Li-groups (Figure 5D). The brain tissue control (C) and MEUL extract groups had normal histological architecture (Figure 5A & B).

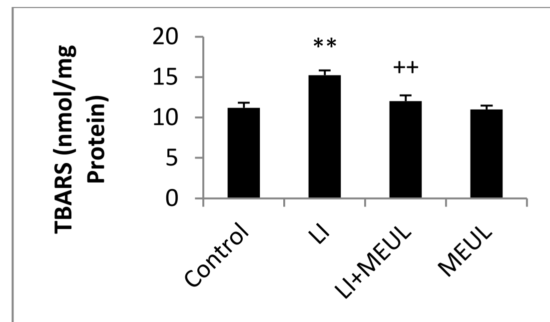


Figure 2: Effects of methanolic extract of *Ulva lactuca* (MEUL) on TBARS in Li-intoxicated rats. TBARS: thiobarbituric acid reactive substance. Data are presented as mean ± SD, n = 6. *p≤ 0.05, **p≤ 0.01 compared with the control group. +p ≤0.05, ++p ≤0.01 as compared with Li-treated rats.

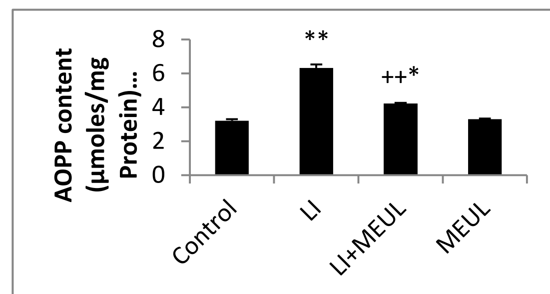


Figure 3: Effects of methanolic extract of *Ulva lactuca* (MEUL) on advanced oxidation protein products (AOPP) content in Li-intoxicated rats. Data are presented as mean ± SD, n = 5. *p≤ 0.05, **p≤ 0.01 compared with the control group. ++p ≤0.01 as compared with Li-treated rats.

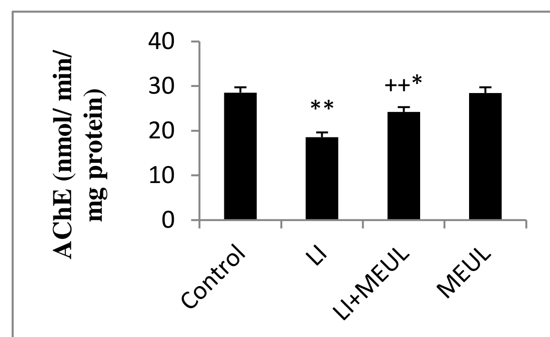


Figure 4: Effects of lithium (Li), methanolic extract of *Ulva lactuca* (MEUL, and their combinations (Li+ MEUL) on brain acetycholinesterase activity (AChE) of control and experimental rats. Values are mean ± SD for six rats in each group. Li and Li+ MEUL treated animals vs control group: *p<0.05, **p<0.01. Li group vs (Li+ MEUL) group: ++p<0.01.

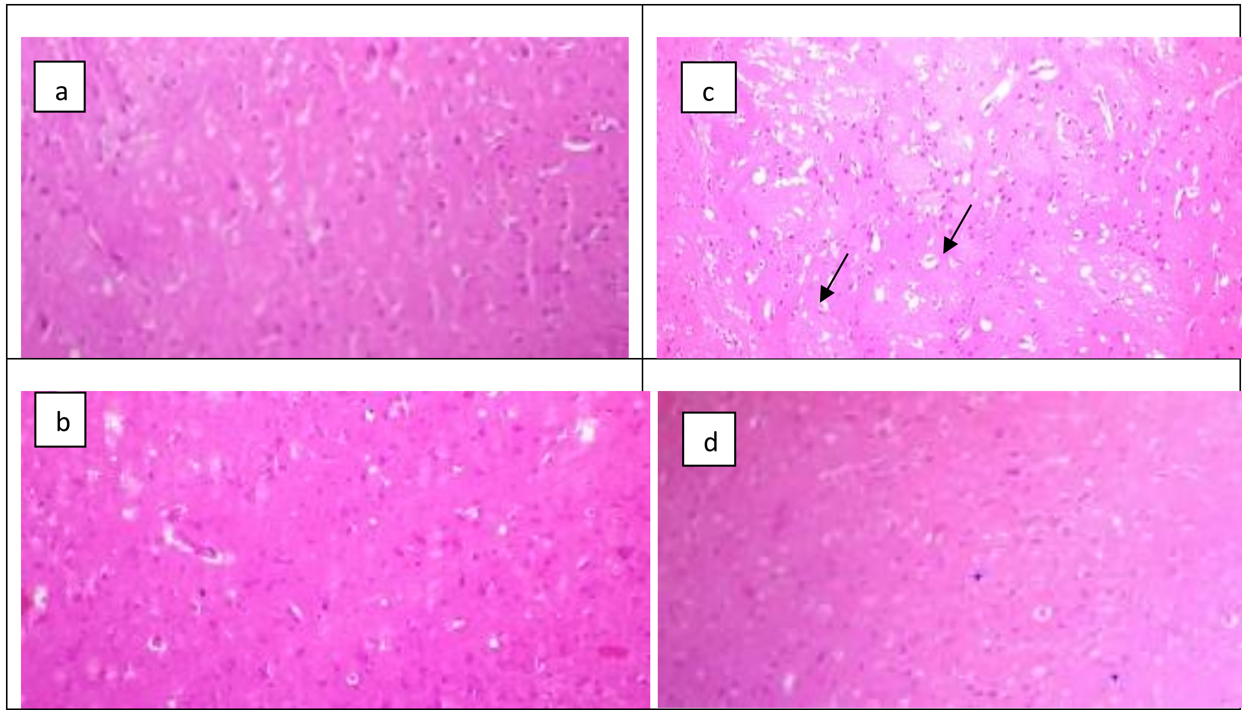


Figure 5: Photomicrographs examination of all experimental groups' rat brain stained with haematoxylin and eosin viewed at original magnification (200). (a) normal architecture of the brain; (b) MEUL alone administered rats indicating normal architecture in brain; (c) abnormal cellular morphology accompanied by severe vacuolisation and necrosis of neurons observed in the brain of lithium induced neurotoxicity; (d) MEUL+Li co-treated rats showing protected cellular morphology moderate presenting degenerative improvement in brain compared to Li-groups.

Table 2: Changes of body weight and brain weight during one month of the experimental period in rats treated with Li, (Li+MEUL) and MEUL.

Parameters	Control	Li	Li+MEUL	MEUL
Initial body weight(g)	190±1.06	191.4±3.32	194±2.25	189.9±3.23
Final body weight (g)	220±2.15	183±3.2**	205±2.03^{*+}	222±4.1
Brain weight (g)	1.71±0.03	1.15±0.02*	1.53±0.07^{*+}	1.74±0.04
Body weight index	0.77±0.02	0.62±0.01*	0.74±0.01^{+}	0.78±0.002

*Body weight index (BWI) was computed as BWI (%) = brain weight (BW)/body weight (BW) *100. Values are given as mean±SD for group of six rats each. Statistically, values are presented as follows: *P<0.05 significant differences compared to control. + P<0.05 significant differences compared to lithium (Li).*

Table 3: Effect of methanolic extract of *Ulva lactuca* (MEUL) administration in rats treated or not treated with lithium (Li) during 1 month on, the time spent (seconds) and number of entries in open arms and the time spent and number of entries in closed arm in the Elevated Plus Maze (EPM) test during 5 min.

Treated groups	Control	Li	Li+ MEUL	MEUL
Time spent in the open arms	255±8.32	58±3.11	148±7.32	260±5.33
Time spent in the close arms	74±3.60	274±10.13**	196±7.5**++	78±3.54
Number of entries in open arms	9±0.42	4±0.24**	5.42±0.32**++	8.96±0.53
Number of entries in close arms	2.05±0.12	3.43±0.21**	2.43±0.32*++	2.12±0.02

*The data are mean ± S.D. for six rats in each group. * p < 0.05, ** p < 0.01 significantly different as compared to the control. + p < 0.05, ++ p < 0.01 significantly different as compared to the Li-treated rats.*

Table 4: Effects of lithium (Li) and methanolic extract of *Ulva lactuca* (MEUL) supplementation on total distance travelled (meter/5min), total time immobile (seconds), number of rearing and number grooming of by rats as determined in the open field test during 5 min.

	Control	Li	Li+ MEUL	MEUL
Total distance travelled	5.04±0.32	2.87±0.12**	3.73±0.42*++	5.10±0.8
Total time immobile	49.81±7.82	245±6.13**	158±4.9**++	52±4.52
Number of rearing	11.05±2.02	5.39±0.52**	9.95±0.46++	10.98±1.08
Number of grooming	13.95±2.53	4.35±0.63**	8.32±0.41**++	13.65±2.30

*The values are mean ± S.D. for six rats in each group. * p < 0.05, ** p < 0.01 significantly different as compared to the control. + p < 0.05, ++ p < 0.01 significantly different as compared to the Li-treated rats.*

Table 5: Effects of methanolic extract of *Ulva lactuca* (MEUL) administration on the activities of antioxidant enzymes (SOD, GPx) and GSH level.

Parameters	Control	Li	Li+ MEUL	MEUL
GPx (nmoles of GSH/min /mg protein)	0.79□0.13	0.51□0.02**	0.76□0.4++	0.80□0.02
SOD (units/mg protein)	4.98□□□□□	.32□□□□□**	4.58□0.52++	5.02□0.23
GSH (µg/mg protein)	0.64□0.17	.36□□□□□**	0.59□□□□□++	0.66□0.19
Ascorbic Acid (µg ascorbic acid/g tissue)	7.21□0.54	4.23□0.18**	6.32□0.41*++	7.32□0.52

*Values are means ±SD for six rats in each group. * P<0.05, **P<0.01 significant differences compared to controls. + P<0.05, ++P<0.01 significant differences compared to lithium (Li) exposed-group.*

Table 6: Effects of methanolic extract of *Ulva lactuca* (MEUL) co-treatment on the activities of Ca^{2+}ATPase and Mg^{2+}ATPase, in all experimental groups.

	Control	Li	Li+ MEUL	MEUL
Ca^{2+}ATPase	1.15±0.01	0.62±0.03**	1.09±0.03*++	1.12±0.02
Mg^{2+}ATPase	2.73±0.23	1.85±0.31**	1.99±0.08*++	2.8±0.15

*Mg^{2+} and Ca^{2+}ATPase activities were expressed as µmol phosphate liberated/mg protein. Values are expressed as mean ± SD of six rats in each group. Statistically, values are represented as follows: * P<0.05, **P<0.01 significant differences compared to controls. + P<0.05, ++P<0.01 significant differences compared to lithium (Li) exposed-group.*

DISCUSSION

In the present work, we have evaluated behavioral anxiolytic effects in tow validated models because an unfamiliar and open environment induces stress which provokes anxiety, inhibiting normal behavior in rats. Following the data provided by the open field test, treatment with Li reduced the loco-motor activity, as shown by a longer immobilized time and shorter distance travelled. This observation was consistent with previous studies showing reduced horizontal loco-motor activity by lithium in an open-field (Johnson, 1972; Smith, 1983; Tenk et al., 2005).

In the EPM test, a statistically significant increase of time spent and numbers of entries into closed arms can show the anxiolytic effectiveness of MEUL. The results showed the algae extract administration could prevent cognitive behavior after lithium treatment and depicting increased anti-anxiety levels. The neuroactive compound is thought to be a family of sulphated polysaccharides, which are highly present in the extract (Violle et al., 2018). It is clearly demonstrated and corroborates the results of the preclinical study evaluating the anti-depressive actions of water-soluble extract derived from *Ulva lactuca* in humans who experience a reduced feeling of motivation and pleasure (Allaert et al., 2018). Changes in the body and relative brain weights are important criteria for organ toxicity assessments and a precious index of lithium-related organ damage which leads to significant reduction of animal growth (Lima et al., 2016).

Lithium treatment led to a significant decrease in acetylcholine hydrolysis. This may cause damage the cholinergic and nervous transmission, which is related to abnormal behavior in rats. Studies show that lithium treatment in certain brain areas can interact with the cholinergic system (Williams and Jope, 1995). Jing et al., (2013) hypothesized that GSK3b may regulate AChE, that the inactivation of GSK3b may lead, by inappropriate post translational modification, to proteasomal degradation of AChE-S. Li-mediated dopamine-dependent neurobehavioral effects on locomotor activity, cognition, emotion and reward were reported for GSK-3 involvement (Beaulieu et al., 2004, O'Brien et al., 2004). Our results have shown that MEUL's antioxidant potential can prevent oxidative neuron damage and improve neuronal dysfunction or neurodegeneration (Kim et al., 2016). MEUL improved cholinergic neural transmission and the memory status of rats with the correction of AChE rate.

Despite widespread use of Li its mode of action is unknown, although effects on biological membranes and synaptic neurotransmission is suspected. It has been suggested that in the brain, the membrane-bound (ATPases) are the targets for lithium action (Dick et al., 1978). In this study Mg^{2+}ATPases and Ca^{2+}ATPases both were significantly decreased in rat brains. A decrease in the activity of Mg^{2+}ATPase reflects a decrease in the ATP synthesis in the mitochondria, affecting all the cellular processes that are ATP dependent (Abipudi and Reddy, 1994). The reduction in the activity of Ca^{2+}ATPase by lithium result in a reduced calcium efflux, an event that would result elevation in intraneuronal calcium concentration in synaptic nerve terminals, this increased Ca^{2+} will cause the release of neurotransmitters and neurological effects of Li (Cho, 1995).

Redox activity is a key to neuronal homeostasis and brain function (Garza-Lombo et al., 2018). These minerals are known to be essential for the function of several enzymes and other proteins and take part in the synthesis of neurotransmitters as well in redox reactions which are required for cellular metabolism, mitochondrial function (Chen et al., 2016). Data obtained within this study suggests that *Ulva lactuca* extract

presented a rich composition of bioactive compounds and mineral compounds (Mg, Ca, Na, K, Fe, and Zn).

Induction of oxidative stress has been proposed as one of the essential pathways by which lithium has its toxic effects (Nciri et al., 2012; Salimi et al., 2017). In reality, some of Li's adverse effects appear to be induced by excessive formation of reactive oxygen species and exhaustion of defense mechanisms and enhancement of lipid peroxidation in rats (Vijaimohan et al., 2010; Musik et al., 2014). The highest levels of TBARS and AOPP observed in the brain tissue after lithium treatment suggest that the brain cells were damaged. Peroxidation of polyunsaturated fatty acids contributes to phospholipids degradation which is considered as an index of cellular deterioration (Abou-Donia, 1981).

In addition, lipid peroxidation (LP) caused in synaptosomes by lithium contributes to modification of synaptic endings and the lipid levels in synaptoplasmatic membranes that subsequently leads to significant disruption in the activity of neurotransmitter uptake systems and depolarisation-dependent calcium channels (Sawas and Gilbert, 1985, Efendiev and Kerimov, 1994). It can contribute to depression, sleep disorders, and other essential indicators of neurotoxicity (Taranova and Nilova, 1986). Following lithium treatment, glutathion (GSH) and ascorbic acid levels have found to decrease significantly in the brain. Reduction of antioxidant enzymes (SOD and GPx) substantiates the occurrence of oxidative damage in brain tissue after lithium treatment (Vijaimohan et al., 2010; Khairova et al., 2012; Joshi et al., 2013).

In this study, MEUL supplementation decreases the brain TBARS level and the protein oxidation process significantly. The observed increase in antioxidant enzymes (GPx, and SOD), non-enzymatic antioxidants (GSH, ascorbic acid) levels may be associated with the antioxidant capacity of the methanolic extract of *U. lactuca*. High levels of these antioxidant enzymes will prevent and scavenge excessive accumulation of free radicals, thus protecting cells against oxidative damage and neurodegeneration. The activities of radical scavenging and metal chelating confirm their antioxidant properties by reducing the intracellular oxidative damage, protecting the brain neurons by protection of lipid oxidation in rats (Chidambararajan et al., 2019; Ghareeb et al., 2019; Olasehinde et al., 2019b). Interestingly, histological study confirms that *U. lactuca* extract has antioxidant properties. Co-treatment with MEUL+Li preserved the brain histological architecture and reduces brain injury, this showed moderate protection of cell morphology with a degenerative brain improvement.

CONCLUSION

In summary, these results show that lithium-induced oxidative stress causes structural and functional damage to the brain. However, the *U. lactuca* methanolic extract has anxiolytic-like effects and could protect Li brain damage by counteracting free radicals by its antioxidant properties and mitigating toxic brain changes.

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