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Potential Impact of Strawberry Leaves Extract on Neurotoxicity in Rats

Issa, G.I.,* Abbas O.A.,* Abdel-GawadE. I*
*Radioisotopes Department, Atomic Energy Authority
Giza, Egypt

Abstract:-In spite of the fact that Strawberry leaves extract (SLE) has an antioxidant and anti-inflammatory capacity, little is known about its effect on brain injury relieve. A rat model of lead poisoning by a daily intraperitoneal injection (i.p.) of lead acetate for 90 days was performed. Thus, this study was designed to investigate whether daily intraperitoneal injection (i.p.) of 200mg/kg SLE for two weeks can inhibit lead-induced brain injury, and whether this effect could ameliorate lead-associated biochemical and pathological changes. The obtained data showed that the injection of SLE caused recover of insulin and neurotransmitters e.g. noradrenalin, dopamine and 5-hydroxytryptoamin and reduced the contents of amyloid beta (Aβ-40), tau protein, calmodulin, calcium adenosine 5′-triphosphatase (Ca^{++}ATPase) and magnesium adenosine 5′-triphosphatase (Mg^{++}ATPase). Furthermore, the histopathological examinations revealed that the injection of SLE could recover the pyknosis and degeneration in the cerebral cortex, focal eosinophilic plagues in the striatum and the vacuolization as well as blood vessels congestion in the matrix. Therefore, it may be concluded that, Strawberry leaves extract can be considered a very promising phytochemical for treating the neurotoxicity due to lead toxicity.

Keywords:-*Strawberry leaves extract, lead poisoning, amyloid beta, tau protein, calmodulin.*

I. INTRODUCTION

There is a considerable emerging evidence suggests that the plant derived polyphenols are known to be the source of useful drugs due to their diverse pharmacological properties [1] [2]. Among these plants, strawberry leaves (Arbutus unedo L.; Ericaceae family), has attracted a great deal of attention because it contains several classes of polyphenols [3, 4, 5]. Previous phytochemical studies have demonstrated that flavonoids and phenylpropanoid glycosides are major bioactive constituents of the strawberry leaves such as gallic acid, flavonol, tannins, anthocyanins, tannins, catechin, quercetin, kaempferol [6, 7, 8, and 9]. The diverse biological activities of polyphenols is attributed to their general free radical trapping capacity, or antioxidant activity *per se*, metals chelation, activation of survival genes, cell signaling pathways and regulation of mitochondrial function [10]. Increasing interest in the application of polyphenols for brain therapy in human and animal has been noted. Whereas, polyphenols may cross the blood brain barrier, accumulate in the brain at nano-molar concentrations and exert neuromodulators effects through interactions with both astrocytes and microglia [7, 11].

Lead is one of the most dangerous environmental pollutants on the brain due to its ability to pass the blood–brain barrier by substituting the calcium ions [12, 13]. The effects of lead are pervasive and often subtle, just entering the blood, more than 95% mobilized by erythrocytes to distribute in all soft organs and only 3% remain in the blood [14, 15]. Lead augments the oxidative stress and directly inhibits glutathione reductase, and other oxidative stress related proteins by binding to their sulfhydryl residues [16, 17]. Excessive oxidative stress not only cause neurotoxicity but also promotes the development of diabetes and inhibits several key components of the insulin signaling pathway [18]. It is worth mention that, insulin signaling have important role in relation to brain structure and function, including myelin integrity and neuronal plasticity and inhibition of apoptosis [19].

Because the endogenous defense systems of the brain have low antioxidant activity, it needs limited resources such as vitamins, bioactive molecules, lipoic acid, antioxidant enzymes and redox sensitive protein transcriptional factors to confront the excessive ROS production [20]. But, the action of these elements requires nutritional antioxidants to be activated. Given these considerations and for a great attention has been paid to health hazards of environmental pollution and usage of alternative medicine, the present study was directed toward the evaluation of the synergistic effect of strawberry leaves extract components on pathological and biochemical neurotoxicity symptoms associated with chronic exposure to lead acetate.

II. MATERIALS AND METHODS

A. Preparation of strawberry leaves extract

Strawberry leaves (*Arbutus unedo*L.) were collected from Banha, Egypt. Mature fresh and healthy leaves were immersed in a five-fold volume of water for 1 hour washed and then air-dried at room temperature and crushed to a mesh size of 1 mm. One kg of the crushed leaves were immersed in four liters of 70% ethanol for 48 h and rinsed for five times with dehydrated ethyl alcohol. The extract consequently filtered and concentrated to dryness using rotary evaporator. SLE was dissolved in water and injected to the rats as aqueous suspension [20].

B. Experimental design

The study was conducted accordance the Guide for the Care and Use of Laboratory Animals (Eighth Edition, 2011, published by The National Academies Press, 2101 Constitution Ave. NW, Washington, DC 20055, USA). This guide was approved by the Ethical Committee at National Center for Radiation Research, Egyptian Atomic Energy Authority, Cairo, Egypt (NCRR- EAEA). Thirty male albino rats, weighted 180-200 grams, were maintained under standard animal house conditions of illumination (12h per day), and ventilation. The animals were provided with water and standard rat chow *ad libitum*, and acclimatized for one week before starting the work. The animals were randomly and equally divided into 3 groups:

- The first group (control group): rats were served as control and received normal saline (0.9 % w/v NaCl).
- The second group (lead intoxicated group): rats were injected daily (i.p.) with lead acetate solution $[Pb(C_2H_3O_2)_2]$ for 90 days at a dose rate of 100 mg/kg b.w.
- The third group (SLE treated group): rats were injected i.p. with 200 mg/kg b.w of SLE for two weeks after lead acetate injection as previous manner.

One day after the last dose of extract injection, the animals were anesthetized by diethyl ether and the blood samples were collected from orbital venous plexus. Sera were obtained after centrifugation and stored prior to the biochemical analyses. The animals were decapitated and the whole brains were obtained for histopathological examinations and additional biochemical analyses.

C. Preparation of brain tissues

After the animals decapitated, the brains were dissected out and rinsed in ice-cold saline to remove the excess blood thoroughly, dried and weighed. The whole brain was homogenized in phosphate buffer (pH 7.0). The volume of the buffer used depends on the weight of the tissue, and usually kept at 10% (brain mass: the buffer volume). The homogenate was centrifuged at 4000 rpm for 15 minutes at 4°C. The clear supernatant was separated for biochemical assays. All the processes were carried out in cold conditions [21]. For histopathological examination, whole brains, representing each group, were fixed in 10% formalin before assaying.

D. Biochemical analysis

Biochemical analyses were performed in both serum and brain tissues of all rats. In serum, the measurements of amyloid beta peptide (Aβ1-40, pg/ml) and tau protein (tau pg/ml) were performed using Rat Amyloid Beta Peptide 1-40 (Ab1-40) ELISA KitCatalog # MBS2022403and Rat Tau Protein ELISA KitCatalog # MBS725098 respectively from MyBiosource, Inc, Southern California, San Diego (USA). Insulin hormone was estimated by RIA technique using EMD Millipore Corporation Catalog # RI-13K (St. Louis, Missouri, USA). In addition to measurement of neurotransmitters activities such as noradrenalin (NA), dopamine (DOP) and 5-hydroxytryptoamin (5-HT) expressed in ng/ml according to the methods of reported by Zagrodzka et al. [22].

In brain tissue homogenate, calmodulin (CaM, mmol/mg), calcium adenosine 5′-triphosphatase (Ca^{++} ATPase) magnesium adenosine 5′-triphosphatase (Mg^{++} ATPase) were estimated according to the method described byVig et al. [23]. The activities of these ATPase enzymes in tissue homogenate were expressed as μmol of inorganic phosphate (PO_4^{3-}) liberated/min/mg protein. The inorganic phosphate was measured according to [24]. The protein content was assayed by the method of Lowry et al. [25].

E. Histopathological examination

The brains were fixed in 10% formalin for 24 h then; they were washed with tap water, and dehydrated with serial dilutions of alcohols (methyl, ethyl, and absolute ethyl). The specimens were cleared in xylene and embedded in paraffin at 56 °C in a hot air oven for 24h. Paraffin – bees wax tissue blocks were prepared for sectioning at 4 μm with a sliding microtome. The tissue sections were collected on glass slides, deparaffinized, and then routinely stained with hematoxylin and eosin [26] for histopathological examination through the electric light microscope.

III. RESULTS

A. Biochemical analysis

Lead acetate intoxicated group showed a significant increase in the activity of Aβ-40, tau and insulin as compared to control group and this adverse effect was more pronounced in level of tau more than Aβ -40 (table 1).In SLE-treated group, serum Aβ-40, tau protein decreased by 24.6 % and 2.2 %, respectively and insulin increased by 0.36 % from lead intoxicated group. Figure (1) showed the effect of SLE administration on the activities of serum neurotransmitters, namely, NA, DOP and 5-HT in lead intoxicated group. The concentrations of neurotransmitters were found to be significantly decreased in lead intoxicated group when compared with controls. The i.p injection of SLE reverted back this alteration to near the normal levels.

Table (2) showed that intoxication with lead caused a significant increase in the concentration of CaM by more than 20 folds compared to the rats in control group. In addition, concomitant decreases in the activities of Ca^{++} ATPase and Mg^{++}ATPase compared to the animals in the control group, were recorded. Treatment with SLE significantly ameliorated these figures, in particular, the tissue contents of Ca^{++}ATPase and Mg^{++}ATPase enzymes.

Table 1:-SerumAβ-40, tau protein and insulin concentration in sera of different groups

Parameters / Groups	Control group	Lead intoxicated group	SLE-treated group
Aβ-40(pg/ml)	10.30 ^{a} ± 0.64	39 ^{b} ± 1.92	35 ^{c} ± 2.37
Tau (pg/ml)	57.57 ^{a} ± 3.21	240.19 ^{b} ± 4.59	110.37 ^{c} ± 3.86
Insulin (µIU/ml)	23.78 ^{a} ± 2.5	11.39 ^{b} ±1.61	19.92 ^{c} ± 1.87

Values represent means ± S.E.
Values bearing different superscript in the same raw are statistically different.

Table 2-: Concentration of CaM, Ca^{++}ATPase and Mg^{++} ATPase in rats of different groups

Parameters / Groups	Control group	Lead intoxicated group	SLE-treated group
CaM (mmol/mg)	2.59 ^{a} ±0.21	45.62 ^{b} ±4.53	26.52 ^{c} ±2.42
Ca^{++}ATPase, µmol *	1.43 ^{a} ± 0.08	0.56 ^{b} ± 0.08	1.20 ^{a} ±0.05
Mg^{++}ATPase, µmol *	1.64 ^{a} ±3.76	0.53 ^{b} ±0.11	1.18 ^{ac} ±0.07

* µmol of Pi liberated/min/mg protein.
Values represent means ± S.E.
Values bearing different superscript in the same raw are statistically different.

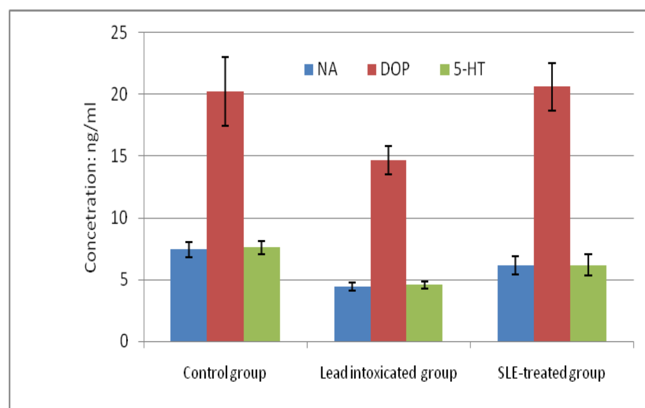

Fig 1:- Levels of neurotransmitters (NA, DOP, 5-HT) in different groups

B. Histopathological observations:

1) Control group

Animal's brain has normal histo-architecture of the meninges, cerebral cortex, hippocampus (subiculum, fascia dentate and hilus), striatum, substantia nigra and cerebellum were appeared in Figure 2(a-f), respectively.

2) Lead intoxicated group

Histopathological examination of different parts of brain revealed that, there was a presence of nuclear pyknosis and degeneration in the neurons of cerebral cortex, (Fig.3, a), subiculum, (Fig., 3b), and few neurons of the fascia dentate, (Fig.3,c), in hippocampus. But, the striatum showed focal eosinophilic plagues formation, (Fig.3, d), congestion in the blood vessels, (Fig.3, e), and vacuolization in the matrix. (Fig. 3, f). There were losses of the nuclei and Nissl's granule in the neurons of the substantia nigra. (Fig.3, g&h). The cerebellum showed mild congestion in blood vessels (Fig.3 i).

3) SLE- treated group

As a result of histological observations, there was no histopathological alteration in cerebral cortex, striatum and medulla oblongata. But, the hippocampus showed few neuronal degeneration and pyknosis as detected in Figure 4, (a-e).

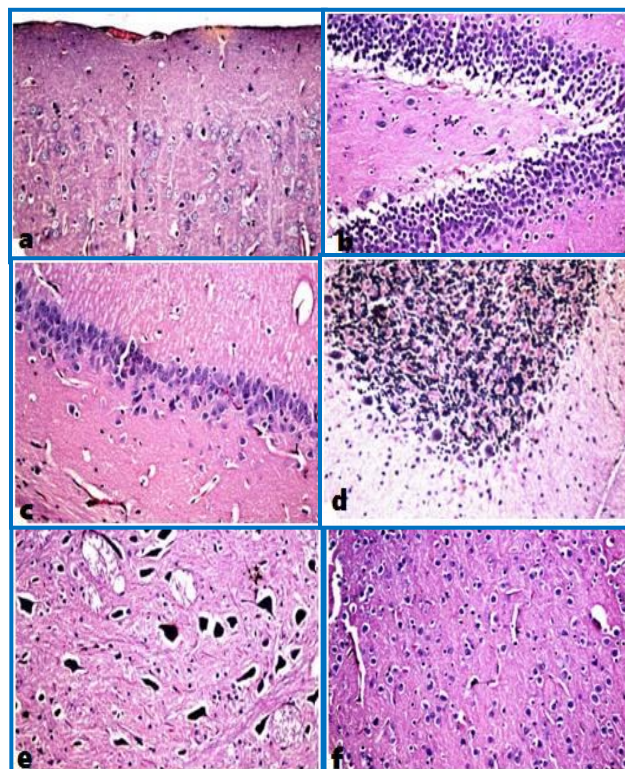

Fig 2:- Photomicrograph of brain section of control rats

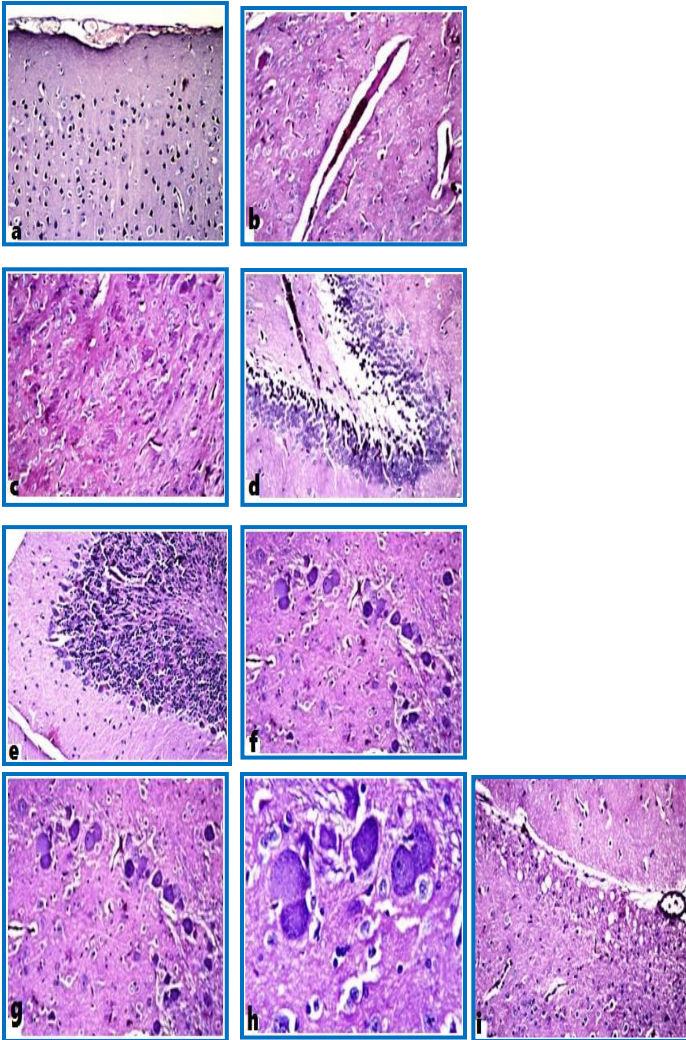

Fig 3:- Photomicrograph of brain section of lead intoxicated group

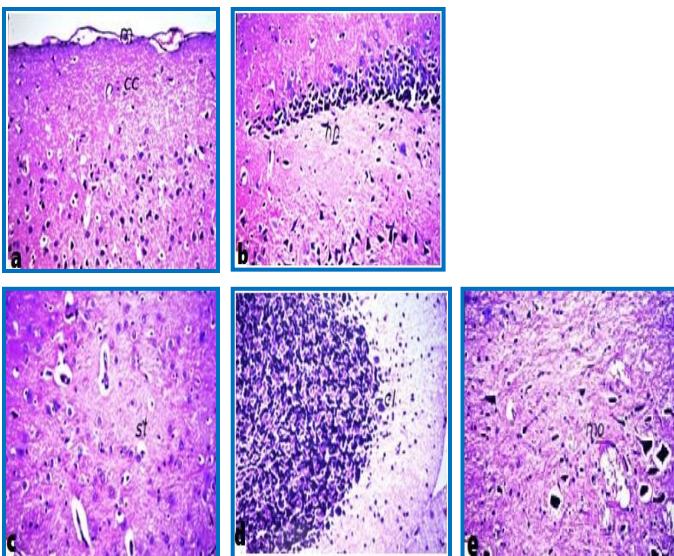

Fig 4:- Photomicrographs of section rat's brain in SLE group

IV. DISCUSSION

Although, the brain has high potential sensitivity to environmental toxicants-induced oxidation, the endogenous defense systems have low antioxidant activity [20]. Lead is considered the most ubiquitous toxic metal detectable in environment and the major health concerns of which relate to its developmental neurotoxicity [27]. This fact was consistent with the present results, since there was an increase in rat's serum tau protein and Aβ-40 post lead intoxication of rats. Both of these proteins are well known as a prognostic biomarker for neurotoxicity represent higher burden in the brain [28]. Tau protein is mainly expressed in the neurons of the central nervous system where it exerts a key role in regulating microtubule dynamics, key components of axonal transport and in signal transduction [29, 30, 31]. In neurodegenerative conditions, tau protein gets hyperphosphorylation due to inappropriate activation of several proline-directed kinases. As a result, tau misfolds and self-aggregates into insoluble fibrillary structures that form neurofibrillary tangles, dystrophic neurites, and neuropil threads [32]. Intraneuronal accumulations of fibrillary tau disrupt neuronal cytoskeletal networks and axonal transport, leading to synaptic disconnection and progressive neurodegeneration. Thus, an increase in serum tau level correlated with severity of brain injury and this phenomenon has been shown in the most prevalent tauopathy [33, 34]. On the other hand, the stimulation of the gene expression and phosphorylation of tau protein is regulated by insulin. Thereby, the reduction in serum insulin level in lead intoxicated rats may be attributed to impact of lead targeted signaling cascade of glucose through interference with certain divalent ions (zinc and calcium) that are known to be essential for pancreatic activity resulted in reduction of β-cell capacity to produce insulin [35]. Consequently, the regulation of neuronal and glial functions which depended on insulin were disrupted such as survival, metabolism, gene expression, protein synthesis, cytoskeletal assembly, synapse formation, neurotransmitter function, and plasticity [36, 37].

In the brain, Mg^{2+}-ATPase plays a role in maintaining high brain levels of intracellular magnesium ions to control the rates of protein synthesis and cell growth [38]. While, Ca^{2+}-ATPase modulates the cellular Ca^{2+} homeostasis which plays a crucial pivotal role in many dynamic processes in nerve cell, when Ca^{++} hemostasis fails; the production of peroxyl radicals threatens the cell viability [39]. The predominant intracellular receptor of Ca^{+2} is calmodulin (CaM) which once bound to Ca^{+2} , acts as part of a calcium signal transduction pathway [40]. To clarify, CaM has dual actions, directly on the vesicles to enhance the transmitter release and to activate Ca^{+2}–ATPase and Mg^{+2}–ATPase to reduce the free Ca^{+2} in the cytosol, and indirect action to inhibit the transmitter release [12]. Thus, the alteration in calmodulin and Ca^{+2}–ATPase and Mg^{+2}–ATPase levels by lead intake suggested either a direct interaction of lead with the Ca^{2+} pump or by permanently destroying the membrane

integrity and permeability [39] or evoked calmodulin conformation and its affinity for select targets [41, 42]. As a consequence, the production of neurotransmitters became insufficient resulting in an impairment of cell signaling and neuronal dysfunction [28]. As report by Gill et al. [43], lead caused impairment of neurotransmitters release via interfering with Ca^{2+}, calmoduline and disrupts the activity of synaptotagmin, which is an important protein mediated release of transmitters [44]. In particular, lead down-regulates dopamine, a neurotransmitter that plays an essential role in the inhibitory pathways of basal ganglia [14, 45]. The incidence of down-regulation of dopamine metabolism in brain may be as a result of lead accumulation in the brain caused disruption of dopamine pools associated with increase in its degradation by monoamine oxidase enzyme [42] and alteration in availability of their precursor amino acid tyrosine [46].

A great deal of evidence supports that polyphenols may cross the blood brain barrier and exert neuromodulatory effects through selective actions on different components of a number of protein kinase and lipid kinase signal cascades [11, 21, 47]. Modulation of these pathways may underlay the ability of SLE derived polyphenols to exert their protective effects, as previously reported for the flavan-3 ol, (–)-epicatechin, which has been observed to stimulate the phosphorylation of the transcription factor cAMP-response element binding protein (CREB), a regulator of neuronal viability and synaptic plasticity [28, 48]. On the other hand, ellagic acid which is one of SLE constituents has an effective role in inhibiting amyloid deposition [49]and in chelating the divalent cations [50, 51]. Along with other authors, polyphenols has the ability to activate the neurotransmitter synthesis in neurons by facilitating neuronal and microglial growth gene pathways [28]. or by modulating enzymatic breakdown/removal rate and all of these pathways were performed via Ca^{++}-ATPase [47, 52]. Polyphenols preserve cerebral vasculature and neuronal loss by enhancing cerebral blood flow, local endothelial repair mechanisms, retarding platelet aggregation and augmenting vasodilatation [7, 53]. These facts were in a harmony with the obtained results, whereas, rats injected with SLE showed a modulation in brain function and structure biomarkers.

On the other hand, the potential bioactivity of SLE depended on the method of solvent extraction and the prepared ethanolic extract was reported as the highest in reducing power, scavenging effect and antioxidant property [52, 53, 54]. Thus, the potential action of SLE may be also rely on its direct action of scavenging pathological concentrations of reactive oxygen and nitrogen species as well as on the ability on chelating the transition metal ions [55] resulted in breaking the vicious cycle of oxidative stress and recovery the development of neurotoxicity [56].Importantly, SLE contained other chemical compositions in addition to polyphenols such as malic and citric acids, sugar, pectin, vitamin C, potassium, manganese, fiber, omega-3, folate, magnesium, and copper [53, 54]. Each of SLE components has a private biological function that may be interacted separately or synergistic ally to mitigate exacerbate of brain injury in case of lead toxicity.

The present findings of microscopic histopathological examination of brain sections of rats were similar to those reported by [57, 58, 59] who found that rats received lead acetate showed degeneration in the neuron cells, nuclear pyknosis and dilatation in the lumen of the blood vessels. i.p. injection of Strawberry leaves extract recovered most of the pathological alterations associated with lead acetate administration.

V. CONCLUSIONS

Based on the experimental results reached, it may be concluded that, Strawberry leaves extract can be considered as promising sources for group of agents with pharmacological activities and is highly recommended phytochemical for targeting the neurotoxicity post lead pollution. However, due to collected contributions of a number of researchers, the field has recently become more receptive to alternative concepts, opening the doors to exciting new avenues of investigations and therapeutic strategies. Therefore, further research is required in order to establish the underlying mechanisms involving the neuro-therapy effects of strawberry leaves extract and focus on the investigation of exact biological activities contributing the well-known beneficial usage.

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