HISTOLOGICAL AND HISTOCHEMICAL EFFECTS OF MORPHINE SULPHATE ADMINISTRATION ON CEREBELLUM OF THE ALBINO RAT

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Abstract

In this experiment the effect of morphine sulphate on the cerebellum of albino rat was investigated by applying histological, histochemical and morphometric techniques. Animals were orally given morphine sulphate tablets (MST) dissolved in sterile saline at a dose level of 5 mg / kg body weight day after day for 10, 20 and 30 days. Histological results revealed that the molecular layer showed cytoplasmic vacuolation in both stellate and basket cells with deeply stained pyknotic nuclei. The Purkinje cells lost their specific flask shaped appearance with the decrease in their size and numbers. In some sections they appeared pyknotic while in others they completely disappeared. The granular cells showed a high degree of deformation, shrinkage, deep staining and degenerated necrotic areas which make them appeared less packed and separated from each other. Histochemical results indicated that the total protein contents showed a progressive depletion in all cells of cerebellar cortex, and the diffusely stained cells as a result of necrotic changes. Most of the

cerebellar cortex cells displayed a conspicuous decrease in the amount of general carbohydrate contents with weakly faint coloured cytoplasm. Also, the white matter revealed a noticeable diminution of stainability compared to the control. The quantitative analysis showed a significant decrease in the diameter of Purkinje cells ($P<0.05$) in all treated groups. Moreover morphine also induced a significant decrease ($P < 0.05$) in the thickness of both molecular and granular layers of the cerebellum of all treated rats.

Introduction

Narcotic analgesics have an ability to relieve pain, thus they play an important role in medical therapy. Although non-narcotic analgesics are capable of alleviating mild to moderate pain, only the narcotics afford relief from severe pain. Unfortunately the administration of narcotic analgesics also may result in physical dependence, no narcotic yet synthesized is completely free of this effect (Craig and Stitzel, 1997). Morphine, the first narcotic analgesic used therapeutically, comes from the unripe seed capsules of Papaver somniferum, the opium poppy. It is still frequently used in the management of severe pain, but suffers from the drawback that it often causes nausea and vomiting. Perhaps for this reason it is not particularly popular as an illicit drug or abuse or dependence (Ghodse, 1989).

The toxicity of morphine has been widely investigated from the physiological, biochemical and histopathological point of view in a variety of experimental animals. Some experimental studies repoted that toxic effects of morphine on different body organs depend not only on the overdose and duration of morphine, but also on the age of treated animals (Abdel-Moneim et al., 1997 and Abdel –Moneim, 2001), suggesting that morphine effects depend on the development of morphine binding sites at different ages. On the other hand, Hassanein (1993 a,b) studied the influence of morphine sulphate on the structure of some organs of mice. He suggested that the

morphological alterations of the liver cells can be attributed to a direct action of morphine on the liver cell receptors and concluded that morphine increases the susceptibility to hepatic disorders and the tendency to develop chronic liver diseases due to oxygen deficiency in the liver lobule. Also, he explained the alteration observed in the lung that resulted from the depressant effect of morphine on the brain respiratory tidal volume and accumulation of carbon dioxide causing the lung tissue suffers from oxygen lack (hypoxic hypoxia) leading to the lung injury. Foster et al., (2001) studied the behavioural and neurochemical responses evoked by morphine and d- amphetamine. Their results suggested that the leterodorsal tegmental (LDT) neucleus plays a critical role in mediating the motoric and neurochemical effects of diverse drugs of abuse, and that the site of the drug action may critically determine whether a drug's efficacy will be enhanced or attenuated by loss of this nucleus.

El-Sherif et al, (2002) studied the histological, histochemical and ATPase localization in the rat liver after morphine sulphate induction. Their results revealed that all doses given resulted in a high degree of increase for carbohydrates with higher granulation and depletion of proteins content and a significant loss of enzymatic activity. Therefore, the present study was carried out to clarify the effect of repeated administration of an addictive drug, morphine on the histological, histochemical and morphometric appearance of the rat cerebellum, in atrial to elucidate the cause of the cereberal injury in narcotic addicts.

Materials and Methods

The experiment was carried out on one hundred rats weighing from 130 – 150 gm. Food and water were available ad libitum. Rats were divided into four equal groups (25 rats in each). The first group(GI) served as a control group and was given oral dose of 5 ml saline, while the other three groups were orally given morphine sulphate tablets dissolved in a strile saline in a dose level of 5 mg / Kg. body weight day after day for 10, 20 and 30 days (GI, GII and GII) respectively. This dose is equivalent to the human therapeutic dose. At

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the end of the intended periods of drug application, animals of different groups were sacrificed by cervical dislocation. Cerebellum was removed and cut into small slices fixed in 10% formalin and carnoy's fluid. Fixed materials were embedded in paraffin wax and sections of 5 microns thickness were cut. Slides were stained by haematoxylin and eosin for histopathological examination. Total proteins were detected using the mercury bromophenal blue method (Mazia et al., 1953). General carbohydrates were demonstrated using periodic acid Schiff's technique (PAS) (Hotchkiss, 1948).

Morphometric techniques:

The changes in thickness of molecular layer, granular layer and the changes in diameter in Purkinje cells were measured in the haematoxylin and eosin stained section, by using caliberated ocular scale grid. The measurments were expressed in micrometers and the mean values were calculated for each group.

Statistical evaluation:

The numerical data obtained in the current study were presented in tables as mean ± standard deviation. The significances of the differences between the means was calculated according to students " t" test (Snedecor and Cochran, 1967).

Results

Effects of morphine sulphate on the rat body weight:

Table (1) showed that treatment of animals with morphine sulphate resulted in a significant decrease in body weight of all treated groups as compared to the control (P < 0.05).

Histological investigations:

Control animals:

The cerebellum consists of an outer cortex of gray matter and an inner white matter. In the gray matter three distinct cell layers can

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be distinguished, an outer molecular layer, an inner granular layer and a central layer of Purkinje cells. The molecular layer contains outer stellate cells whose unmyelinated axons normally course in horizontal direction. Descending collaterals of more deeply placed basket cells arborize around the Purkinje cells. The granular layer contains numerous small granular cells with dark staining nuclei and little cytoplasm. Also, scattered larger stellate cells or Golgi II cells with typical vesicular nuclei and more cytoplasm are present. Throughout the granular layer there are small irregularly dispersed; clear space called the glomeruli (Figs. 1,2). The Purkinje cells are typically arranged in a single row at the junction of molecular and granular cell layers. Their large "flask shaped" bodies give off one or more thick dendrites, which extend throughout the molecular layer giving off complex branchings. The thin axon leaves the base of Purkinje cell, passes through the granular layer, and becomes myelinated as it enters the white matter (Figs. 1,2).The white matter consists of myelinated nerve fibres or axons and also contains dendrites and numerous neuroglia. It forms the core of the numerous cerebellar folds (Fig. 3).

Treated animals:

Upon treatment with morphine sulphate tablets (MST) for 10 days (GII), the cerebellum sections revealed remarkable pathological changes. The molecular layer appeared with highly vacuolated cytoplasm in both stellate and basket cells, some stellate cells showed degenerative changes in some areas (Fig.4). Also, the Purkinje cells were slightly shrinking and reduced in their size showing a mild degree of cytoplasmic vacuolation and faintly stained nuclei. The granular layer cells appeared polymorphic reduced in their size showing a slight degenerative changes and became loosen and separated from each other (Fig.5).The white matter showed some vacuoles (Fig. 6). After treatment with morphine sulphate for 20 days (GIII), the microscopic examination of the cerebellum revealed a conspicuous deleterious changes observed in Purkinje cells, where they lost their specific " flask shaped" appearance and appeared cloudy, deformed, rounded, small in size with pyknotic nuclei and lost their cell boundaries (Fig.7). In the granular layer some areas showed a high degree of deformation, shrinkage with deeply stained cells and loosely packed cells appeared highly separated from each others (Fig. 8), another areas of the granular cells were compact and fused together

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forming degenerated necrotic areas (Fig. 7). The molecular layer appeared to be less affected,where both the basket and stellate cells decreased in their size showing more cytoplasmic vaculation and pyknotic nuclei (Fig.8). The white matter had numerous and wide degenerative foci which were detected in the core of the folia (Fig. 9). After 30 days of morphine sulphate treatment (GIV), The molecular layer showed a progressive degree of fibricity and a wavy appearance of nerve fibres (Fig. 10). Both basket and stellate cell nuclei showed degeneration and pyknotic structures. Evidently, the more striking sign of cerebellar cortex destruction, was well discerned at the Purkinje cell layer which displayed an obvious signs of advanced degeneration and pyknotic nuclei varied in shape and size (Fig. 11). Also, in some sections they decreased in their number or completely disappeared. The granular layer cells revealed severe signs of damage and lost their normal organization and shape like structure, they clearly deformed and appeared polymorphic (Fig. 11). Moreover, both cells types the glomeruli and Golgi II cells were deeply stained, and the neighbouring cells fused together producing eosinophilic sheets representing clear coagulative necrosis. The white matter also manifested distinct histopathological alterations where it showed degenerated nerve fibres and contained large vacuoles (Fig.12).

Histochemical investigations:

Total protein contents :

Control animals :

Total proteins were visualized in the form of positive bluish or bluish green colour. The outer molecular layer of the rat cerebellum exhibited a moderate to strong reaction in these preparations. The cytoplasm of stellate and basket cells was homogenous stained, the nuclei generally exhibited a diffuse weak reaction, while the nucleoli illustrated a strong reaction (Fig. 13). Purkinje cells exhibited a markedly positive reaction in the form of small bluish granular particles randomly distributed or in a diffusely stained cytoplasm. The nuclear structures revealed strong positive reaction. The granular cells, as well as the Golgi cells displayed a moderate reaction with bromophenol blue, the cellular membrane were positively stained while the remaining nuclear inclusions exhibited a

weak reactions (Fig.13). Also, the white matter exhibited a positive reaction in the form of small bluish granules randomly distributed or in a diffused form (Fig. 14).

Treated animals

In rats receiving MST at a dose of 5 mg / kg body weight for 10 days, a slight reduction in the protein stainability was observed (Fig. 15). The constituent cells cytoplasm in the molecular layer showed a reduction of proteinic inclusions, while the Purkinje cells appeared with a heterogenous distribution of the total protein contents. However, some cells appeared deeply stained due to their shrinkage, but in general Purkinje cells showed a reduction in protein reactivity. An obvious decrease of protein stainability in the ground cytoplasm of the granular cells. Also, the nuclei and chromatin particles of these cells were lightly stained. The white matter exhibit a strong positive reaction due to thickness of the nerve fibres (Fig. 16). After 20 days of MST administration, the above description of protein depletion was more pronounced as in (Fig.17). The molecular layer displayed a weaker protein reactivity and the protein elements become less in the amount in the cytoplasm of the constituent cells. However, some nuclei appeared deeply stained due to their pyknotic features. The Purkinje cells showed further reduction in proteinic inclusions defusely stained. The degenerated cell cytoplasm was devoid of these proteinic inclusions to a large extent. The granular layer cells showed a high degree of depletion of protein elements while the necrotic cells cytoplasm exhibit a deeply stained in a diffuse pattern. In the white matter the proteinic inclusions were more pronounced in the thick condensed nerve fibers between the large vacuole (Fig. 18). After 30 days of MST treatment, a remarkable depletion in the protein content was well discerned in the most of cerebellar cortex cells (Fig. 19). In addition most of degenerated cells exhibited clear signs of a negative protein stainability, and the diffusely stained cells as a result of narcotic changes. While the white matter still exhibited pronounced positive reactions due to the condensation of thick nerve fibres between the large vacuoles (Fig. 20).

General carbohydrates:

Control animals:

PAS – technique utilization revealed a distinct distribution of the polysaccharides in the control cerebellar layers as illustrated in (Fig. 21). The molecular layer exhibited a moderate to strong PAS – reaction. The cytoplasm and the cell processes, as well as their individual fibres were positively stained and the nuclei were negatively stained . The Purkinje cell layer manifested positive reaction for PAS as seen in the same above figure, the ground cytoplasm of these cells displayed a strong magneta red colouration, in which case carbohydrate inclusions appeared as diffused form in the ground cytoplasm. The nuclear structures as well as the cell membrane were positively stained. The granular layer cells exhibited a strong PAS reaction. The cytoplasm showed dense positive diffuse reaction, indicating the presence of high carbohydrate contents. The nuclear structures, the cell membrane as well as the cell processes and their individual fibres were positively stained. The white matter showed a highly strong PAS reaction (Fig.22). The thick myelinated fibres displayed a strong magenta red colouration indicaling the presence of high carbohydrate contents.

Treated animals

Treatment with morphine sulphate induced a gradual decrease in the amount of the general carbohydrate contents in the different treated groups. After 10 days of oral administration, the PAS preparations revealed a slight decrease of carbohydrate contents in the cerebellar cortex and the medulla as compared to the contral group (Figs. 23, 24) . After 20 days treatment with MST, the cerebellar cortex showed marked decrease of carbohydrate contents, while the Purkinje cells as well as the white matter were moderately stained (Figs: 25, 26). With continuous treatment with MST for 30 days most of the cerebellar cortex were faintly stained and displayed a conspicuous decrease in the amount of general carbohydrates. The white matter revealed a noticeable diminution of stainability in a comparison with the control (Figs: 27, 28).

Morphometric results:

The quantitative decrease in the diameter of Purkinje cells of cerebellum under the effect of oral administration of morphine sulphate tablets is shown in (table 2). The results revealed a significant decrease (P < 0.05) in the diameter of Purkinje cells of all treated groups. The diameter mean values were 12.240; 9.600 and 7.440 after 10, 20 and 30 days respectively compared to the control group.

In addition, morphine caused significant reduction in the thickness of molecular layer of cerebellum as shown in (table 3).The results showed a significant decrease (P< 0.05) compared to the control. The thickness means were 144; 129.2 and 117.2 after 10, 20 and 30 days respectively compared to the control. Also, morphine caused marked reduction in the thickness of the granular layer when compared with the control (P <0.05) (table 4).

Discussion

In the present study, the most impressive alterations in the cerebellum of treated rats with oral morphine sulphate administration at a dose of 5 mg / kg body for different periods of times was vacuolated cytoplasm of both stellate and basket cells in the molecular layer, some of them showing degeneration and pyknotic structures. Also, the molecular layer showed a progressive degree of fibricity and wavey appearance of nerve fibres after 30 days of morphine treatment. Moreover, the Purkinje cells revealed a conspicuous deleterious changes where they lost their specific " flask shaped" appearance, reduced in their size, pyknotic nuclei and lost their cell boundaries. Also, the granular layer cells appeared polymorphic reduced in their size with deeply stained cells and loosely packed appeared highly separated from each others in some sections and after 20 days of treatment. Moreover, both cells types, the glomeruli and Golgi II were deeply stained and the neigbouring cells fused together produced eosinophilic sheets representing clear coagulative necrosis. These observations were clearly seen after 30 days of morphine sulphate treatment. Our findings are in agreement with those of EL- Banhawy et al., (1993a), who reported that morphine sulphate had frequently produced prominent alterations in the histological structure of the

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et al., (1993a), who reported that morphine sulphate had frequently produced prominent alterations in the histological structure of the cerebellum of rat. The major impairments encountered in such cases comprised mainly submeningeal oedema and haemorrhage as well as dilatation and marked congestion of the submening blood vessels. This was beside lymphocytic infiltration and inflammatory changes in this tissue. In addition , pyknosis of the nuclei and necrosis of the Purkinje cells. Tawfek *et al.,* (1997) studied the histological effects induced by indomethein on rat cerebellum. Their observations reveated vacuolation of some nerve cells , necrosis and a tendency of the molecular layer to be more fibrous than control in addition to contraction of the blood vessels of cerebellar cortex. Also, Atta (2002) studied the depressant effects of morphine on cellular composition of lymphoid tissues and cerebellum in rats. His study revealed an evident degeneration of the cerebellar layers especially Purkinje cell layer, appearance of empty areas and disturbance of the arrangement of dendritic nerve fibres. He also, reported that the Purkinje cells were reduced in size, became irregular in shape and deformed. These results were parallel to those of Wang *et al.,* (1999); Katsorchis *et al.* (2001), Seo and Suh, (2001); Light *et al.,* (2002) and in our present study. In the present study, it has been also noticed that treatment with morphine sulphate for 10, 20 and 30 days had exerted a marked diminution in the protein content in the cells of the cerebellar cortex layers. EL-Banhawy *et al.,* (1993b) concluded that the destruction of the Golgi appartatns by Ketamine anaesthesia had resulted in the inhibition of protein synthesis and consequently the reduction of the observed total protein contents. Tawfek *et al.,* (1997) reported that the histochemical effects induced by indomethein on rat cerebellum were decrease of plasma protein contents and elevation of blood glucose. Protein synthesis is greatly affected by morphine sulphate,where depletion of proteins was found to be directly proportional to the dose and duration which agrees with Gulyi *et al.,* (1992); Matthew and Ray (1999) and EL Sherif *et al.,* (2002).

The present investigation showed also a decrease in the general carbohydrate contents after treatment with morphine sulphate. These results are in agreement with the results of Lux *et al.,* (1989), who illustrated that morphine caused a time – and dose dependent decrease in liver glycogen levels and was more potent in causing

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glycogenolysis. Also, Tawfek *et al.,* (1997) found that carboydrate content decreased in all cerebllum especially in molecular layer. While, our results are in disagreement with Ashry *et al.,* (1988) who found that morphine adminie administration elevates glycogen stores, both quantitatively and qualitatively. Such elevation exhibited a dose dependent characteristic with higher dose exerting a double fold increase. Also, El- Sherif *et al.,* (2002) found that all doses given of morphine sulphate resulted in high degree of increase of carbohydrates and a significant loss of the enzyme activity.

It may be interpreted that contradiction between our results and those of aforementioned investigators is due to interlaboratory variabilities of dosing, duration of exposure, route of administration which make comparison of toxic response to various narcotics difficult.

Regarding the effect of morphine administration on morphometric and quantitative analysis, the present results showed that morphine induced significant decrease (P< 0.05) in body weight of treated rats as compared to the control. Also, it induced a significant decrease (P< 0.05) in the diameter of Purkinje cells and in the thickness of molecular layer as well as granular layer in all treated animals. In this respect histological and morphometric study demonstrated a relationship between the changes in cells of cerebellar cortex and morphine consumption. These results are in agreement with the results of Bhargava *et al.,* (1995) who indicated that morphine depressed weights of lymphatic tissues and cellularities in mice and suggested that the effects of morphine on the immune system are at least partially mediated through opioid receptors. Demiri *et al.,* (2002) revealed that morphine caused significant decrease in the molecular layer thickness of the rat cerebellum and lowered Purkinje cell numbers in unit length of the Purkinje cell layer. Granular layer of the males was affected more profoundly than those of the females. While Metwally and Zaghloul (2000) concluded that numerus and significant histo pathological and cytological alterations were recorded after three weeks of exposure to acute noise stress and revealed signs of degeneration and karyolysis of Purkinje cells. Also, a significant (P< 0.05) increase in the mean diameter and the mean number of Purkinje

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cells was observed, while the neuroglia revealed a highly significant (P<0.01) increase in the mean number.

From these results, it is speculated that the histological lesions found in the cerebellum tissue of rat treated with morphine may be interpreted as a result of toxic effect of morphine on the oxidative enzyme systems of the cerebellar tissues, therefore the tissue metabolites will not be oxidized and the cerebellar tissues suffer from oxygen lack (histotoxic hypoxia) leading to the cerebellar injury. The histological alterations observed in the present study may also be explained as a result of the depressant effect of morphine on the brain respiratory centers that lead to a decrease in the respiratory tidal volume and accumulation of carbon dioxide causing that the cerebellar tissue suffer from oxygen lack (hypoxic hypoxia) leading to the cerebellar injury.

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TABLE 1: Showing effect of MST on body weight of rats.

Statistical analysis	Control rats			Treated rats		
	10 days	20 days	30 days	10 days	20 days	30 days
Mean	142.72	145.56	147.15	133.64	122.12	110.56
SD	5.3	4.2	6.1	5.63	4.68	5.90
SE	1.06	0.84	1.22	1.13	0.94	1.18
P				< 0.05*	< 0.05*	< 0.05*

N=25   *: Significant   SE: Standard error.   SD: Standard deviation.

TABLE 2: Showing effect of MST on the diameter of Purkinje cells of cerebellum.

Statistical analysis	Control rats	Treated		
		10 days	20 days	30 days
Mean	17.640	12.240	9.600	7.440
SD	1.578	2.185	1.555	1.158
SE	0.315	0.437	0.311	0.232
P		< 0.05*	< 0.05*	< 0.05*

N=25   *: Significant   SE: Standard error.   SD: Standard deviation.

TABLE 3: Showing effect of MST on the thickness of molecular layer of cerebellum.

Statistical analysis	Control rats	Treated			
		10 days	20 days	30 days
Mean	182.4	144	129.2	117.2
SD	6.633	7.071	7.593	6.782
SE	1.327	1.414	1.519	1.357
P		< 0.05*	< 0.05*	< 0.05*

N=25   *: Significant   SE: Standard error.   SD: Standard deviation.

TABLE 4: Showing effect of MST on the thickness of granular layer of cerebellum.

Statistical analysis	Control rats	Treated			
		10 days	20 days	30 days
Mean	136	112.4	97.2	86.8
SD	5	4.359	6.782	6.271
SE	1	.872	1.357	1.524
P		< 0.05*	< 0.05*	< 0.05*

N=25   *: Significant   SE: Standard error.   SD: Standard deviation.

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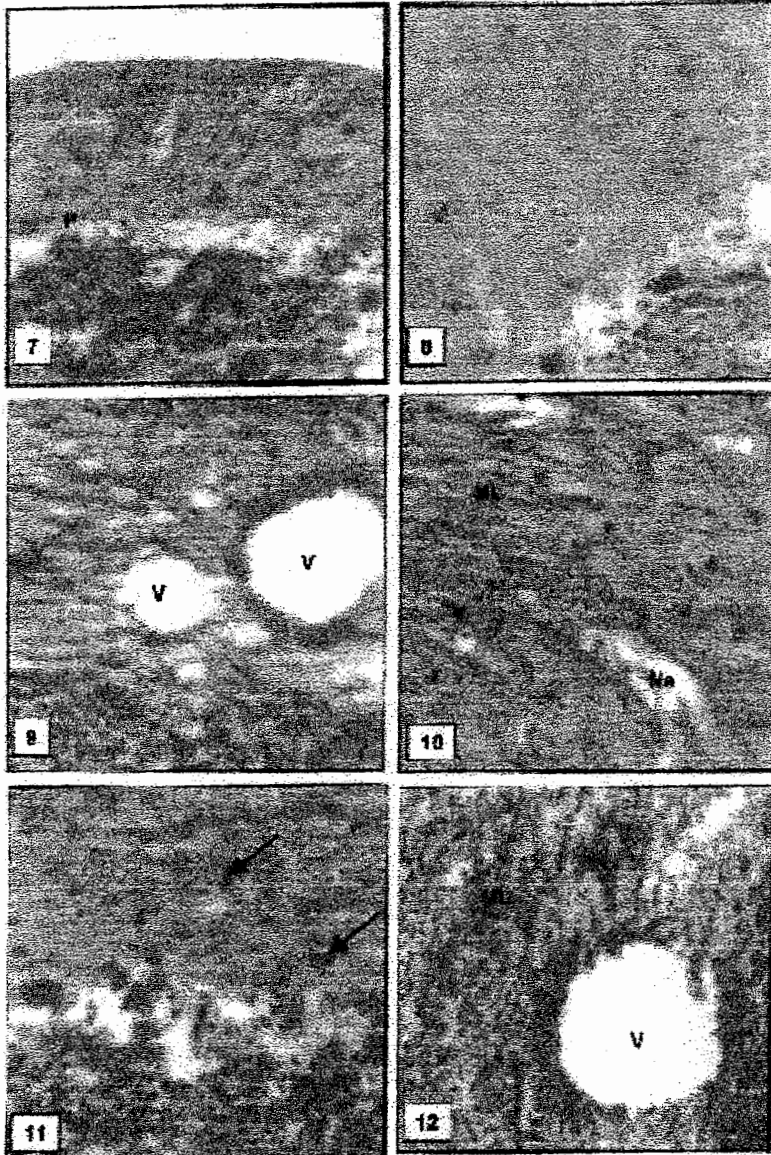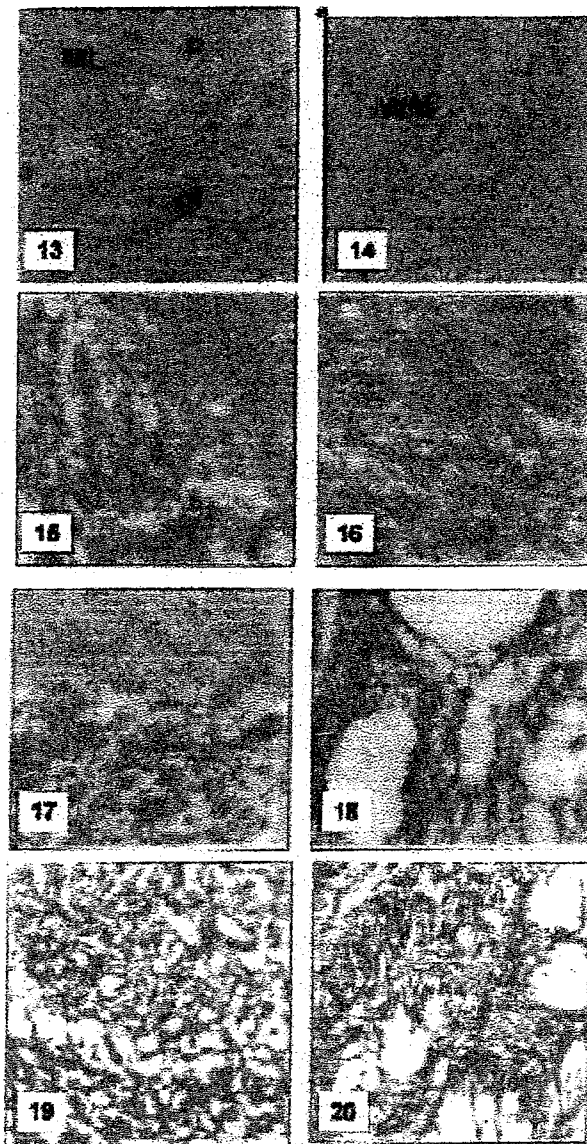
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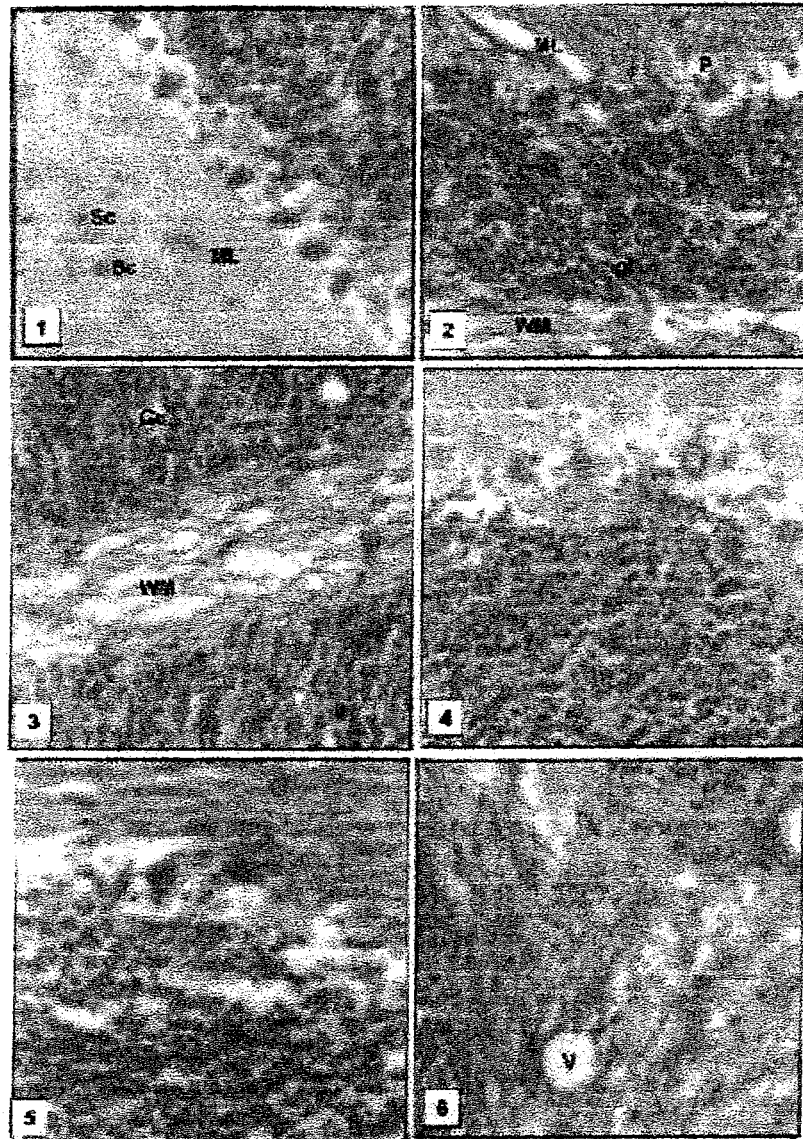
PLATE2.JPG (510x660x16M jpeg)

PLATE3.JPG (510x660x16M jpeg)

PLATE4.JPG (510x660x16M jpeg)
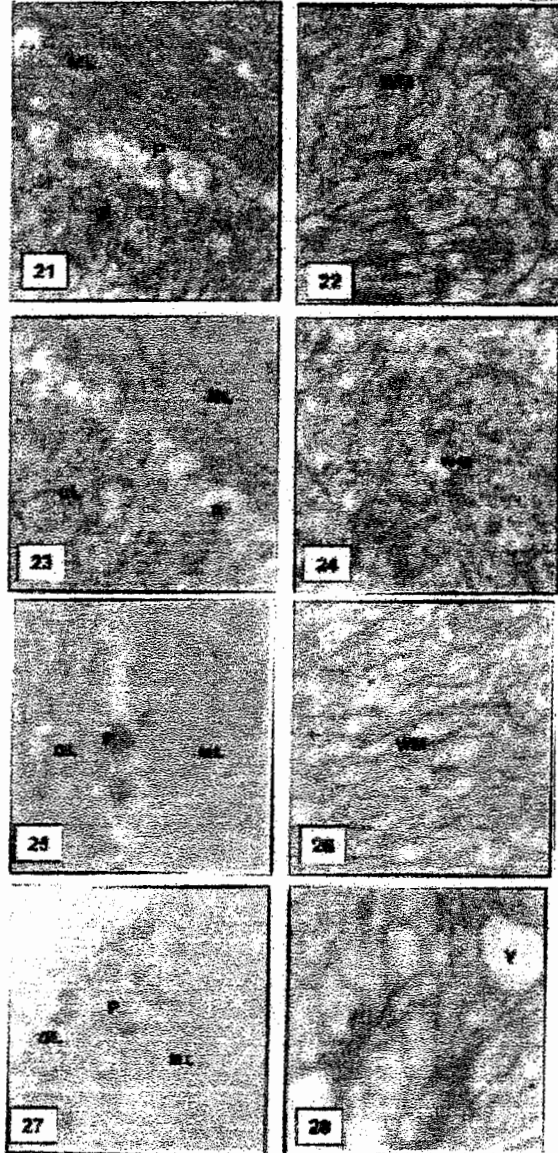
التأثيرات الهستولوجية والهستوكيميائية لعقار كبريتات المورفين على مخيخ الجرذان البيضاء

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ملخص البحث :

فى هـذا البحـث تم دراسة التغيرات النسيجية والنسيجوكيميائية والمورفومترية التى تحدث فى مخيخ الجـرذان البيضاء نتيجـة المعالجـة بعقار التخدير كبريتات المورفين وقد قسمت حيوانات التـجارب إلى أربعـة مجموعـات : تركت المجموعـة الأولى للمقارنة بينـما تم حقن الثلاث مجموعات الأخرى يوم بعد يوم بجرعة قدرها ٥ ملى جرام من وزن الجسم لمدة ١٠ ، ٢٠ ، ٣٠ يوم .

تم فحص العينـات بعد الـتوقف مـن اعطاء العقار وقد لوحظ تأثر المخيخ نسيجيا بصورة ملموسة نتيجـة المعاملـة بهـذا العقار ممـا أدى ذلك إلى حدوث فجوات سيتوبلازمية فى خلايا الطبقة الجزيئية وأصبحت أنويـتها داكنه الاصطباغ وفقدت خلايا بركنجى شكلها الكمثرى وصغرت فى الحجم وشوهت واختفت زوائدهـا امـا خلايا الطبقة المحببه فقد اصبحت اقل تماسكاً واصبحت انويتها داكنة الاصطباغ وشـوهدت ضمور خلاياها فـى بعـض المناطق كما شوهدت علامات تخريبية فى المادة البيضاء انعكست بصورة واضحة عـلى هيئـة فجـوات منتشرة فى تلك الطبقة وقد زادت حدة هذه التغيرات بزيادة مدة المعالجـة . أيضاً احـدث هـذا العقار انخفاضاً ملحوظاً فـى محـتوى الـبروتينات الكـلية ومحـتوى الكربوهيدرات وازدادت حدة التغيرات بازدياد فترة المعالجة .

كمـا تضـمن البحـث دراسـة إحصائية كمية لمتوسط أوزان الفئران أثناء فترة المعالجة وكذلك أقطار خلايا بركنجى وسمـك كل من الطبقة الجزيئية والطبقة المحببة وقد تم جدولة تلك البيانات ومقارنتها بالمجموعة الضابطة .

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