



Remedy Efficiency through Paclitaxel on Rat (*Rattus norvegicus*) Brain Injury and Risk Premium of N- Diethyl nitrosamine (DEN) Injection

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Abstract

The current study deals with the effect of N-Diethylnitrosamine (DEN) induced traumatic brain injury on male albino rats, as well as the outcome results of treatment with paclitaxel nanoparticles for a period of 8 weeks with two-week intervals is the concern of the present study. Mean body weight, as well as brain weight, was considered as the main parameters whereas a detailed immunohistochemical study on rat brain sections was performed. Astrocytic biomarkers for the diagnosis of astrocytes by fibrillary glial acidic protein (GFAP). Neuronal GFAP staining used for various broke sections were forwarded. Comparison and Contrast of all these parameters in all steps of the experiment had been discussed. The efficiency of the treatment was found to be evident. The effect on body weight was significant whereas brain weight results showed insignificant differences. A longer duration of treatment may well reflect more significant results.

Keywords: Paclitaxel Nanoparticles, N-Diethylnitrosamine, Immunohistochemistry, Neuron, Fibrillary Glial Acidic Protein (GFAP), Brain injury, Rats.

1. Introduction

Cancer is a major public health problem as it is one of the most prevalent disorders around the globe due to unavailability of the effective drugs so far however mortality rate associated with this disease seems to exceed globally even that of cardiac disorders [1].

"N-Nitrosodiethylamine (NDEA)" which is an organic compound with the empirical formula of "C₄H₁₀N₂O" with IUPAC name of "Diethyl nitrous amide, it is known as "N-Ethyl-N-nitrosoethanamine" or "Diethyl nitrosamine [2]. This compound is highly light-sensitive, and yellow in color, it is soluble in water and several organic solvents, as well as in lipids [3]. This compound is widely used in several industrial processes. Still, by heating, it discharges nitrogen oxide which is a highly toxic fume [4]. Biologically it negatively affects the DNA integrity through

that is alkylation [5]. It is widely used in different research projects to induce cancer in the liver cell because of its carcinogenic nature [6]. Diethyl nitrosamine has also been reported to affect the disturbance of the structure of arginine (amino acid) [7]. Among all carcinogenic known agents, NKK is considered one of the strong cancer-causing agents which induce cancer in a laboratory to rat lungs and liver. Further, it was also found to induce cancer in the aerodigestive tract and lungs in several animals like a hamster, mice, and rats [6,8]. Paclitaxel was reported to be an efficient novel drug against cancer [9]. However, Paclitaxel as a drug was successfully used as an anticancer drug and for the management of the deadliest and most prevalent ovarian cancer, as well as the advanced stage of breast cancer. After administration of this drug, it was found that it can be absorbed by the spleen and liver in large quantities still trace amounts may be found in the brain, kidney, lungs, and heart [10].

Lipid peroxidation that is caused by Diethyl nitrosamine [11,12], showed the possible effect on crossing or breaking the blood-brain barrier, which might increase brain oxidative stress and may lead to neurotoxicity. Diethyl nitrosamine (DEN) is common for its hepatotoxicity and nephrotoxicity effect, but so far only limited studies performed on its a few research have examined its neurotoxicity effect and cytotoxicity against breast cancer [11,13,14]., who proposed more investigation on the degree of oxidative stress and morphological alterations in rats, and cerebellar cortex after chronic exposure to DEN. Some common examples of TBI-related biomarkers are neuron-specific enolase, brain-derived neurotrophic factor, tau protein, neurofilament, myelin basic protein, S100 calcium-binding protein B (S100B), interleukin 6 (IL-6,) and glial fibrillary acidic protein (GFAP) [13,15].

The present study aims to investigate and follow up the effect of the single dose of N-Diethyl nitrosamine against one dose concentration of treatment with Paclitaxel Nanoparticles (PX NPS), in rat brain and body weight through chemotherapy. All variations and effects have been monitored and examined for a period of 8 weeks on two weeks' intervals. actually, the investigation is to point out the most suitable period of time for the applied dose on brain injuries in albino rate.

2. Materials and Methods

2.1 Animals studies

The present investigation dealt with thirty (30) male Albino Domestic rats, (*Rattus norvegicus*) weighting around 250-300g B.W. at the beginning of each experiment. animal house of the Department of All animals were maintained at a constant temperature ($23 \pm 1^\circ\text{C}$), on a 12h dark/light cycle, rats were maintained on standard rat pallets manufactured to a formula supplied by the Medical Research of Soran University. All experimental protocols were approved by the Ethical Review Committee ERC- 26-113, Soran University, Soran-Iraq.

2.2 Experiment design

Experimental animals (rats) were divided into six groups each of five (n=5). Table 1 shows the treatment schedule of each group. Distill water (DW) were used as a solvent for reconstitution of DEN, and also for Paclitaxel suspension. The rats were divided into five groups (n=8). Table 1 showed the treatment schedule of each group. Distil water (DW) used for both as a solvent for reconstitution of extract, and also for gliopalamide suspension. The project was designed to examine and find out the effect of a single dose (80) mg/kg of carcinogenic ability of N-Diethyl nitrosamine "C₄H₁₀N₂O" (13). with a single dose concentration on the whole rat as and on rat brain as an organ before and after treatment with paclitaxel C₄₇H₅₁NO₁₄(14) cared out for a period of 8

weeks with 2week interval as follows: Out of the total of 30 rats, five rats as one group used as negative control. Whole remaining 25rats were injected with 80mg/kg(0.5ml). Five rat was scarified after two weeks of injection and regarded as positive control. Then after the remained 20 rats were faced treatment with paclitaxel 80mg/kg daily for a period of 8 weeks every 2weeks scarification of (5 rats) as a group took place whole animal weight and brain weight took place and recorded fortnightly.

Table 1: Experimental design of the treatment group.

Groups	Name of group	Treatment and dosage
I	Normal control(NC)	Rats treated with 0.5ml of Distil Water
II	0.5 ml (DEN) (PC)	80mg / kg BW (DEN) injected intraperitoneally for 2 weeks
III	0.2ml (Paclitaxel)	(0.2ml) paclitaxel injected intraperitoneally after 2 weeks
IV	0.2ml (Paclitaxel)	(0.2ml) paclitaxel injected intraperitoneally after 4 weeks
V	0.2ml (Paclitaxel)	(0.2ml) paclitaxel injected intraperitoneally after 6 weeks
VI	0.2ml (Paclitaxel)	(0.2ml) paclitaxel injected intraperitoneally after 8 weeks

2.3 Determination of rat body and brain weight

An average of numerous readings was calculated in order to estimate mean weight of the whole body and brain weight. Multiple replications were performed to ensure accuracy. Then mean value was estimated and used in this paper. This procedure took place using a sensitive balance (Mettler, PN1 21 0). That have a sensitivity up to 0.01 mg.

2.4 Histological tissue processing

2.4.1. Histopathological and Immunohistochemical study

The brains were quickly removed from the skull, postmortem dissection was used to cut part the fresh brain, which were subsequently fixed in formal saline solution, brain tissues dehydrated in ascending alcohol (80-100), cleared in xylene, and embedded in paraffin. Then paraffin blocks were sliced into 4µm serial cross- sections using semi-automated microtome (Thermos scientific). Following deparaffinization and rehydration, the sections were stained with hematoxylin- eosin finally, they were mounted with D.P.X. [16]. Stained slides were photographed using the CCD digital camera (Olympus DP-12) which was attached to the light microscope (Olympus CX41) at different magnification. Histological examination for brain tissue were carried out in order to follow up any alterations or disposition that were induced by (DEN) and chemotherapy Paclitaxel injection. Histological changes in the brain tissue sections of all groups were observed and assessed by pathologist [17]. for inflammation into four categories as follows [18].

Score zero (0) = normal cells with no inflammatory in filtrate, score 1 =1-5 mononuclear cell in filtrates (minimal grade), score 2 =6-20mononuclear cell (mild grade), score3=more than 20 mononuclear cell (moderate grade), score4=multi focal coalescent inflammatory infiltrates (severe grade).

The details of Immunohistochemical analysis for brain tissues were summarized in table 2. sections were deparaffinized, rehydrated in alcohol, and then transferred to diluted target retrieved solution in PT link (Dako North American Inc) for 1hr. Next, sections were incubated with it is specific Glial fibrillary acidic protein (GFAP) Antibody for 1 hr. at room temperature. After washing samples were incubated with corresponding HRP-labeled secondary antibody for 20 min. Samples were then wash with buffer and the peroxidase activity was visualized by treating the slide with Di Amino benzidine plus chromogen for 6 min. Finally, the slides were counterstained with Mary Hematoxylin and cover slipped for examination using light microscope. successive sections from cerebral cortex of rat brain were performed. Few studies stated an accumulation of neuronal GFAP that was observed in various brain pathologies, including traumatic brain injuries [15,19].

Table 2: Characteristics of immunohistochemistry staining study.

Organ	Antibody	Type	Dilution
Brain	Glial Fibrillary Acidic Protein (GFAP)	Mouse Monoclonal	1:50

2.5 Statistical analysis

All data are expressed as means ± standard error of means (M ± SEM) and Statistical analysis was carried out using statistically available software (SPSS Version 11.5). Data analysis was made using one-way analysis of variables (ANOVA). Comparisons between groups were performed using Duncan test and unpaired student t-test. P< 0.05 was considered as statistically significant.

3. Results

3.1. Body weight

Present experiment was performed on a group of 30 albino rats with a mean body weight (M.B.W.) of about 271 gm. Results of (M.B.W) after two weeks of (DEN) injection was declined to an average of about 254.2 gm. Body weights of each group of animals (group of 5rates) was estimated after treatment with a dose (0.2 ml) of paclitaxel for two-, four-, six- and eight-weeks. And was 264, 264.6,270.8 and 253 gm respectively. The results of the present study are shown in **Table.3**.

Results of mean body weight after two, four, six and eight weeks is illustrated in **Figure(1)**.

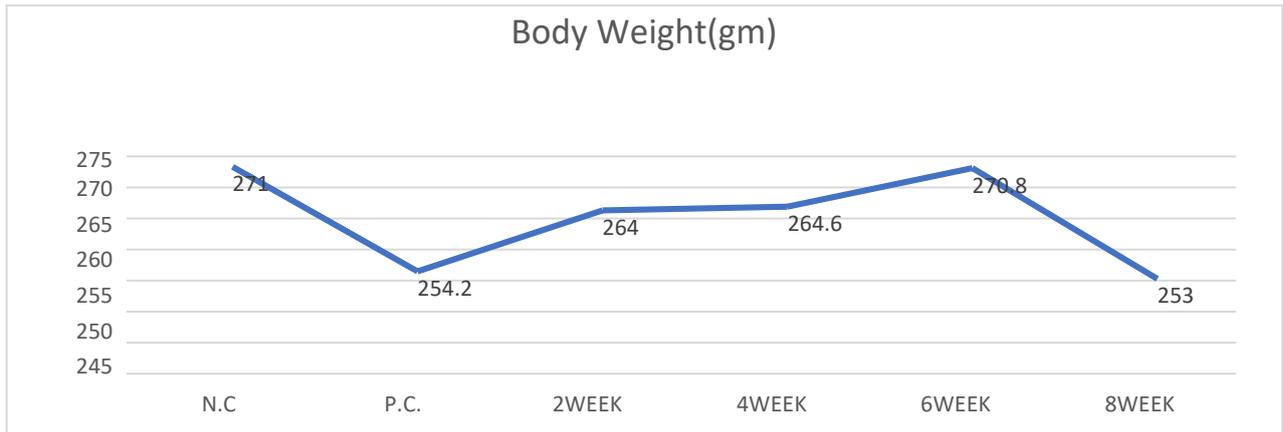


Figure 1. Body weight (gm) negative control, positive control, (0,5ml) DEN injected for 2,4,6,8 weeks and treatment with paclitaxel (0.2ml) for 2,4,6,8 weeks.

Table 3. Rat means body weight throughout experimental.

NegativeControl		Positive control injection D.E.N after 2-week injection(0.5ml)	Paclitaxel treatment			
			2 weeks	4 weeks	6weeks	8weeks
Dose		80mg/kg 0.5ml	0.2ml	0.2ml	0.2ml	0.2ml
Mean	271	254.2	264	264.6	270.8	253
M.B.W						
Gm						

3.2. Brain weight

Five rats were scarified and the mean fresh brain weight has been taken .and the results of the brain weight of the experimental rates throughout present investigation is tabulated in table 4, the weight of negative control as well as positive control group of rates that was injected by toxic DEN in (80 mg/kg 0.5 ml) is showed in **Figure 2**. fresh brain weight samples were found to be around 1.09 gm. The mean brain weight of positive control after treatment with 0.2 ml of paclitaxel was about (1.23, 1.23, 1.16 and 1.03) gm respectively for 2,4,6 and 8 weeks of treatment (Table. 4) and **Figure. 2**,

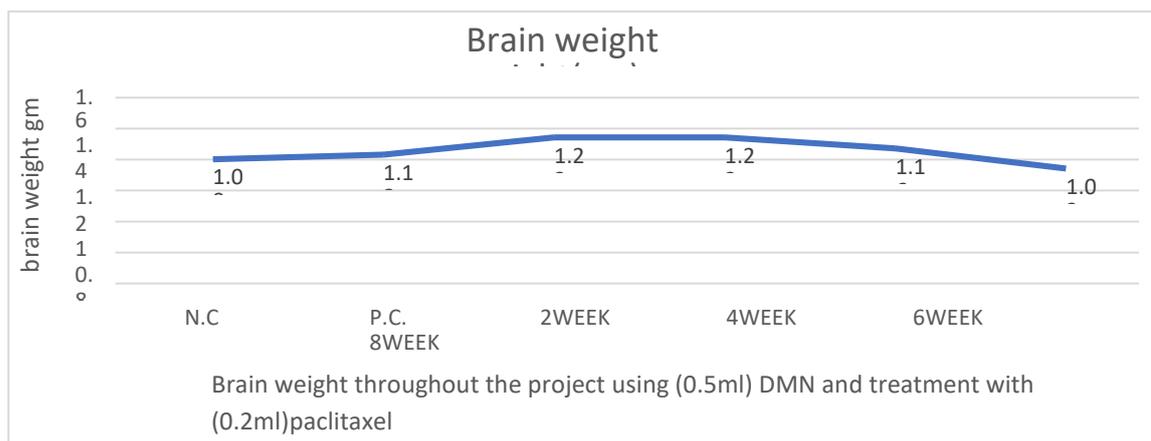


Figure. 2. Mean brain weight of all group of rates used in the study.

Table 4. Mean body weight negative control, DEN positive control, and treatment of paclitaxel.

Negative control		Positive control injection D.E.N after 2-week Injection	Paclitaxel treatment			
			2 weeks	4 Weeks	6weeks	8weeks
Dose		80mg/kg 0.5ml	0.2ml	0.2ml	0.2ml	0.2ml
Mean Gm	1.09	1.12	1.23	1.23	1.16	1.03

3.3. Histopathological findings

The brain was examined and the result indicated that injected animal with N-Diethylnitrosamine (DEN) after 2 week showed clear inflammation and glial apoptosis, necrosis of neurons, it means the brain injury appeared, that is a good indicant **Figure (3, b)** illustrated with hypertrophy, vasculature and irregular shape of neuron in granular layer. The congestion of blood vessel between the pia matter and pyramid layer. was evidence. whereas the animal after treatment with dose (0.2ml) paclitaxel **Figure (3,c)** shows that most of neuron and glia cell attend to repeal this under magnification (400X) where stain with hematoxylin and eosin where as inflammation and dis organized cortical layer shows in outer pyramidal and granular layer which bean mor crowded.

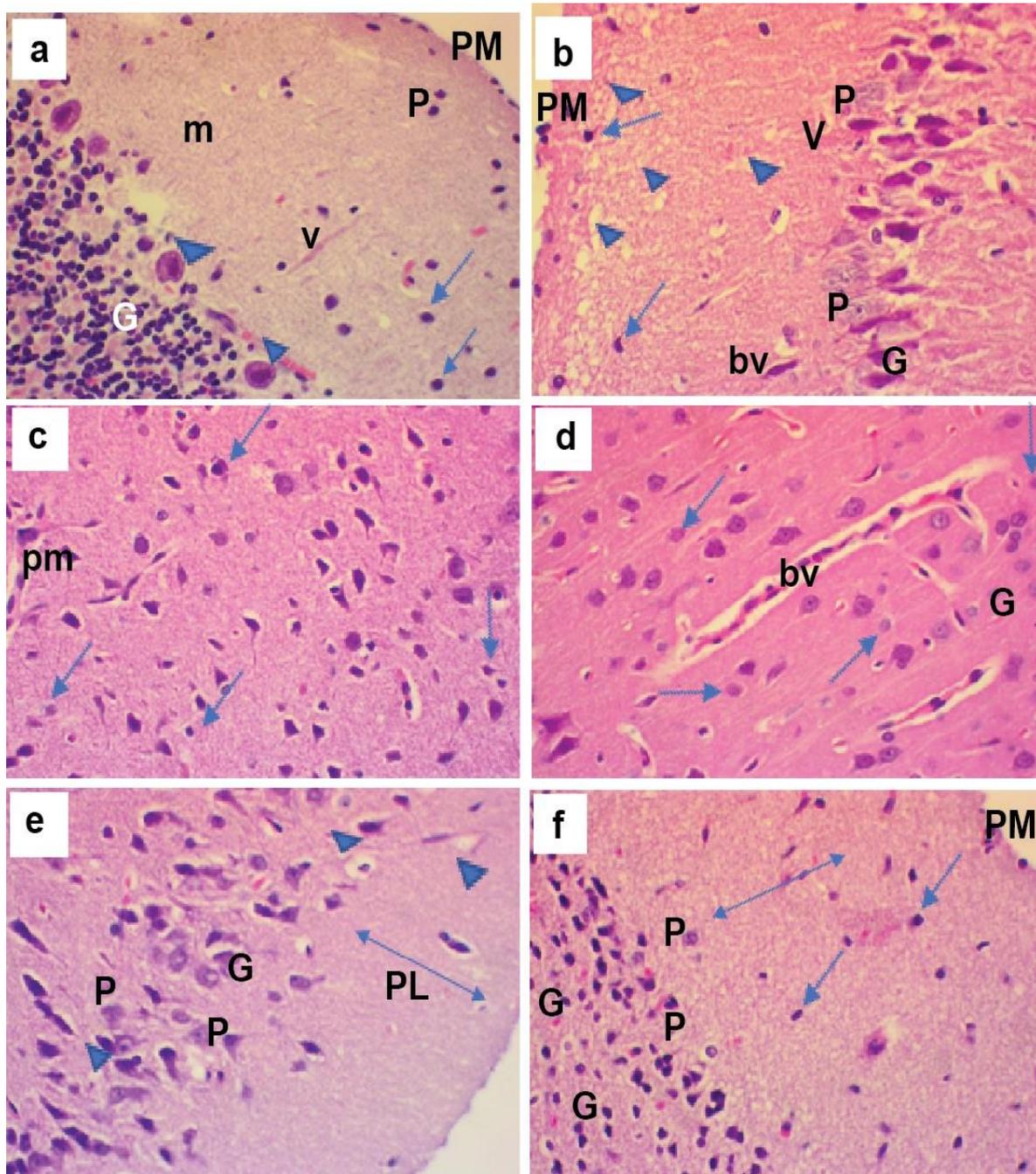


Figure 3. Representative photographs of cerebral cortex from the brain of the rats, sections of different groups staining with Hematoxylin and Eosin (400 X magnification).

Note: Photomicrographs of a section from cerebral cortex of control group rat **(a)** (H and E) 400X, showing the normal external molecular layer (M), middle purkinj cell neuronal cells layer (Head arrow) and inner granular layer (G) glial cells (black arrow) in the outer molecular layer. the granular layer shows aggregation of small granule cell(G)with pale areas of the cerebellar is land in between (*), the cerebral cortex surrounded by pia matter (PM), Hematoxylin and Eosin shows pyramidal cells (P) have multipolar shape, also shown blood vessel, (V) H & E 400X. **Figure (3b)** section from cerebral cortex injected with single dose of (0.5 ml) N-Diethylnitrosamine (DEN) after two week, it was observed that edema (arrow head), inflammation were seen and glial apoptosis ,necrosis of neurons(arrow),also the granular cell(G),neuronal cell astrocytic cell,

damage and glial apoptosis ,with hypertrophy and vasculature, irregular shape aggregated chromatin in their nucleus of neuron in granular layer also shown congestion of cerebral cortex blood vessel (bv) surrounded by pia matter (pm). **Figure (3c)** section from cerebral cortex of rat brain treated with (0.2ml paclitaxel) injected intraperitoneally after two weeks (H& E 400X) staining showed most of the neuron or glial cells ripper but still there is inflammation and some disorganized cortical layer. shown granular and outer pyramidal layers crowded and become mor cellular they are irregular shape, with darkly stained nuclei, neuroglial cells some of these cells are shrunken. **Figure (3d-f)** The group given paclitaxel same dose after received (0.5ml DEN) 4,6,8 weeks it was observed that the inflammation and edema were seen but is less when compared with the control group and there is no specific change in microglial cell, astrocytic cell damage and glial apoptosis(P), the edema become less and still there is neuron and astrocyte with some inflammation neuron cell, pyramidal cell (P) (H&E) 400X.

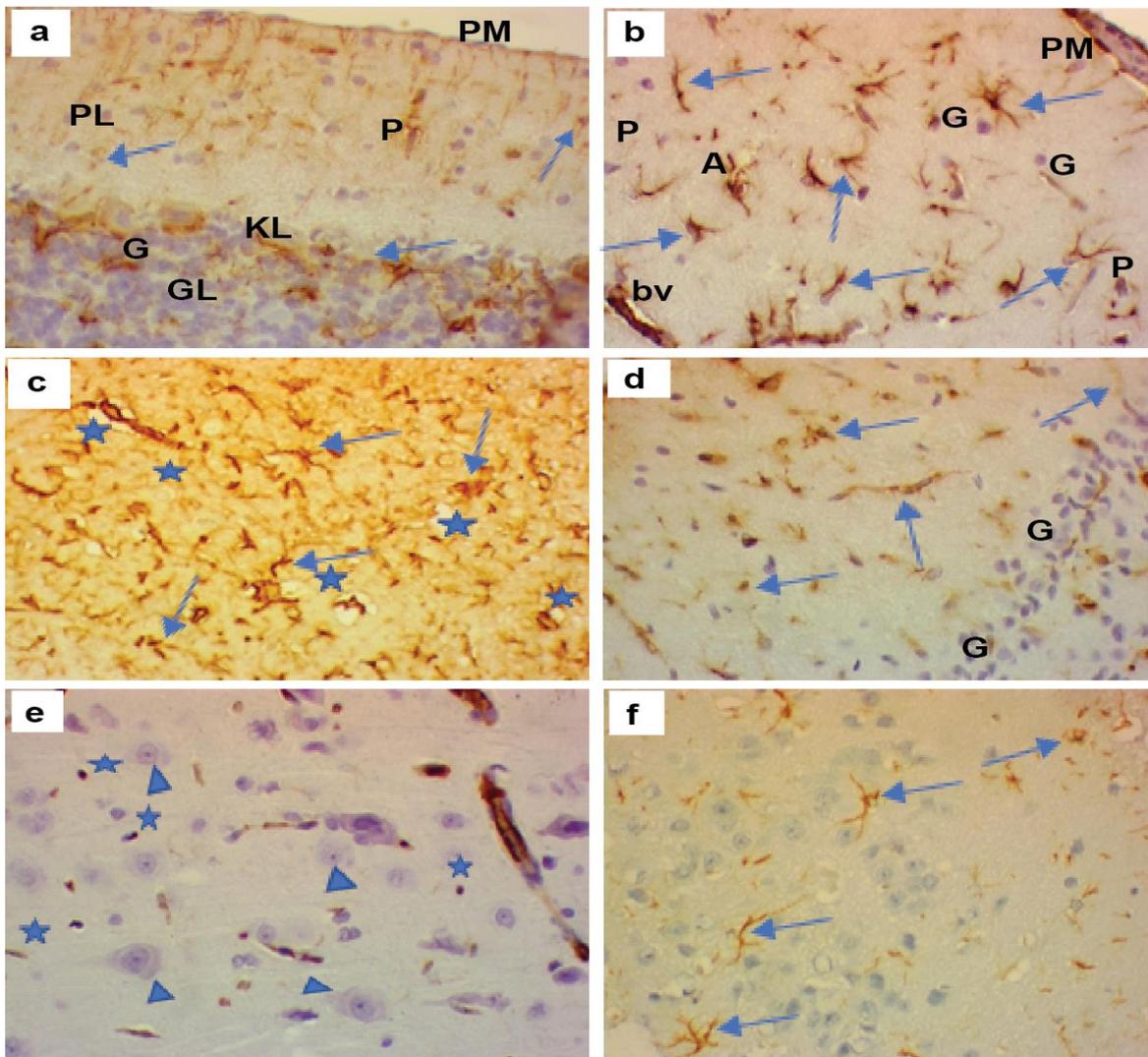


Figure 4. Representative immunostaining sections of cerebral cortex from the brain of the rats. Stained with the glial fibrillary protein (GFAP) antibody from the following groups.

Note: Photographs of cerebral cortex from the brain of the rats, sections of different groups staining with Hematoxylin and Eosin (400 X magnification).Brain rat immunohistochemistry for glial fibrillary protein

(GFAP) (a) control group shows normal layer for cerebral cortex, outer layer is pia matter (PM) inner pyramidal layer (PL) inner granular layer (GL) purkinji layer (KL) pyramidal cells(P) have multipolar shape while granular cell(G) has large nuclei and little cytoplasm. The categories for inflammation cell are score 1 (%2) a little microglia cell damage and astrocytic cell damage (400x). **Figure (4b)** glial fibrillary acidic protein immunohistochemistry (GFAP) positive astrocytes (arrows) show in different regions of cerebral cortex in rat which is treated with (0.5ml) a single dose of (DEN), the disappearance and distortion of normally cerebral outer pyramidal layer crowded and become mor cellular with neuroglial and astrocyte neuron called reactive astrogliosis which become pathological hall mark of brain injury tissue. The inflammation cells average is score 3 moderates to server grate around %40 damage of astrocyte cell (400X). **Figure (4c)** paclitaxel injected rats (0.2ml) after 2- and 4-weeks treatment the effect of inflammation and edema (*) were evident specific change astrocyte (arrow) and neuroglial cell damage and glial apoptosis were observed the inflammation categories it seen be moderate to score 3 severe grade (400X). **Figure 4(c-f)** with (paclitaxel) dose (0.2ml) for 8-week treatment (2week interval) the effect of inflammation and edema (*) were evident no specific change in astrocyte (arrow) neuroglial cell damage and glial apoptosis were observed disturbance of purkinji cell (arrow head) arrangement nonlinear random distribution particularly in late stage of inflammation score is moderate grade score 2 (400X).

Figure (4a). Showed the immunopositively for GFAP in neuron was detect in brain injury cases and rat control brain glial fibrillary acidic protein was used to display GFAP immunoreactive astrocytes, the study showed an increase in astrocytes in treated rat with (DEN) dose (0.2ml) injection rat compared with the control rates **Figure (4 b-c)** after 2-week inflammation.

However specific change in astrocyte as well as damage glial neurons and apoptosis were illustrated most of the purkinje cell are scattered with lost leaving empty spaces and lost their arrangement on the purkinje layer **Figure (4e)**. However, the inflammation categories it seen to be score 2 moderate grades finally the effect of injection with (DEN) as well as the treatment of paclitaxel thought out different period have been observed and illustrated in Figure (3f H&E) and Figure (4f GFAP) after treating the brain with paclitaxel.

4. Discussion

The current study carried out in reference to a well-known carcinogen chemical (DEN) that is known to be strong cause causing agent (2,5,11,9) however often it was applied to induce cancer to animals' rats in laboratory (6,12,19,20) never the less paclitaxel on the other hand is known to be efficient drug against cancer (13,21) in fact both drugs were applied is investigation. As it was also reported by(21,22,23) the different in mean body weight (M.B.W) was noticed after treatment with paclitaxel .the point is a significant difference in body weight were reported by through different intervals of treatment that was surprisingly linear in other word after 2&4 weeks of treatment around 264 gm the weight sustained around 264gm whereas after 6weeks the weight raised up-significantly to 278gm in contact after 8week treatment the body weight fall to approach the original positive control weight (253)gm such phenomena an undoubtedly will be clarified much more after more intensive investigation in years come . Apart from body weight of rats the brain weight was also considered as another parameter in present study however after to rat scarified the mean brain weight were estimated and each interval then after it was found out from the results that no significant difference in brain weight were evident throughout present study. However, all results before and after injection with DEN as well as they treatment with paclitaxel throughout 8 weeks with the illustration. Quite little and insignificant difference in the rate led to propose different dose of injection and treatment should be considered in future for such studied. however, brain weight was also used. Histopathological examination of various sections of rat brain throughout the duration of current investigation clearly reflect the variation in different

cells and tissues, their arrangement and interred structure, all these variations were observed through staining with H&M) as well as DFAP protein. Such variation in brain and other organs in other animals had also been referred to by (25,27).

The variation and changes in rat brain cells after staining with H&E. Can be noticed in the inflammation, glial apoptosis and other change. After injection with (DEN), where as sections after treatment with paclitaxel shows the repair of cells and tissues glial cell for example, but still inflammation is remained after two weeks. However, the effect of treatment after 4 weeks and six weeks on inflammation and edema still were evident, whereas after 8 weeks of treatment No specific change- in astrocytes, Neuroglial cell damage were observed. Such phenomenon were also reported by (12,18,19). Histopathological result through treating with GFAP shows quite clear variation in various interval before and after injection with Carcinogen agent as well as before and after treatment also the variation between different intervals (2,4,6 & 8 weeks) were forwarded and discussed.

Present experiment on albino rat brain resulted in finding out clear changes in adaptation, brain cell injures, after the rat been injected with (DEN). All such alterations and variation can easily be noticed.

5. Conclusion

- *In conclusion, current study showed that the treatment with Paclitaxel is quite effective.
- * As long as treatment duration extended the out com become much more effective as it is clean from the Histopathological and immunohistochemical examination of Brain tissue sections.
- * Using stronger Concentration may well result in faster treatment out come in future.
- *It become evident that Paclitaxel "Provides neuroprotection against brain injury from focal cerebral Ischemia

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