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RESEARCH ARTICLE

Sustained Release of Drugs from Prepared Polymeric Systems for Alzheimer Disease Protection

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Abstract

Objective: Slow release technique is a trial for long-acting protection from Alzheimer. Drug delivery systems, polymer based, can be designed to improve the pharmacological and therapeutic properties. Slow release technique gave the availability of drugs to deliver the right location in the body. The main principle of this technique is holding and keeping the drug on the polymer carrier for a sustained release of medicine in the body for a long period of time. The present work aimed to prepare polymeric materials from starch cellulose acetate coacrylate (SCACA), containing sodium bentonite nano clay and tween 80 as a surfactant for improving drug loading. The objective of this formulated natural polymeric product is to obtain practical effective sustained release drug systems by evaluating its potency in vivo for protection from Alzheimer disease. SCACA biodegradable polymeric system formulations containing anti Alzheimer drugs (such as Curcumin, Rosemary or Donepezil) were investigated through oral administration in experimental animals (rats). Methods: The characterization of the prepared polymeric system was carried out by FTIR and SEM. The drug release was measured spectrophotometrically. The in vivo evaluation of the released drug against Alzheimer disease was an assessment of the effect in rats through biochemical and histopathological studies. Results: The slow release of the drug according to the study time illustrated that Rosemary, Curcumin and Donepezil estimated values through the tested time gave promising results, especially for releasing Rosemary, Curcumin and Donepezil. In the present investigation, the biochemical findings were also confirmed by histopathological observations which showed improvements in most biochemical parameters accompanied with amelioration in histological architectures in rats treated. Conclusions: Slow release technique is a good means for long-acting protection from Alzheimer's disease. Starch cellulose acetate coacrylate (SCACOA) is a good carrier for drugs such as Donepezil as a conventional drug while Rosemary, Curcumin as bioactive natural products. The in vivo study for Alzheimer's protection gave promising results.

Keywords: *Release, Alzheimer disease, Biodegradable polymer, In vivo study.*

Introduction

Alzheimer's disease (AD) is the most common form of dementia, a general term for memory loss and other intellectual abilities serious enough to interfere with daily life [1]. Although the parameters that cause Alzheimer's aren't yet fully understood, its effect on the brain is clear [2]. AD damages and kills brain cells and currently affects 35 million patients across the world, which is expected to double in the next 20 years [3]. It is a neurological disorder that results in cognitive and behavioral impairment [4].Considerable progress has been made in the treatment of (AD), but all available strategies focus on alleviating symptoms rather than curing, which means that AD is viewed as an unresolvable neurodegenerative disease [5]. In spite of screening numerous drug candidates against various molecular targets of AD, only a few candidates -such as

acetyl cholinesterase inhibitors are currently utilized as an effective clinical therapy [6]. However, targeted drug delivery of these drugs to the central nervous system (CNS) exhibits several limitations including meager solubility, low bioavailability, and reduced efficiency due to the impediments of the blood-brain barrier (BBB) [7- 8].

The development of Nano metric drug delivery systems may permit a targeted and sustained release of old and new treatments offering a novel strategy to treat these neurodegenerative disorders [9]. In this view, at the cutting-edge of innovation, Nano carriers such as polymeric nanoparticles, liposomes, Nano assembly and dendrimers, have been studied and investigated in order to ameliorate the detection (in vitro and in vivo) and/or the therapeutic options in AD [10].

The particle size below 200nm is a very important prerequisite for crossing BBB. Several methods have been developed to prepare polymeric nanoparticles of hydrophilic drugs like nanoprecipitation and emulsion solvent evaporation. In spite of its low entrapment efficiency values for hydrophilic drugs nanoprecipitation offers an easy and reproducible technique with narrow unimodal particle size distribution (100-300nm) [11]. A potential alternative to currently used materials is starch-based polymers, which are characterized as biodegradable materials which have been recently proposed as biomaterials [12].

The starch used in the industry usually contains between 20 and 30 % amylose, with the remainder being amylopectin (80-70 %) and minor components (less than about 1 %) such as lipids and protein [13]. Various starches and their derivatives have been used to prepare biodegradable materials for controlled release applications in medicine, pharmacy, and environmental remediation and protection [14].

This technique depends on controlling the sustained release of a drug for long time periods to overcome the drawback of a conventional application of drugs. The main goal of the present work is to study the release of the active ingredients from controlled release of Starch cellulose acetate coacrylate polymeric materials holding drugs such as Donepezil, Rosemary, Curcumin in neutral aqueous media. Also evaluate the in vivo bioassay for protection of human beings from Alzheimer disease through the study of the effect of releasing active agents on experimental animals (rats).

Materials

Potato starch was supplied as a neutral white powder by El Nasr Pharma Central Chemical Company, Abu Zaabal, Egypt, Cellulose acetate containing 40% acetyl group was supplied by Sigma-Aldrich, Acrylic acid with molecular weight of 72, freezing point of 13°C, boiling point of 141°C, density at 20°C of 1.046 g cm-3, and relative index nD at 20°C of 1.42-1.421 was supplied by Sigma-Aldrich, Ethyl alcohol with density of 0.789 g cm-3 and boiling point of 78°C was supplied by Aldrich Company, Germany, Sulfuric acid with density 1.84 g cm-3 and boiling point 337°C was supplied by Aldrich Company.

Germany, Sodium hydroxide pellets are odorless, white, solid hemispheres of uniform diameter and thickness with melting point 318.4 °C was supplied by Sigma-Aldrich, Dicumyl peroxide (DCP), pure grade, melting point (39-41°C), Mwt= 270.37g/mol, Sigma-Aldrich product, Sodium bentonite clay powder (mesh size 300 mm), with a cationic exchange capacity (CEC) of 90 mequiv per 100 g, Tween-80 (polyethylene sorbitol ester) non-ionic viscous liquid and average molecular weight 1,310 Da purchased from Sigma-Aldrich, Donepezil hydrochloride was purchased from Across Co, Curcumin and Rosemary were purchased from Across Co, Aluminum Chloride ALCL3 was purchased from Sigma Chemical Co. (St. Louis, MO, USA).

Instruments

Ultra Violet Spectroscopy

The amount of drug released was determined spectrophotometrically. The spectrophotometer used was a UV-240 1PC Visible VIS.

Morphology Study

- Scanning electron microscopy (SEM, Quanta FEG 250, FEI)
- High-Resolution Transmission Electron Microscopy (HRTEM), JEOL JEM 2100 T.E.M HR Japan.

Fourier-Transform Infrared (FTIR) Spectroscopy

The FTIR spectra of samples were obtained using a Jascow (Japan) FTIR 430 series infrared spectrophotometer equipped with Potassium Bromide (KBr) discs.

Experimental Techniques

Preparation

- **Nano Curcumin**

Curcumin solution was prepared by dissolving Curcumin powder (commercial) in ethanol. The solution was added to boiling water in a drop-wise manner under ultra sonication condition with an ultrasonic power and frequency of 50 kHz. The solution was sonicated for about 30 minutes. After sonication, the mixture was stirred at 800 rpm for about 20 min till the orange colored precipitate was obtained. Thereafter, supernatant was discarded and the pellet obtained was used.

- **Extraction of Rosemary**

100 g of raw plant leaves was extracted with 50% ethanol by percolation (24 h) at room temperature (24 ± 1 °C). After filtration, the extract was concentrated under vacuum to eliminate the ethanol content. The concentrated extract was frozen and freeze-dried. The final product yielded 7.4 g of solid extract.

- **SCACA/Modified Nano-clay Loaded with different Anti-Alzheimer Materials (nano Curcumin, Rosemary and Donepezil)**

SCACA/modified nano-clay loaded with Curcumin, Rosemary and Donepezil nanocomposites (0.5%) were prepared under vigorous stirring [15]. The final products were cast in a cylindrical mold for shaping.

In Vitro Release Profile

The release of drug from the prepared polymeric nanoparticles was conducted by subjecting the disks (containing 0.5% of drug) in 10 ml of distilled water (pH 7). The tubes were kept in a shaker at 37◦C. At predetermined time intervals, 2ml were withdrawn and replaced with fresh distilled water. The amount of released drug present was determined spectrophtometrically at a wavelength of 416, nm for the investigated drugs Curcumin, Rosemary and Donepezil in distilled water respectively [16].

In Vivo Evaluation for the Potency of the Prepared Controlled Release System Loaded with Drugs Against Alzheimer Disease

Rats were divided into 8 groups; each group contains 6 animals as follows:

- Group 1 (untreated control): animals were fed on a standard diet and given water throughout the course of the experiment.
- Group 2 ($ALCL_3$ treated): Rats were injected orally with $ALCL_3$ at a dose of (100 mg/kg b.w) for 42 days.
- Group 3 ($ALCL_3$ and Donepezil): Rats from Group 3 were injected orally at a dose of (100 mg/kg. $ALCL_3$ + 10 mg/kg Donepezil) for 42 days.
- Group 4 ($ALCL_3$ and Rosemary): Rats from Group 4 were injected orally at a dose of (100 mg/kg. $ALCL_3$ + 100 mg/kg Rosemary) for 42 days.
- Group 5 ($ALCL_3$ and Curcumin): Rats from Group 5 were injected orally at a dose of (100 mg/kg. $ALCL_3$ + 100 mg/kg Curcumin) for 42 days.
- Group 6 ($ALCL_3$ and Polymer loaded with Donepezil): Rats from Group 6 were injected orally at a dose of (100 mg/kg. $ALCL_3$ + 200 mg/kg polymer) for 42 days.
- Group 7 ($ALCL_3$ and Polymer loaded with Rosemary): Rats from Group 7 were injected orally at a dose of (100 mg/kg. $ALCL_3$ + 200 mg/kg polymer) for 42 days.
- Group 8 ($ALCL_3$ and Polymer loaded with Curcumin): Rats from Group 8 were injected orally at a dose of (100 mg/kg. $ALCL_3$ + 200 mg/kg polymer) for 42 days.

Assessment of Rat's Cognitive Abilities using Rewarded T-maze Test

A cognitive ability of rats in this study was assessed by using the rewarded T-Maze test (locally constructed in the National Research Centre, Giza, Egypt) according to Deacon and Rawlins [17].

Biochemical Studies

Sample Preparations

Blood was collected from each animal by puncture of a sublingual vein. Blood samples were divided into two parts. The first part was collected on EDTA for hematological analysis. The second part was collected into dry test tubes and then centrifuged at 3000 rpm in order to separate the serum. The sera were kept at -20EC for further biochemical analysis. In order to collect the hepatic tissues, rats were immediately dissected. The liver was homogenized with 10% w/v ratio in ice-cold 50 mM Tris HCl buffer at pH 7.4 and then centrifuged at 10,000 rpm for 20 minutes at 4 ECs. The supernatant was collected and kept in deepfreeze at -20EC for further analyses.

Estimation of Serum Biochemical Parameters

In the serum of all the experimental groups, the levels of total lipids (TL), total cholesterol (TC), triglycerides (TG), low density lipoprotein cholesterol (LDL-C), high density lipoprotein cholesterol (HDL-C), total proteins (TP), albumin (A), globulin (G), aspartate aminotransferase (ASAT), alanine aminotransferase (ALAT), alkaline phosphatase (ALP), total bilirubin (TBil) and direct bilirubin (DBil) were measured colorimetrically using Biodiagnostics kits (Dokki, Giza, Egypt). Lipid peroxide assay: The level of malondialdehyde (MDA) in the liver homogenate was assayed according to the technique described by Ohkawa et al (18). The principle of this method depends on the reaction of the liberated MDA after lipid peroxidation (LPO) of the cell membranes with thiobarbituric acid in an acidic medium.

Non-enzymatic and Enzymatic Antioxidant Assay

The concentrations of non-enzymatic (glutathione, GSH) as well as enzymatic (catalase, CAT, superoxide dismutase, SOD, glutathione reductase, GR) antioxidants were estimated in the homogenate of the liver of control and treated rats [19]. The method by which GSH content was measured was based on the reaction of 5, 5'Dithiobis-2-nitrobenzoic acid with GSH [20]. The CAT activity was estimated in accordance to the method described by Aebi [21]. The SOD activity assessment was based on the ability of SOD to inhibit the reduction reaction of nitrobluetetrazolium dye mediated by phenazine methosulphate [22].The principle for measuring the GR activity was based on its ability to catalyze the reduction of glutathione (GSSG) as described by Goldberg and Spooner [23].

The Comet Assay

Comet assay was performed referring to the protocol developed by Blasiak et al. [24], with minor modifications. Rat's hepatic cells of each treatment were mixed with low-melting-point agarose (ratio of 1:10v/v), then pipetted to precoated slides with normal-melting-point agarose. The slides were kept flat at 4°C for 30 minutes in a dark environment. The third layer of low melting point agarose was then pipetted on slides, left to solidify at for 30 minutes at 4°C. The slides were transferred to a pre-chilled lysis solution, kept for 60 minutes at 4°C.

After that, slides were immersed in freshly prepared alkaline unwinding solution at room temperature in the dark for 60 minutes. Slides were subjected to an electrophoresis run at 0.8 V/cm, 300mAmps at 4°C for 30 minutes. The slides were rinsed in neutralizing solution followed by immersion in 70% ethanol and then air-dried. Ethidium bromide is used for slides stain then and visualized by using a Zeiss epifluorescence microscope (510-560 nm, barrier filter 590 nm) with a magnification of ×400. 100 cells per animal were scored then analyzed with DNA damage analysis software (Comet Score, TriTek corp., Sumerduck, VA22742).

Statistical Analysis

All values were presented as means of standard error (mean S.E). Data were statistically analyzed with the aid of Statistical Package of the Social Sciences, SPSS version 23 (copyrighted by IBM SPSS software, USA).

Histopathological Study

Brain and liver tissues were excised from sacrificed animals, individually weighed, and, from them, 5 μm thickness slices were cut, fixed in 10% paraformaldehyde, and embedded in paraffin wax blocks. Tissue sections of 5 μm thick were stained with hematoxylin and eosin (H&E).

Results and Discussion

FTIR Characterization

Figure (1) shows the IR spectra of the prepared starch cellulose acetate co-acrylate

polymer when containing the synthesized anticancer organic compound. Fig. (1a) (Curcumin) shows the strong C=O stretching peak observed for Curcumin at 1623 cm^{-1}. A broad band at 3410 cm^{-1} is attributed to vibrations of the free hydroxyl group of phenol (Ar-OH). An intense band at 1630 cm^{-1} attributed to vibrations of the carbonyl bond (C=O) accompanied by a smaller shoulder at 1742 cm^{-1} due to keto-enol tautomerism of Curcumin compound.

Three bands at 1590, 1506, 1457 cm^{-1} are attributed to the vibrational mode of C-O elongation of alcohol and phenol groups. The spectrum presents characteristic bands in the case of Fig. (1b) (Rosemary) at 1700 – 1500 cm^{-1} corresponding to C=C Bending, the signals which appeared between 3150-3050 cm^{-1} corresponding to C-H (aromatic) stretching, groups. For Carboxylic Acid O-H Stretch with characteristic absorption 3000 - 2500 cm^{-1}.

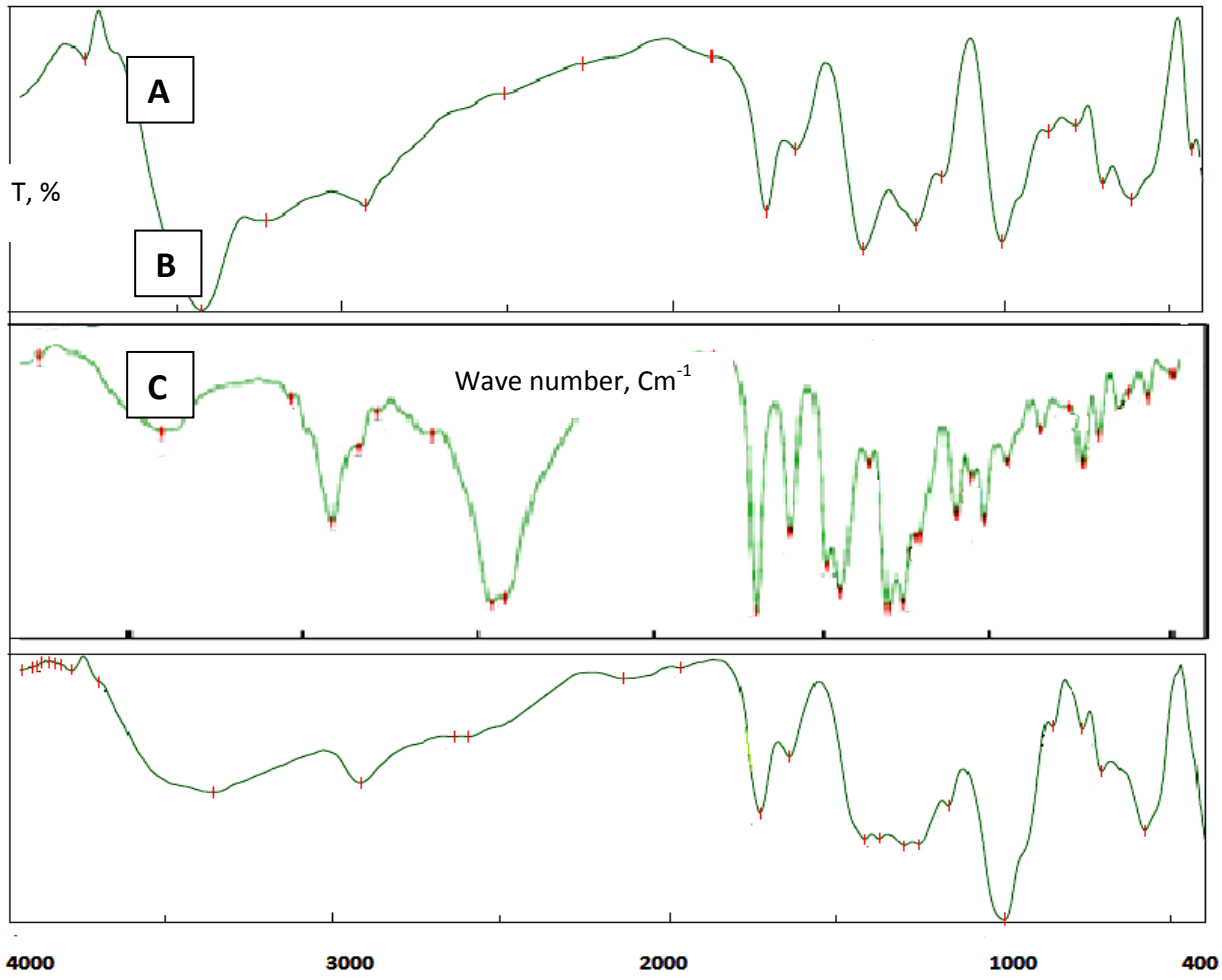


Figure 1: FTIR of the prepared polymer SCACA/SB/tween containing
(a) Curcumin (b) Rosemary (c) Donepezil

The FTIR spectra of the Donepezil HCl with starch cellulose acetate co-acrylate shows in Fig.(1 c) from the spectra of the Donepezil HCl, it was observed that the main functional groups of the compound are aromatic phenone and para- substituted aromatic hydrocarbon. The most intensive absorption band around 1682 cm-1 in the spectra was attributed to the stretching vibrations of the C=O group in the structure of Donepezil HCl. Following bands were observed in the spectrum C-N (1262 cm−1), C-O (1081 cm-1), C-H bending aromatic (855), C=C Aromatic (1589 cm−1), C-H stretching in CH3 (2923 cm-1).

The Release Rate of Anti Alzheimer Materials from the Investigated Polymer

The results of the leaching rates of the anti Alzheimer materials (Curcumin, Rosemary and Donepezil) in distilled water at 25C expressed as µ gm/ day are represented in Fig. 2.

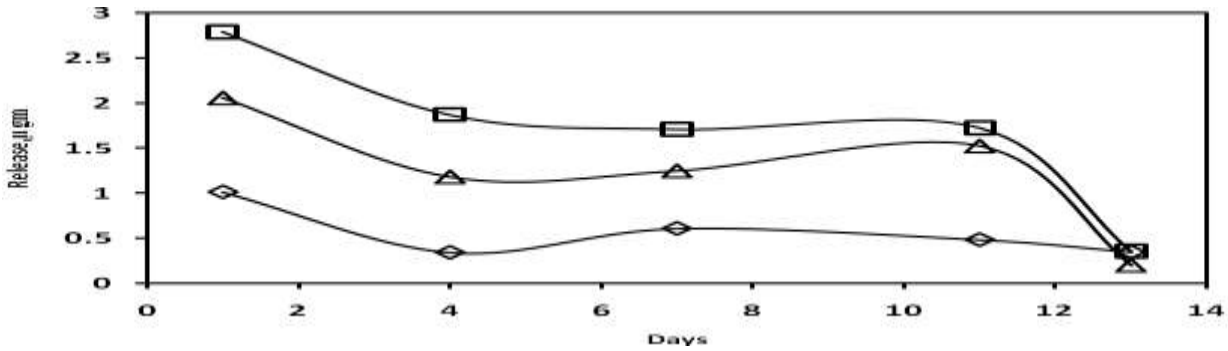


Figure 2: the release rate of curcumin, Rosemary and Donepezil in a distilled water from SCACA

It was found that the highest amount of release was observed in the initial stage for all the anti Alzheimer materials, then the amount of release decreased and a steady state of sustained release was obtained up to the period of study of 11 days. The release rate was changed according to the type of the anti Alzheimer materials which depends on the structure and the nature of these materials. The estimated release rate of the investigated Alzheimer materials can be arranged according to the following order:

Rosemary > Donepezil > Curcumin

Scanning Electron Microscopy (SEM)

Figure 3 (a, b, c) illustrated the investigated polymer loaded with clay/ tween as a surfactant and drugs; Curcumin, Rosemary and Donepezil respectively; it was found that the surface texture of the tested samples is homogeneous and had a good distribution of all ingredients, also smooth with no evidence of aggregations.

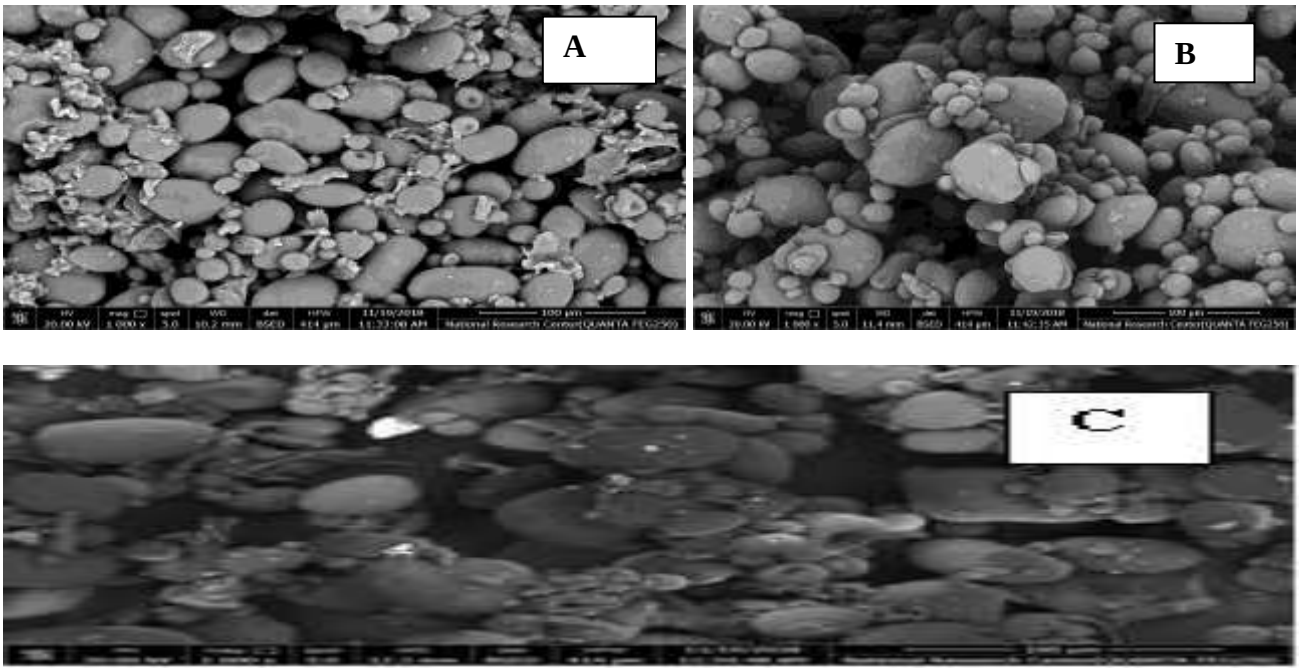


Figure 3: Scanning electron microscope imaging for the prepared polymer SCACA/SB/tween containing: (a) Curcumin (b) Rosemary (c) Donepezi

Transmission Electron Microscopy (TEM)

It is important to provide some information about the particle size of the prepared investigated nano-sized controlled release polymeric materials containing drugs. This was done and estimated by Transmission electron microscope (TEM) technique.

Figure 4 (A, B, C, D) illustrates the particle size of the prepared polymer before and after loading drugs. It was shown that the particle size diameter of SCACA/SB/tween was found to be from 14 to 146 nm; for SCACA/ SB/ tween/ Curcumin was from 15 to 34 nm. For the SCACA/SB/tween/ Rosemary the particle size range from 227 to 570 nm and for

SCACA / MSB / Donepezil the particle size range from 27 to 37 nm. The variation of the values of the nano sized depending on the type and the nature of the material applied [25].

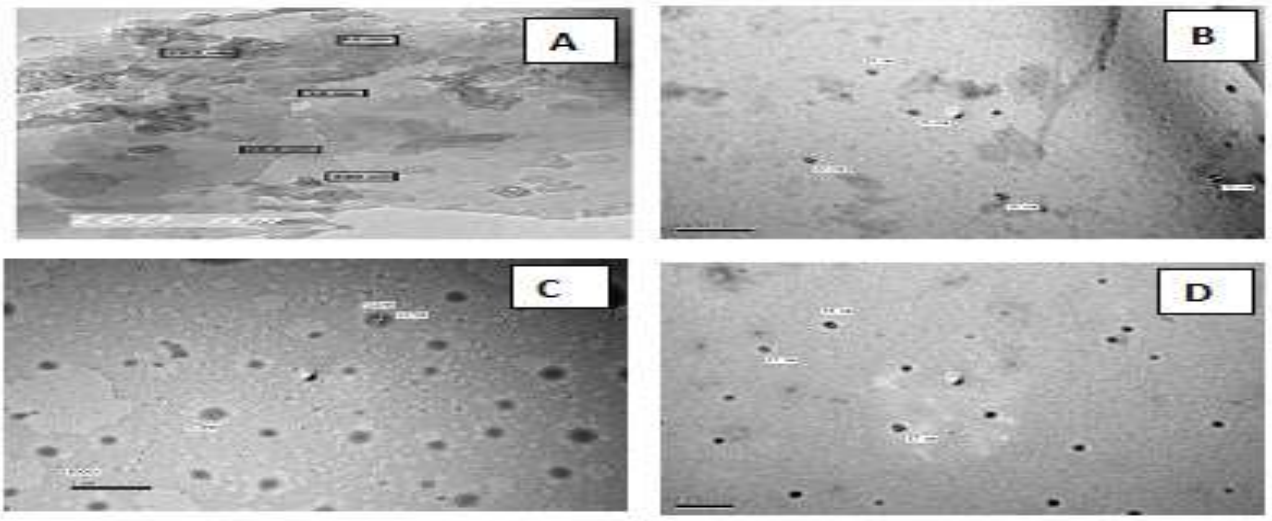


Figure 4: Transmission electron microscope (TEM)
(a) SCACA/SB/tween (b) SCACA/SB/tween/ Curcumin (c) SCACA/SB/tween/ Rosemary (d) SCACA/SB/tween/ Donepezil

In Vivo Evaluation of the Potency of the Prepared Controlled Release Systems Loaded with different Drugs for Protection against Alzheimer Disease

In Vivo Testing of the Released Active Materials and Drugs in Rats

This section deals with the induction of Alzheimer disease in male albino rats and the effect of the investigated polymer formulations of SCACO which were loaded with different active materials and drugs such as: Rosemary (R) & Curcumin (C) extracts as well as the conventional drug Donepezil (D) were performed according to the experimental techniques. In vivo testing of the released active materials and drugs was carried out on six groups of rats as demonstrated in methodology part.

The Results and Observations are as Follows:

Assessment of Cognitive Abilities using Rewarded T-maze Test

The results obtained in Table (1) showed significant increase in time in seconds (denoting deteriorated cognitive abilities), taken by rats to reach food in the T-Maze for the positive control AD grouping comparison with baseline of each of this group (before giving ALCL3). On the other hand, all groups treated with the prepared polymers holding drugs in combination with ALCL3 for 4 successive weeks exhibited a significant reduction in time in seconds (denoting improved cognitive abilities), taken by rats to reach food in the T-Maze in comparison with the positive control AD-group.

Table 1: Evaluation of protective effects of different prepared polymers using Rewarded T-Maze test in AD-disease induced in rats by $ALCL_3$

Time Duration Group	Baseline, (0 weeks)	2 weeks Pre-treatment	2 weekspre-treatment, then 4weeks treatment with $ALCL_3$.
Control	13.44 + 0.91	14.1 + 0.88	15.56 +1.3b
$ALCL_3$	16.66 +1.07		109.2 + 4.83a
Curcumin/ Polymer	15.33 +1.63	51.16 + 1.5a	49.4 +1.4ab
Rosemary/ Polymer	12.33 + 1.7	50.2 + 13.6a	56.3 + 2.95ab
Donepezil/ Polymer	13.57 + 0.48	47.6 + 6.3a	48.6 + 3.47ab

All data Results are in seconds expressed as Means +SEM. (a) Significantly different baseline duration of the same group at $P < 0.05$. (b) Significantly different from $ALCL_3$ after 4 weeks induction at $P<0.05$

Rate of DNA damage in brain tissues of Alzheimer induced rat groups treated with different extracts using a comet assay:

Results illustrated in Table (2) and figure 5 (a, b) showed that the prepared controlled release polymer SCACA containing Rosemary (R) & Curcumin (C) and Donepezil (D) gave promising protective effect on ALCL3 induction of Alzheimer in rats due to controlling the slow release of these drugs. The effect of drugs may be arranged according to the classes of comet assay as follows:

- For the 3rd class: Rosemary (R) > Curcumin (C) > Donepezil (D).
- For the 2nd class: Rosemary (R) > Donepezil (D) > Curcumin (C).
- For the 3rd class: Donepezil (D) > Curcumin (C) > Rosemary (R).

These results depend on the release of the investigated drugs.

Table 2: Effect of oral administration of $ALCL_3$ alone or with different prepared polymers, on the rate of DNA damage in brain tissues of rats using comet assay

Treatment Groups	No. of cells		Class¥ of comet				DNA damaged cells (mean ± SEM)
	Analyzed	Total comets	0	1	2	3	
Control	500	37	463	26	11	0	7.4 ± 0.18^{c}
$ALCL_3$	500	109	391	32	36	41	21.8 ± 0.63^{a}
Curcumin/ Polymer	500	63	437	21	25	17	12.6 ± 0.2^{b}
Rosemary/ Polymer	500	54	446	24	18	12	10.8 ± 0.52^{b}
Donepezil/ Polymer	500	49	451	19	19	20	11.6 ± 0.41^{b}

¥: Class 0= no tail; 1= tail length < diameter of nucleus; 2= tail length between 1X and 2X the diameter of nucleus; and 3= tail length > 2X the diameter of nucleus.(*): No of cells analyzed were 100 per an animal

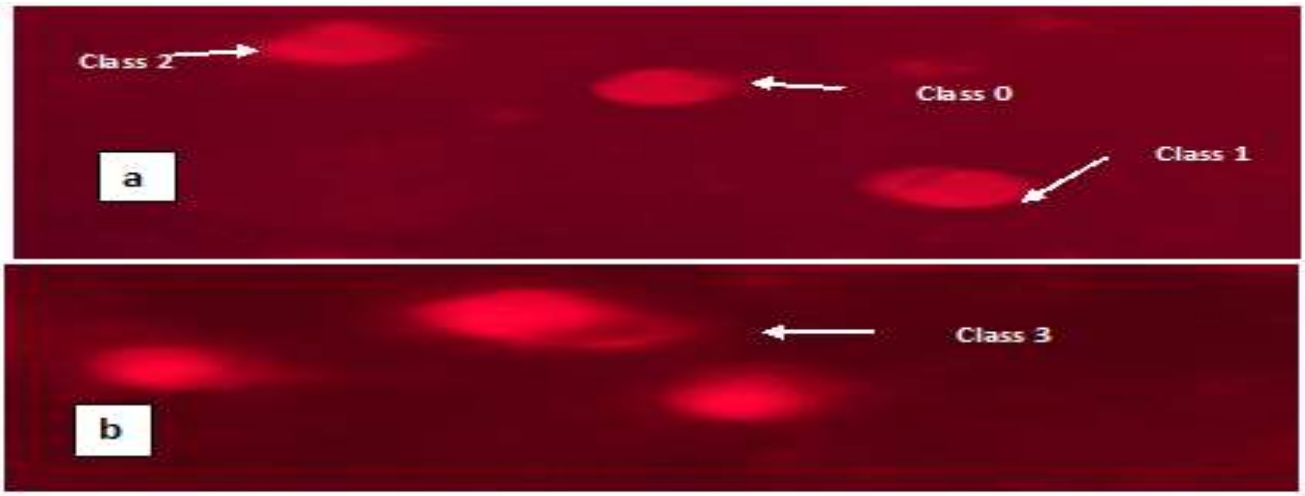


Figure 5: Visual score of DNA damage: a: classes 0, 1 and 2; b: class 3, using comet assay in brain tissues of rats

Effect on Serum Biochemical Parameters

Effect of oral administration of ALCL3 alone or with different prepared polymers containing different drugs, on different biochemical parameters in male albino rats is illustrated in Table 3. Results showed that the lipid profile of the experimental animals as affected by the administration of ALCL3 alone, the different prepared polymers containing Rosemary (R) & Curcumin (C) and Donepezil (D) plus ALCL3 are shown in Table 6-3. Levels of TL, TC, TG, LDL-C and HDL-C of the rats were markedly influenced by the type of treatment.

In comparison to the control group, all the studied lipid profile parameters of the ALCL3 treated group were significantly elevated except the levels of HDL- C that were notably reduced. On the other hand, rats treated polymers containing Rosemary (R) & Curcumin (C) and Donepezil (D) plus ALCL3 exhibited a marked reduction in the levels of TL, TC, TG and LDL-C, as compared with the ALCL3 treated group.

The results of the present study have also established that ALCL3 treatment could have affected the lipid metabolism of the liver (triglyceride and cholesterol levels).

This is evidenced from the present observations in which ALCL3 caused a significant ($p < 0.05$) increase in the levels of lipid parameters. However, rats treated with polymers containing Rosemary (R) & Curcumin (C) and Donepezil (D) plus ALCL3, showed a significant ($p < 0.05$) decline in triacylglycerol and cholesterol values compared to CCl4- intoxicated rats.

The mechanism of lipid lowering effects of different prepared polymers might be attributed to an inhibitory activity of the microsomal acylco enzyme A: cholesterol acyltransferase in vitro. This enzyme is responsible for the acylation of cholesterol to cholesterol esters in the liver.

Moreover, the serum protein profile of different groups of rats was noticeably affected by the type of treatment, as rats administered ALCL3 alone exhibited marked reductions in the levels of albumin simultaneous with a significant increase in the levels of globulin, as compared to the controls.

Thus, the A/ G ratio of this group was remarkably reduced. On the other hand, the rats of polymers containing Rosemary (R) & Curcumin (C) and Donepezil (D) plusALCL3, treated groups displayed a marked increase in the levels of albumin and A/G ratio but a marked decrease in the levels of globulin, as compared to the ALCL3 treated group.

In this study the significant ($p < 0.05$) decrease in serum albumin of rats treated with ALCL3 as compared to control may indicates poor liver functions or impaired synthesis, either primary as in liver cells damage or secondary to diminished protein intake and reduced absorption of amino acids caused by a mal absorption syndromes or malnutrition, or loss protein in urine, due to nephritic syndrome and chronic glomerulonephritis. On the other hand, a significant ($p < 0.05$) increase in concentration of serum albumin was observed in rats received polymers containing Rosemary (R) & Curcumin (C) and Donepezil (D) plusALCL3, in comparison to rats received ALCL3 alone. The increase of albumin concentration after treatment with prepared polymers may be attributed to the decrease in lipid peroxidation processes and increase in the activities of plasma protein thiols as a result of the treatment.

Liver function markers, as influenced by the administration of ALCL3 alone and mixed with prepared polymers, were also presented in Table 3. The activities of ASAT, ALAT and ALP and TBil, were significantly affected by the type of treatment where these serum levels of DBil were not affected by any of the studied factors.

In comparison to the controls, the ALCL3 treated rats showed significant elevations in the activities of ASAT and ALAT and ALP as well as the levels of TBil. On the contrary, the activities of ALP, ASAT and ALAT as well as the levels of TBil and DBil of prepared polymers plus ALCL3treated rats were not significantly different from those of the control group.

In the present study, serum hepatic biomarkers, AST and ALT activities were greatly increased ($p < 0.05$) in rats treated with the ALCL3 alone compared to the control.

As in the present investigation, previous studies have shown that ALCL3 alone significantly increased serum ALP levels, and total protein and albumin levels. The increased serum levels of hepatic markers have been attributed to the liver injury because these enzymes are found in the cytoplasmic area of the cell and they are released into circulation in case of cellular damage.On the other hand, treatment with prepared polymers plus ALCL3 was found to suppress ($p < 0.05$) the increase of serum AST and ALT activities.

Table 3: Effect of oral administration of $ALCL_3$ alone or with different prepared polymers containing different drugs, on different biochemical parameters in male albino rats

Parameters	Experimental groups				
	Control	$ALCL_3$	Curcumin Polymer	Rosemary Polymer	Donepezil Polymer
TL ($mgdL^{-1}$)	512.04 ± 43.6	658.8 ± 50.38	488.40 ± 38.07	440.80 ± 31.76	432.20±18.2
TC ($mgdL^{-1}$)	118.20 ± 2.97	228.8 ± 20.31	122.40 ± 13.68	103.80 ± 4.54	113.8±4.4
TG ($mgdL^{-1}$)	104.40 ± 7.34	164.80 ± 14.5	106.00 ± 9.39	101.40 ± 8.33	106.4±9.3
LDL-C ($mgdL^{-1}$)	61.20 ± 9.87	159.02 ± 16.7	55.60 ± 8.03	43.80 ± 4.49	59.8±3.9
HDL-C ($mgdL^{-1}$)	36.60 ± 6.40	27.06 ± 3.95	40.00 ± 5.52	39.80 ± 4.73	37.8±2.3

TP (g dL^{-1})	6.68 ± 0.22	6.52 ± 0.30	6.24 ± 0.05	6.19 ± 0.08	6.24±0.05
A (g dL^{-1})	4.42 ± 0.13	3.42 ± 0.15	4.12 ± 0.09	4.36 ± 0.07	4.2±0.08
G (g dL^{-1})	2.46 ± 0.24	3.70 ± 0.18	2.62 ± 0.19	2.59 ± 0.11	2.62±0.09
A/G ratio	1.72 ± 0.16	0.85 ± 0.09	1.38 ± 0.16	1.36 ± 0.13	1.62±0.07
ASAT (UL^{-1})	33.02 ± 1.30	118.7 ± 24.49	48.20 ± 8.01	52.7 ±11.2	42.4±3.1
ALAT (UL^{-1})	25.60 ± 1.50	75.60 ± 2.77	39.02 ± 5.52	38.9 ± 7.63	23.6±1.2
ALP (UL^{-1})	55.30 ± 3.84	70.02 ± 8.08	53.22 ± 5.72	56.14 ± 7.61	53.2±2.7
TBil (mg dL^{-1})	0.66±0.02	0.89 ± 0.03	0.73 ± 0.05	0.77 ± 0.03	0.69±0.04
DBil (mg d L^{-1})	0.11 ± 0.005	0.14 ± 0.006	0.10 ± 0.008	0.10 ± 0.004	0.12±0.02

Data are represented as mean ± standard error

Effect on the Hepatic Lipid Peroxidation and Endogenous Antioxidants

The effects of ALCL3 alone or with different prepared polymer administrations on the levels of hepatic MDA and GSH and the activities of endogenous antioxidant enzymes were shown in Table 4. The hepatic levels of MDA and GSH as well as the activities of CAT, SOD and GR were significantly influenced by the type of treatment.

In the liver of rats administered ALCL3 alone, there was a meaningful elevation in the levels of MDA accompanied by a marked reduction in the GSH content, SOD and GR activities as compared to those of the controls. In the rats of different prepared polymers plus ALCL3 treated groups, the mean values of hepatic MDA concentration were significantly lower than those of ALCL3 treated rats and were not significantly different from those of the controls.

On the other hand, the mean values of hepatic GSH content of different prepared polymers plus ALCL3 treated rats were significantly higher than those of the ALCL3 treated group. As compared to the ALCL3 treated group, the rats administered different prepared polymers plusALCL3 showed a marked elevation in the activities of CAT and SOD and GR that did not significantly differ from those of the controls.

The present study revealed that different prepared polymers decreased ($p < 0.05$) ALCL3, which induced elevated enzyme levels in tested groups, indicating the protection of structural integrity of hepatocytic cell membrane or regeneration of damaged liver cells. Our observations and findings can be attributed to the antioxidant ingredients of different prepared polymers that probably inhibit lipid peroxidation and consequently, inhibition of oxidative stress. Therefore, the cell membranes remain. So the cells are prevented from entering the necrosis tip.

Table 4: Effect of oral administration of $ALCL_3$ alone or with different prepared polymers containing different drugs, on the levels of hepatic malondialdehyde (MDA) and glutathione (GSH) and the activities of catalase (CAT),superoxide dismutase (SOD) and glutathione reductase (GR) of male albino rats

Parameters	Experimental groups				
	Control	$ALCL_3$	Curcumin/ Polymer	Rosemary/ Polymer	Donepezil/ Polymer
MDA (nmol g^{-1} liver)	4.48 ± 0.11	9.18 ± 0.26	4.15 ± 0.22	4.78 ± 0.34	4.37±0.3
GSH (mg g^{-1} liver)	40.04 ± 5.1	19.72 ± 0.9	37.34 ± 2.84	38.91 ± 2.31	37.34±2.8
CAT (U g^{-1} liver)	104.3 ± 17.1	39.40 ± 8.2	99.03 ± 13.38	101.5 ± 14.74	100.6±10.3
SOD (U g^{-1} liver)	9.56 ± 0.17	4.36 ± 0.19	9.41 ± 0.16	10.23 ± .35	9.41±1.2#
GR (U g^{-1} liver)	73.20 ± 6.7	27.80 ± 1.2	68.40 ± 3.4	69.76 ± 3.9	68.5±7.8#

Data are represented as mean ± standard error

Histopathological Examination of different Groups of Rats

The Effect of different Treatments on Brain

Figure 6 (a-e) represents the photomicrographs for brain cortex sections of control and treated groups of rats. Microscopic investigation of brain sections for a normal control rat group shows highly active neurons which have huge pale-stained nuclei, nuclear chromatin and prominent nucleoli disappeared. The glial cells surrounded the neurons and support it. These cells have small densely stained nuclei with condensed chromatin and invisible nucleoli. Neuropil or background substances are shown in the cortex (Figure 6 a).

Examination of sections of brain cortex of rats administered with Al Cl3 alone showed

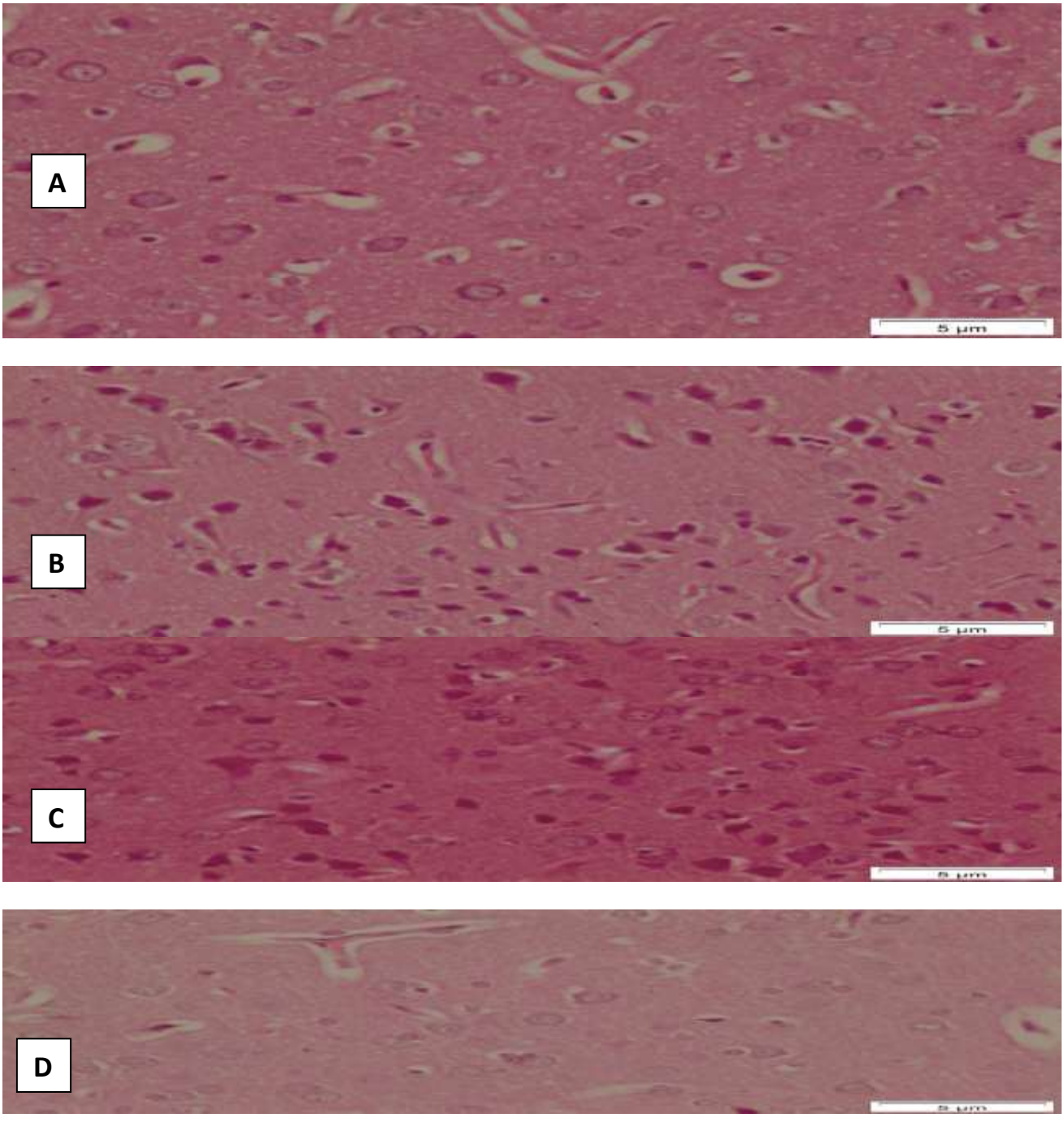
dark neurons with irregular shape and glial cells that appeared inside white vacuoles.

Neurofibrillary tangles stained with magenta color and looking like flames were found. The tangle appears as long pink filaments in the cytoplasm. The neuropil appeared vacuolated (Figure 6b).

Photomicrograph of sections in brain cortex of rats administered with Al Cl3 and the prepared SCACA polymer loaded with the Donepezil drug showed dark neurons with irregular shape and glial cells. No neurofibrillary tangles were found. The neuropils were appeared to be vacuolated. Some rats, cortex sections showed the normal structure of neurons with regular shape and glial cells. Few dark neurons and neurofibrillary tangles were found (Figure 6c).

In case of the administration of Al Cl3 and the investigated polymer included Curcumin, sections showed the appearance of neurons more or less like normal and regular shape. Also, a few dark neurons were found in sections of other rats (Figure 6d).

For rats administered with Al Cl3 and the polymer containing Rosemary, the neurons appeared more or less like normal and regular shape with a few neurons surrounded by precellular halos and no extracellular vacuoles were found in the neuropil (Figure 6e).



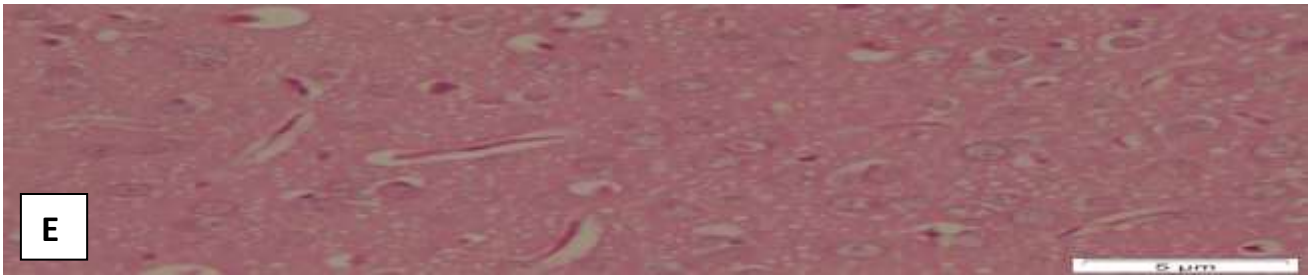


Figure 6: Photomicrograph of section in brain cortex of rats administered with: a- control rat ; b- Al Cl_3 ; c- Al Cl_3 + polymer + Donepezil ; d- Al Cl_3 + polymer + Curcumin ; e- Al Cl_3 + polymer + Rosemary

Effect of Different Treatments on Rat's Liver

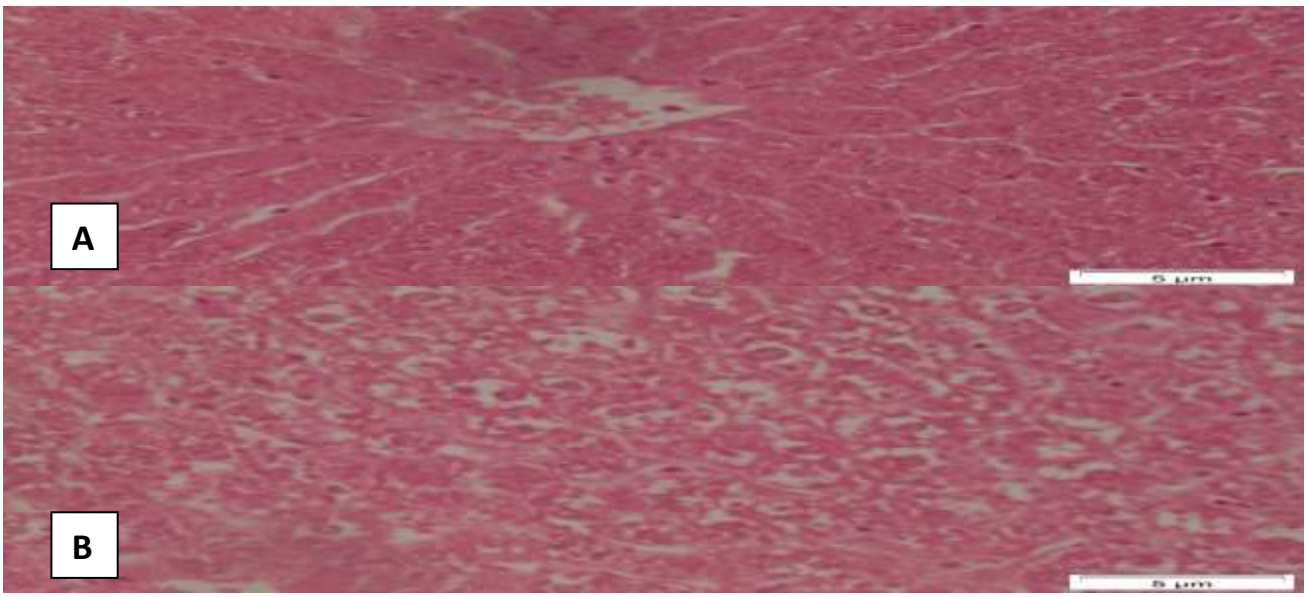
Figure 7 (a-e) showed the microscopic examinations of liver sections from non-treated (control) and treated groups of rats (administered with Al Cl3 alone and the prepared SCACA polymer loaded with different bioactive materials). In case of normal control rats, liver sections showed the normal architecture of hepatic lobules. The central veins lay at the center of the lobules surrounded by cords of hepatocytes. Between the strands of hepatocytes, the hepatic sinusoids are seen (Figure 7 a).

Histopathological investigation of liver from rats administered with Al Cl3 alone showed different lesions. These lesions were in the disturbance of normal architecture of hepatic lobule, hydropic degeneration and foci of nectotic hepatocytes with large areas of focal necrosis of the hepatocytes, micro, and macro vesicular changes in the hepatocytes were shown in other sections (Figure 7b).

Figure (7c) illustrated liver sections of rats administered with Al Cl3 and a polymer included Donepezil. It showed the normal structure of the hepatic lobule and a few regenerative hepatocytes were noticed.

In case of rats administered Al Cl3 and investigated a polymer loaded with Curcumin, the sections of the liver showed an improvement in the histological feature of the hepatic lobule as compared with rats given AlCl3 only (Figure 7d).

In rats administered with Al Cl3 and a polymer containing Rosemary, liver sections demonstrated a little improvement of the hepatic lobule structure as compared with rats given Al Cl3 only (Figure 7e).



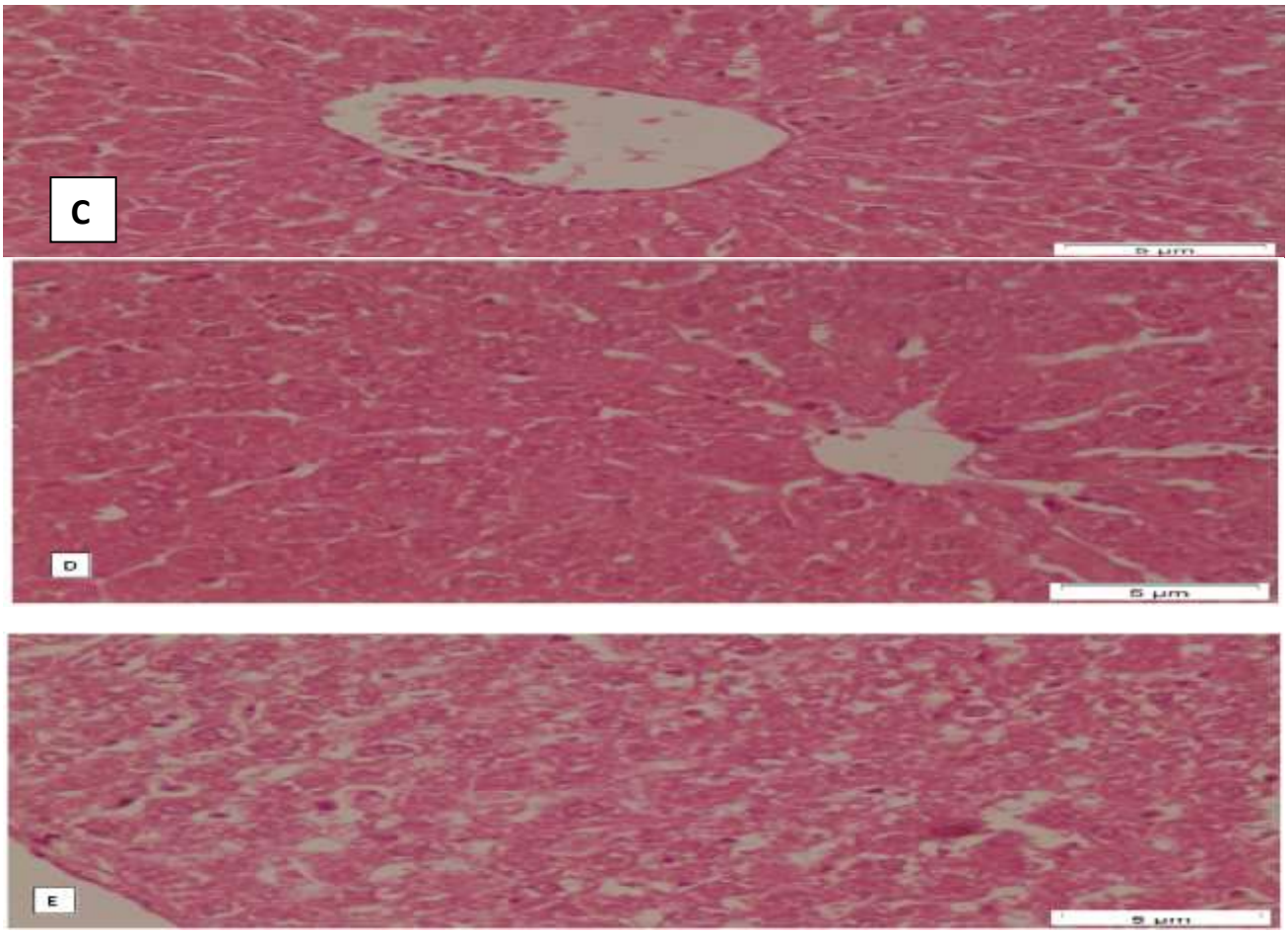


Figure 7: Photomicrograph of section in liver from rats administered with: a- control rat ; b- Al Cl_3 ; c- Al Cl_3 + polymer + Donepezil ; d- Al Cl_3 + polymer + Curcumin ; e- Al Cl_3 + polymer + Rosemary

In the present investigation, the biochemical findings were also confirmed by histopathological observations. The changes mostly include hepatocellular necrosis or apoptosis, fatty accumulation, inflammatory cells infiltration and other histological manifestations which were also consistent with the findings of other authors [26].

Conclusions and Recommendations

The slow release technique is considered a good means for long-acting protection from Alzheimer. Depends on the time of exposure. Moreover, this technique reduces the side effects of the conventional drug (Donepezil). Starch cellulose acetate coacrylate SCACA is considered a good binding polymer matrix for holding anti Alzheimer drugs such as Rosemary and Curcumin as bioactive natural products or Donepezil as a conventional drug used for treatment of this disease. The in vivo study for protection from Alzheimer disease gave promising results, especially for Rosemary, Curcumin and Donepezil.

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