PROTECTIVE EFFECT OF *MYRTUSCOMMUNIS L.*ON ARSENIC-INDUCED PATHOLOGICAL CHANGES ON BRAIN TISSUE OF WHITE RATS.

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ABSTRACT

The study was performed on twelve white rats of approximately of the same body weight (200-220 gms). It divided equally into four groups.The 1st group was injected with (10mg/kg B.W) arsenic chloride intraperitoneally, 2nd group was injected with (10mg/kg B.W) arsenic chloride intraperitoneally and (3 mg/ml) *myrtle extractL*. 3rd group was normal control, rats treated orally with(3 mg/ml) *myrtle extract* only .4th group was injected with (0.2ml) distal water as a control group**.**At the end of the experiment, the animals were sacrificed,and small specimens(2cm³) were taken from brain to histopathology preparation. The microscopic examination of histopathological sections of the brain of the1st group was showed severe pathological changes characterizedby an area of degeneration,vacuolation of microglia and pyknosis of neuron also there are edema and hemorrhage. On the other hand, the 2ndgroup showed less pathological changes compared by 1st group which characterized by less gliosis, less vaculation of microglia with few number of astrocyte.The neuron showed normal.while, the 3rd and 4th groups showed normal neurons, microglial, and astrocyte

INTRODUCTION

Arsenic is a common element in the earth. It is widely distributed in the land, water and air (1).It enters the food chain by dissolution in groundwater, burnings of fossil fuels, fertilizers pesticides, rivers, rain, and industrial wastes(2).Long period exposure for arsenic resulted in cancers of the bladder, skin, lungs and liver, as well as it causes dysfunction of nervous and renal tissues (3).It considers a dangerouseffect to the animals, human and environment, It has oxidative effects and causes anincrease in hydrogen peroxide production that represents oxidative stress factor (4).Many Studies documented that arsenic generates ROS in brain tissue (5) and it inducesmany of pathological changes such as destruction of DNA,in the brainespecially. The cortex of the cerebral and cerebellar considered main target by arsenic.Arsenic causes lipid peroxidation as primary mechanisms for toxicity, loss of mitochondrial membrane potential and it induces (ROS) (6&7).The putative neuroprotective action of antioxidants has been verified in rat models of ischaemia-induced memory impairment (8) and Alzheimer's disease (9).Many of medicinal plants used for treatingmany of diseases. Medical plants have antioxidant action which play a veryimportant role ininhibitionof free radicals andtheyprotect against the infections (10). The medicinal plants represent sources of natural antioxidants .They have great benefit against arsenic toxicity in the brain tissue (11).

Myrtus communis L. known as True myrtle is extensively used in biomedical and aromatic industry(12).*Myrtus communis L*is a medicinal plantused worldwide asan alternative medicine. *Myrtus communis L* leaves, fruits, and berries used in folk medicine for a long time. Also, They used in treating some diseases such as diarrhea,skin diseases, peptic ulcer,inflammation and haemorrghic .Many studies suggest *Myrtus communis L.*hastherapeutic and pharmacological effects such as antiviral,anticancer,anti-diabetic, antioxidative, antibacterial, antifungal, neuroprotective,and hepatoprotective activity(13).Arsenic is an element spreading in the environmental, water and

food could contain contaminant by Arsenic,this work aimed to discuss therapeuticeffects of the *Myrtus communis L.* in arsenic-induced neurological deficits in rats.

MATERIALS AND METHODS

Twelve white rats (200-220gm) were obtained from animal house of Veterinary Medicine,College of AL- Qadisiya University.Animals were acclimatized for seven days at 12h. Light/dark cycle. The animals were housed in plastic cages in an airconditioned room with thetemperature maintained at 25±2 C. Rats were given food pellets and water ad libitum. They were divided randomly into four groups (3 rats each) and were treated for 30 days.

Chemicals:

Arsenic chloride is a heavy metal obtained from acentral laboratory atALQadisiya University. Arsenic chloride(BDH chemical Ltd(England)). The rats were administered 10 mg/kg B.W(14)**.**

Myrtle ethanol extracts preparation:

*MyrtusCommunis*L. (1 kilogram)were obtained from the Iraqilocal markets. Leaves of *MyrtusCommunis*L.were converted to powder by grinding. The powder was extracted by use hydro-ethanol mixture (one litter) (80/20, v/v) for 8 h. Thisstep was recureseveral times. Afterwards, the filtrate was pooled and concentrated under the vacuum at a temperature not exceeding 60°C. The obtained *MyrtusCommunis L.* alcoholic extract was stored at -20°C before being used(15). The concentrated extract was then poured into sterile Petri dishes and dried at 40˚C. The dried powder was then collected and mixed with distilled water at concentrations of 3 mg/ml(16).

Experimental design:

Twelve white rats, both sexes were divided into four groups (3 rats each) randomly and treated as follows:

-1st group: injection (10mg/kg B.W) arsenic chloride intraperitoneally.

-2nd group : injection (10mg/kg B.W) arsenic chloride intraperitoneally+ (3 mg/ml) *myrtle extract*

-3rd group: normal control rats weretreated orally with(3 mg/ ml) *myrtle extract* only

-4th group : injection (0.2ml) distal water as acontrol group**.**

Tissue samples:

The histopathology is done by taking (1-2) cm^3 slices from brain tissue. The sample fixedwith formalin10% for 2 weekand processed in series of graded ethanol solution They were embedded in paraffin, serially section at (5Mm) for each sample by using microtome apparatus (juny 4291, west Germany) stainned with Eosin and Haematoxylin and examined underunder light microscope(17).

RESULT

Eosine and haematoxylin used in staining the samples to see histopathological changes in nervous tissue.In the 1st group, there were sever pathological changes characterizedby an area of degeneration (Fig.2). vaculation of microglia, pyknosis of neuron&cerebral odema(Fig.3&4). Also, there are hemorrhage in brain tissue (Fig 5). On the other hand, the 2nd group showed fewer changes compared by 1st group which characterized by less number of microglia(less gliosis) with few numbers of astrocyte, the neuron showednormal with angular shape also there is mild vaculation with neuron tissue.(Fig.6&7). while the 3rd and 4th group revealed normal multipolar neurons, microglialand fibrous astrocyte(Fig.8&1)

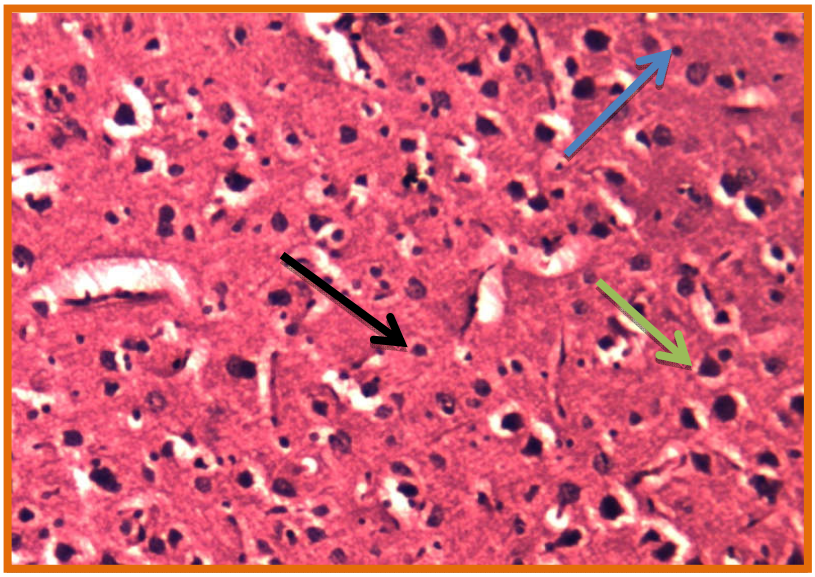


Figure (1): Histological section of brain(cerebrum) in control rats showing normal multipolar neurons with ganglion appears to be haphazard (green arrow), microglial(black arrow) and fibrous astrocyte(blue arrow) 40H&E.

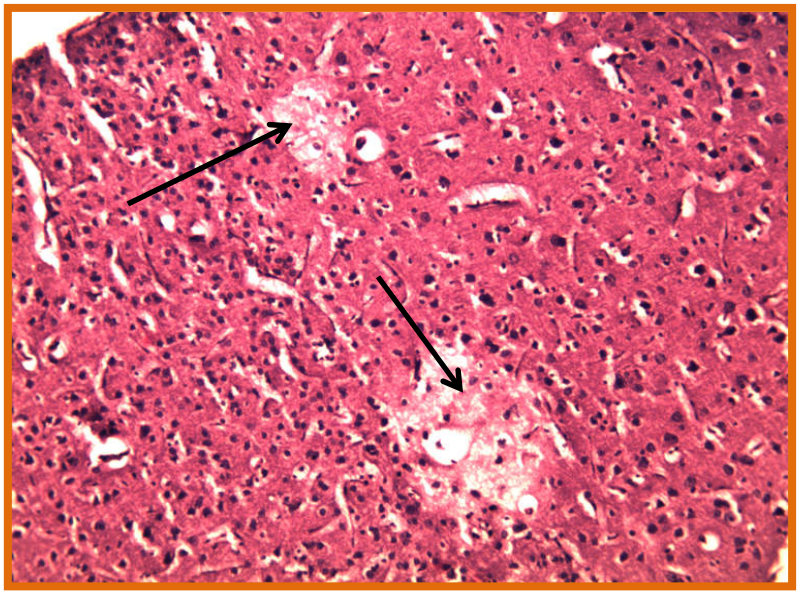


Figure (2): Histopathological section of brain(cerebrum) in rats treated with arsenic chloride (10mg/K.W) showingdegeneration of neuron(black arrow) 10H&E.

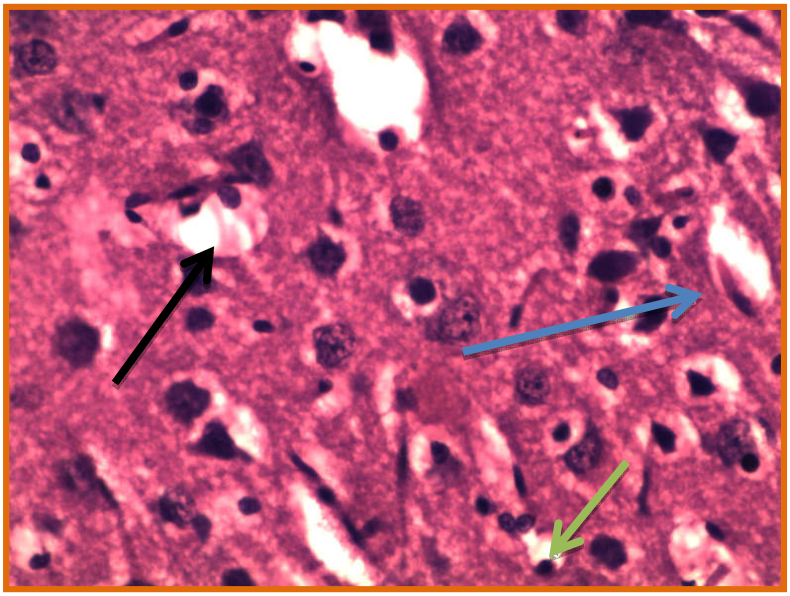


Figure (3): Histopathological section of brain(cerebrum) in rats treated with arsenic chloride (10mg/kgB.W) showingvacuolation of microglia (black arrow)pyknosis of neuron(green arrow) and cerebral edema (blue arrow).40XH&E

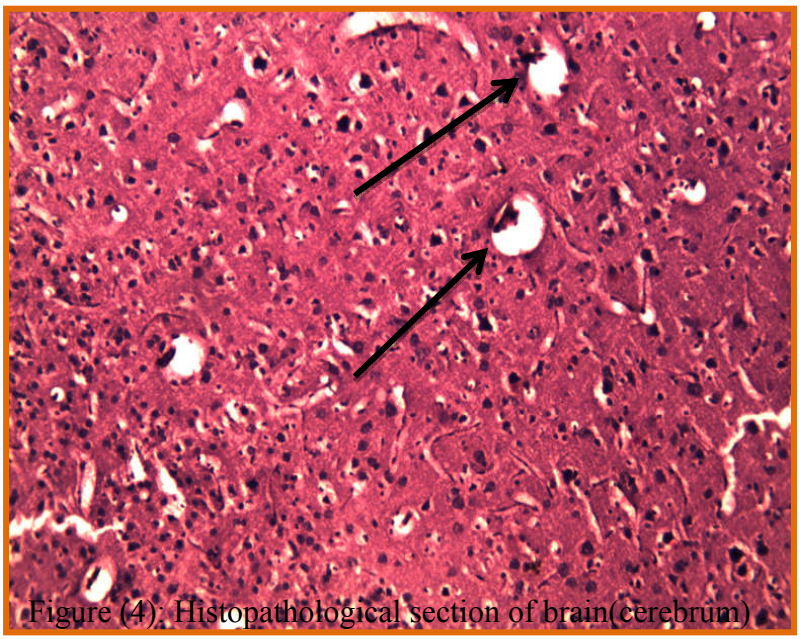


Figure (4): Histopathological section of brain(cerebrum) in rats treated with arsenic chloride (10mg/kgB.W) showing extravascular edema (black arrow)10XH&E

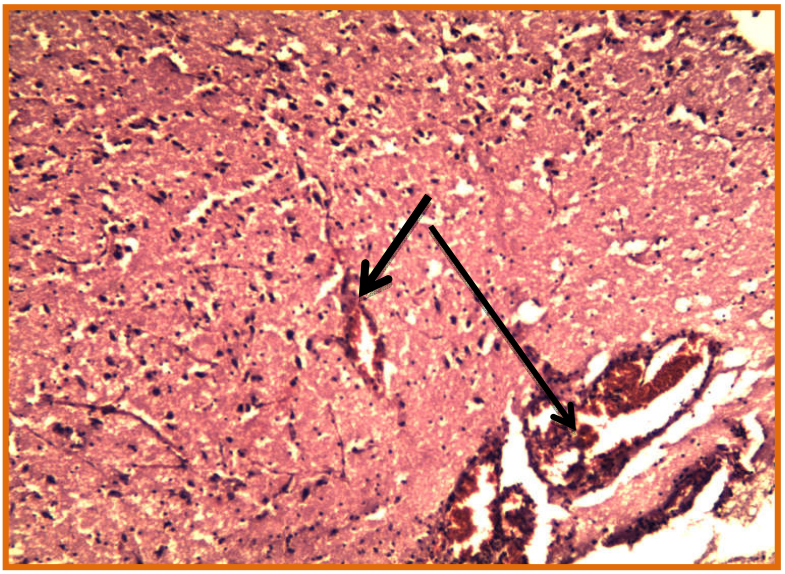


Figure (5): Histopathological section of brain(cerebrum) in rats treated with arsenic chloride (10mg/kgB.W) showing hemorrhage in brain tissue(black arrow)10XH&E

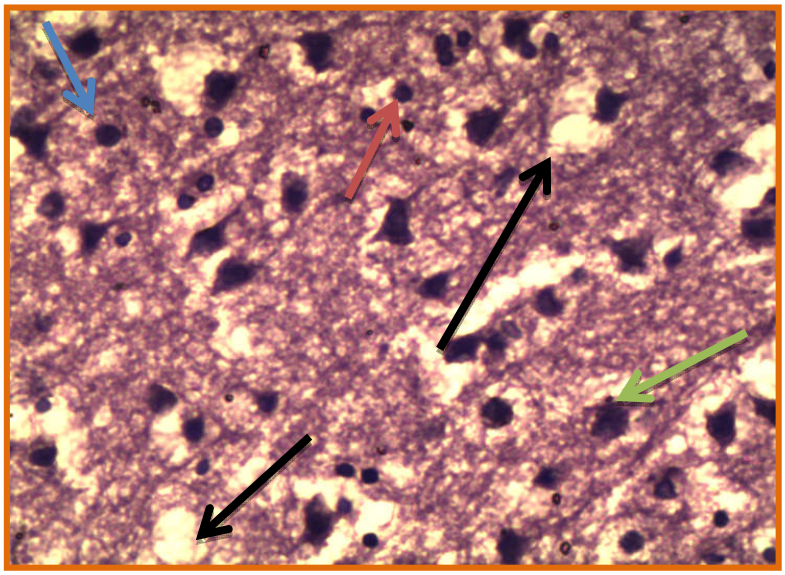
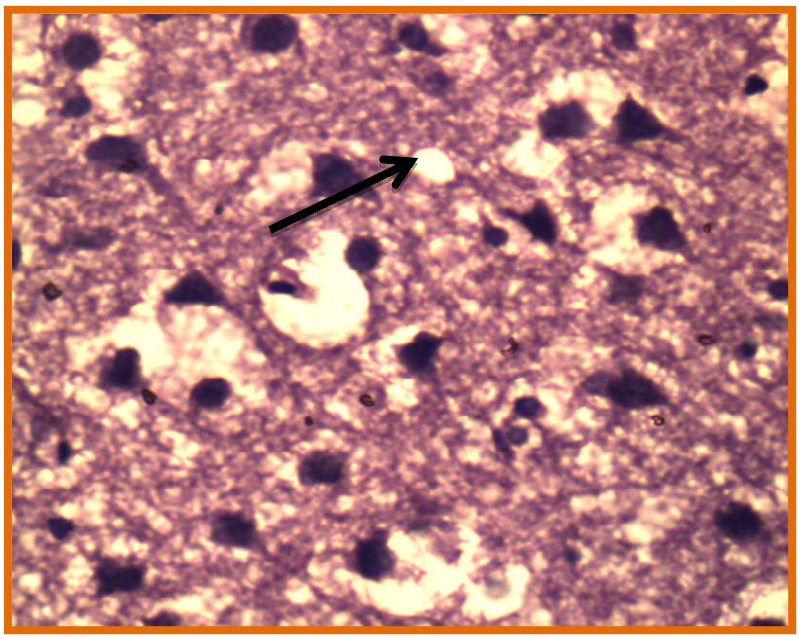


Figure (6): Histopathological section of brain(cerebrum) in rats treated with arsenic chloride (10mg/kgB.W) and *myrtuscommunis L*(3mg/kg B.W)showing less number of microglia(less gliosis)(red arrow) with few numbers of fibrous astrocyte(blue arrow) the neuron show normal with angular shape(green arrow), also there is mildvaculationwith neuron tissue(black arrow)40XH&E



Figure(7):Histopathological section of brain(cerebrum) in rats treated with arsenic chloride (10mg/Kg B.W) and *myrtuscommunis* L (3mg/Kg B.W) showing less vaculation of microglia(black arrow) .x40 H&E

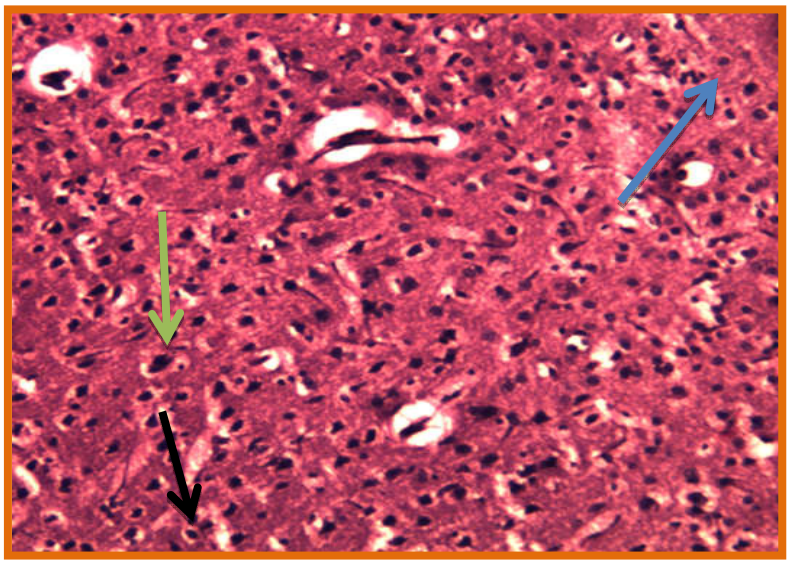


Figure (8): Histopathological section of brain(cerebrum) in rats treated *myrtuscommunis L*(3mg/kg B.W)showing normal multipolar neurons(green arrow), microglial(black arrow) and astrocyte(blue arrow). 40H&E.

DISCUSSION

There are some studies documents brain damage by arsenictoxicity(18). Arsenic can pass through the blood-brain barrier.It has effects on white matter in the brain (19). The current study evaluated the actions of alcoholic extract of *Myrtuscommunis* L. (MCL) on arsenic-induced pathological changes in brain tissues of white rats.The histopathological examination revealed degeneration and pyknosis of neuron in the 1st group.Also, there were vaculation of microglea.Thisfinding is in agreement with previous study (20) ,in which goats brain tissues that intoxicated by arsenic. Arsenic can increase lipid peroxidation by oxidative stress, that will cause impairment in a dynamic balance between antioxidant and peroxidant(21).Previously, it has been found that increased oxidative stress and very little of glutathione playan important role in pathological changes in the brain tissue due to arsenic toxicity(22,23).It also has been found thatdecreasing in GSH triggers the activation of neuronal 12-lipoxygenase, which leads to ultimately cell death and the production of peroxides (23).In addition, it has found that there was edema, intracellular space, andhemorrhage ,this finding is agreement with previous studies (24,25)in which that rats received 100ml/L with sodium arsenate after six weeks revealed congestion of cerebral blood vessel and edema. On the other hand, rats treated with both arsenic chloride and extract of *MCL*.revealed anameliorate in histopathological alteration induced by arsenic after 30 days.

Histopathology of thebrain in the 2nd group showed normal sized glial cellswith mild cytoplasmic vacuolization, aneuron showed normal with angular shapeand few number of astrocyte. It has been found that the effectiveness of MCLis attributed to its antioxidant properties. Therapeutic use of extract reduced MDA levelmarkedly, which might be related to extracting free radical scavenging properties (26).

In conclusionthe current studyrevealed that arsenic poisoning leads to severe oxidative and impairment in the brain tissues in the rats and that could decrease the toxicity by *MyrtusCommunis* L. administration. Treatment with *MyrtusCommunis* L. accelerates recovery of antioxidant enzymes and reduce lipid peroxidation in experimental animals that intoxicated by arsenic.

MyrtusCommunis L. extractedcan react with free radicalsand convert it to final inactive products,remove all radical substances, and prevent damage toproteins, DNA and lipids against free radical (27).

Control groups showed normal glial cells,normal nucleated neurons, and pyramidal cells consist of multilayers.

التاثير الواقي لاوراق نبات الياس على التغيرات النسيجية التي يحدثها الزرنيخ في نسيج الدماغ في الجرذان البيضاء

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الخلاصة

أجريت الدراسة على (١٢) جرذ ابيض وزن ٢٠٠-٢٢٠ غم مقسمة بالتساوي الى اربع مجاميع على النحو التالي: المجموعة الاولى اعطيت كلوريد الزرنيخ بجرعة ١٠ ملغم/ كغم من وزن الجسم داخل البريتون بينما اعطيت المجموعة الثانية كلوريد الزرنيخ بجرعة ١٠ ملغم/ كغم من وزن الجسم داخل البريتون مع المستخلص الكحولي لاوراق الياس ٣ملغم / لتر من الماء فمويا ، المجموعة الثالثة اعطيت مستخلص اوراق الياس فقط بجرعة ٣ملغم/لتر فمويا اما المجموعة الرابعة اعتبرت كمجموعة سيطرة وأعطيت ٢و٠ مل من المحلول الدارئ المتعادل مره واحده يوميا لمدة ٣٠ يوم داخل البريتون. تم قتل جميع الحيوانات وتم اخذ قطع صغيرة من الدماغ وارسلت للتقطيع النسيجي .اظهر الفحص ألمجهري للتقطيع النسيجي للدماغ في المجموعة الاولى تغييرات نسيجية شديدة تميزت بتنكس في خلاي الدماغ ، وجودتجويف بالخلايا الدبقية كذلك لوحظ نزف وتجمع للسوائل حول الاوعية الدموية للدماغ،،اما المجموعة الثانية فقد كانت التغيرات النسيجية اقل مقارنة بالمجموعة الاولى حيث لوحظ قلة بالخلايا الدبقية مع وجود فجوات بسيطةفيها كذلك قلة في خلايا النجمية . بينما لوحظ بالمجموعة الثالثة والرابعة خلايا عصبية طبيعية.

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