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PHARMACEUTICAL AND HISTOLOGICAL STUDIES OF NEW SOLUBLE DIAZEPAM

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من المعروف أن عقار الديبازيبام غير قابل عمليا للذوبان فى الماء لذلك تهدف الدراسة إلى محاولة زيادة إذابته فى محاليل للحقن وذلك بإستخدام طريقة التجفيف بالتجميد.

وأستخدم عديد فينيل البيروليدون ٤٤,٠٠٠ وبلورنيك – ١٢٧ بنسبة مختلفة كأمثلة للبلمرات المحبة للماء والتى ثبت إستعمالها بسلام فى محاليل الحقن.

وقد أعطت التركيبة ديازيبام : عديد فينيل البيروليدون (١:٥) كذلك التركيبة المكونة من ديازيبام : بلورنيك – ١٢٧ (١:٦٠) أفضل النتائج.

وأثبتت النتائج أن التركيبة المحضرة بطريقة التجفيف بالتبريد يمكن أن تعد فى محاليل للحقن عند أس ايدرزجينى ٧,٢ كما يمكن أيضا إستخدامها فى صورة زيدس يذوب فى الحال فوق اللسان.

كما برهنت النتائج الهستولوجية أن المركب المحضر بطريقة التجفيف بالتبريد المجمد ليس له أى تأثير واضح على أنسجة الجسم بعد حقنة سواء فى العضل أو تحت الجلد.

Lyophilization technique has been adopted to enhance the aqueous solubility of diazepam. Two hydrophilic polymers namely; polyvinyl pyrrolidone 44000 (PVP) and pluronic F-127 (PF-127) in different ratios were used as the adjuvents in preparing the freeze-dried samples. Of the tested ratios, diazepam: PVP (1:10) and diazepam: PF-127 (1:60) were found to gave the highest solubility of the drug.

The dissolution studies revealed that lyophilized diazepam samples required from 1-3 minutes to dissolve compared to 20 minutes in case of untreated drug.

Histological evaluations were performed after S.C. and I.M. injection into mice. A slight histological changes were induced. Consequently, the prepared lyophilized-diazepam product can be reconstituted with ease using phosphate buffer (pH 7.2) for parenteral administration, as well as it can met the requirement of zydis.

INTRODUCTION

Diazepam, is a well known benzodiazepine tranquillizer. It was introduced as a parenteral preparation in the early of 1960, by Roche pharmaceuticals. The poor water solubility of diazepam accounts for the difficulty in the development of injection solution formulation. This necessitates the use of organic solvent vehicles such as polyethylene glycol, propylene glycol and ethyl alcohol to dissolve diazepam in the aqueous formulation.

However, there have been many reports that the toxic and side effects observed following diazepam injection could be attributed to these solvents or to the drug itself[1-8]. Propylene glycol for example, decreases the systemic vascular resistance, increase the LD_{50} of diazepam in mice and produced pain, inflammation and frequent thrombophlebitis at the injection site[3].

On the other hand, diazepam precipitates locally as a result of formulation dilution in the blood and tissue fluid which reduced drastically its solubility in the microenvironment[5-8].

However, Ghorab at al[9] reported that, diazepam in a cosolvent vehicle composed of

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50% propylene glycol, 5% ethyl alcohol, 2% benzyl alcohol and 43% water did not produced any sign of erythema or inflammation at the injection site of rabbits. Moreover, Saleh at al[10], suggested the use of 30% sodium salicylate solution as a suitable vehicle for diazepam injection. However, diazepam-sodium salicylate solution induced a higher degree of hemolysis in-vitro and less bound to bovine serum albumin than commercial diazepam injection[10].

Injectable diazepam has recently been formulated as a submicronized emulsion[3-8]. Although these preparations have been proved successful in reducing the incidence of venous sequelae of diazepam, it was found that larger doses of "Diazemuls" were needed to produce the same level of sedation as valium[11]. As explained by Mclean *et. al*:[12], the higher clinical potency of valium injection is due to the higher plasma free fraction of diazepam, where, greater affinity of diazepam for non-plasma constituents in case of "Diazemuls" was existed.

The development of parenteral formulation of water insoluble drugs often requires the use of lyophilized (Freeze-dried) powders in order to achieve better solubility and stability of water insoluble drugs[13,14]. Thus, the aim of the present study was:

1- to attempt the freeze-drying technique for enhancing the water solubility of diazepam.
2- Solubility and dissolution studies of the prepared lyophilized product to judge the efficacy of the technique.
3- Histological studies to judge the safety of the lyophilized samples following S.C. and I.M. injection into mice.

EXPERIMENTAL

Materials:

Diazepam was kindly supplied by Wyeth laboratories U.K., Pluronic F-127 (a gift from Ato Chem.), Polyvinyl pyrrolidone 44000 (BDH, Pool, England), and all other chemicals are of pharmaceutical grade and were used as received.

Table 1: Solubility of Different Lyophilized Diazepam Samples in Phosphate Buffer pH 7.2 at 30°C.

Sample	Composition	Drug:Polymer ratio	Solubility mg/ml.
A	Diazepam	1 : 0	0.0410
B	Diazepam:PVP44000	1 : 3	0.0820
C	Diazepam:PVP44000	1 : 5	0.1090
D*	Diazepam:PVP44000	1 :10	0.2240
E	Diazepam:PVP44000	1 :15	0.0700
F	Diazepam:PF-127	1 : 3	0.0713
G	Diazepam:PF-127	1 :10	0.0693
H	Diazepam:PF-127	1 :15	0.0797
I**	Diazepam:PF-127	1 :60	0.4280

* The solubility of corresponding physical mixture = 0.0737 mg/ml.

** The solubility of corresponding physical mixture = 0.0960 mg/ml.

Methods:

1- Preparation of lyophilized samples:

Two hydrophilic polymers viz; PVP 44000 and PF-127 in different ratios (Table 1) were used. The required amounts of diazepam and the selected polymer were weighed and then dissolved in a mixture of alcohol and phosphate buffer pH 7.2 (The least amount of alcohol is used just to dissolve the drug in the aqueous buffer). The solution samples were freeze-dried using the freeze dryer model (No. F.D.500160 Birchover Inst. Ltd, Herts, England). The total cycle time was 48 hr. After completion of the cycle the hardened product was powdered and sieved. The fraction size between 90-60 μm was selected. All samples were stored in a desiccator over silica gel at 25°C.

2- Solubility study

An excess amount of the lyophilized diazepam powder was added to 10 ml of phosphate buffer pH 7.2 in 25 ml screw-capped amber glass bottles. The tightly closed bottles were placed in a mechanical shaking water bath adjusted at 30°C. Preliminary experiments indicated that equilibrium was established within 3 hours.

After equilibrium, the test solutions were subjected to filtration. The samples were diluted with phosphate buffer pH 7.2 and measured spectrophotometrically at 230 nm (Shimadzu Double-Beam spectrophotometer 150-02, Japan).

3- Infrared:

The I.R. spectra for PVP 44000, diazepam, and diazepam-PVP lyophilized samples (1:10) were performed in the range 1800-1600 cm^{-1} using the Unicam Sp 1025 IR spectrophotometer and KBr disk method.

Powder X-ray diffractometry:

The X-ray diffraction patterns of diazepam, PVP, diazepam-PVP Freeze-dried sample; PF-127 and the diazepam-PF-127 Freeze-dried samples were determined. The powder X-ray diffraction patterns were taken by an X-ray diffractometer (Philips diffractometer PW 1710, Netherlands) with Cu-K as α-radiation (1.5418 A°) nickel filter 40 KV, 30 mA and a rate of 0.06°/ 5 min.

Dissolution study:

This was performed using the USPXXi paddel apparatus at 37°C and 50 r.p.m. The dissolution medium was 900 ml of 0.1 N HCl. An amount from the lyophilized samples equivalent to 5 mg diazepam was dispersed in the dissolution medium. Aliquots 5 ml each were withdrawn at appropriate time intervals and assayed spectrophotometrically at 286 nm (λ_{max}) in 0.1 N HCl.

Histological study:

Thirty six adult male mice, were divided randomly and evenly into six groups as follows:
Group 1: Used as control and injected with saline.
Group 2 and **3**, were used for testing the effect of diazepam-PVP freeze-dried sample (1:10) and the corresponding polymer respectively.
Group 4 and **5**, were used to test the effect of diazepam-pluronic freeze-dried sample (1:60) and the corresponding polymer respectively.
Group 6: was injected by the hydroalcoholic preparation [5% ethyl alcohol, 2% benzyl alcohol, 50 PG and 43% water][9].

Each group was injected S.C.(0.1 mg/kg) in one hind limb with the tested preparation, on the other limb, the same preparation was injected I.M (0.1 mg/kg). The injections were carried out daily for 7 days. The animals were then sacrificed and the injected parts (either skin or muscle) were taken and treated for histological examination. The specimens were examined by the light microscope after staining with hematoxylin and eosin (H & E).

RESULTS AND DISCUSSION

The lyophilization technique was adopted to enhance the water solubility of diazepam. The technique is efficient and the products produced were of high yield and lack of tackiness. Two hydrophilic polymers were utilized in this respect. PVP 44000 is commonly used as a blood expander which consequently make it safe for I.V formulation. The other polymer, PF-127 has been recommended for use as a vehicle for injectables by I.V, I.M and S.C routes[15,16].

Solubility:

The aqueous solubility of diazepam lyophilized powder was determined at 30°C in phosphate buffer pH 7.2. Table 1 shows the solubility of lyophilized diazepam using different ratios of PVP or PF-127. The solubility of pure diazepam as well as the physical mixtures was also performed.

As expected, the presence of either PVP or pluronic F-127 increased to different extent the solubility of diazepam from its lyophilized powder. It is evident from the results presented in the table, that the solubility of diazepam depends on both type and amount of the polymer used.

On using PVP the solubility of the drug was increased from 81.5 to 223.5 mg/L as the drug to polymer ratio was changed from 1:3 to 1:10 respectively. However, further increase of PVP content (1:15) resulted in a decrease of the solubility of diazepam. This decrease in solubility may be attributed to an increase in viscosity of the stagnant layer around the drug particle, or may be due to salting out effect.

With respect to PF-127, the solubility of diazepam was increased ten-folds from its lyophilized sample and two-folds from its corresponding physical mixture (drug-PF-127 ratio 1:60) as compared to pure drug. It is obvious that there is an abrupt change in the solubility of the drug from the lyophilized sample at this ratio. This abrupt change might due to the pluronic content at this ratio which was chosen around the CMC of this polymer[17].

Fig. 1 displays the IR spectra of PVP, diazepam and diazepam-PVP lyophilized sample (1:10).

The spectrum of diazepam (Fig. 1B) characterized by one sharp peak between 1600 and 1800 cm^{-1} corresponding to C=O functional group at 1680 cm^{-1}.

For PVP 44000 (Fig. 1A) the spectrum shows different peaks in this region. The spectrum of diazepam-PVP freeze dried sample (Fig. 1C) revealed that the peaks of the carbonyl groups of diazepam and PVP appears as if they were bound as indicated by the low-peak intensity in this region.

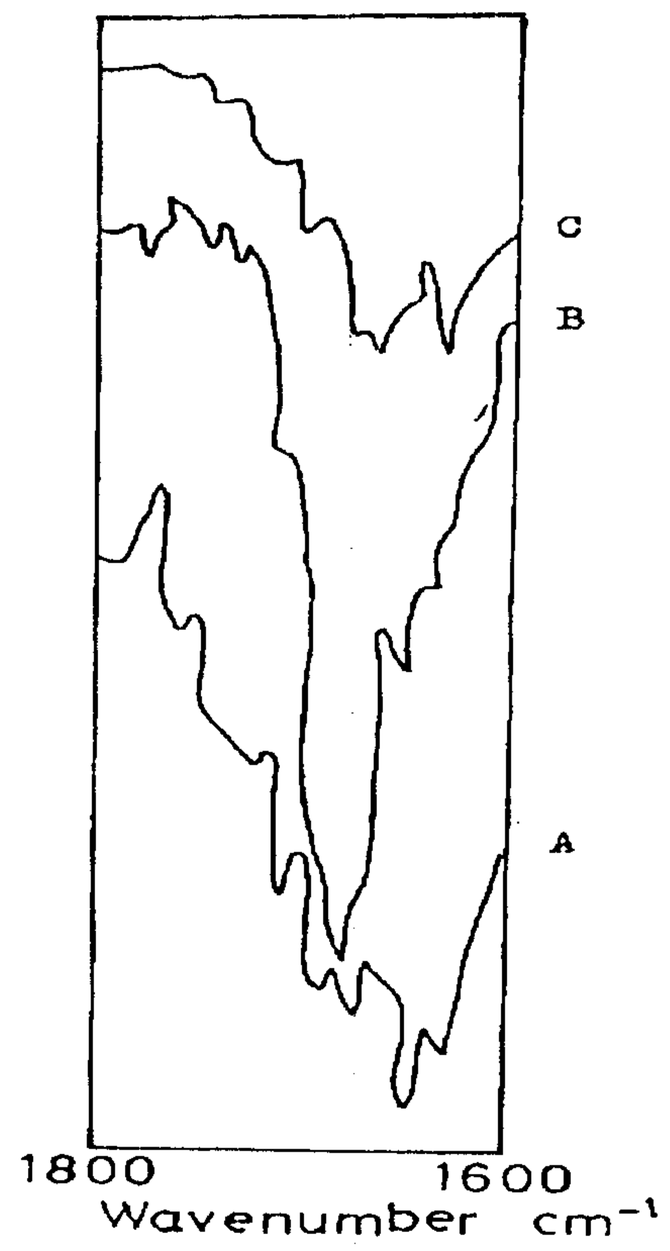

Fig. 1: Infrared Spectroscopy of (A) PVP, (B) Diazepam, (C) Freeze-dried sample (drug : PVP 1:10)

With respect to PF-127 lyophilized samples, they could not be handled (sticky) using KBr disc or nujol method. This may be related to the intrinsic physico-chemical nature of PF-127.

Fig. 2 shows the X-ray diffraction spectra of diazepam, PVP 44000, diazepam-PVP lyophilized sample, PF-127 and diazepam-PF-127 lyophilized sample. It is evident that diazepam (Fig. 2A) exhibited characteristic peaks of diazepam crystals at 9.45, 13.60, 18.89, 22.06, 22.78, 23.99 and 29.71 (2θ degree). Some of these peaks in diazepam-PVP freeze-dried sample (Fig. 2B) showed either decrease in intensity or disappeared. However, in case of diazepam-pluronic freeze dried sample (Fig. 2D), most of them were vanished. This finding indicated the presence of diazepam in less crystalline form in case of diazepam-PVP lyophilized preparation or entrapped in micelles in case of diazepam-PF-127.

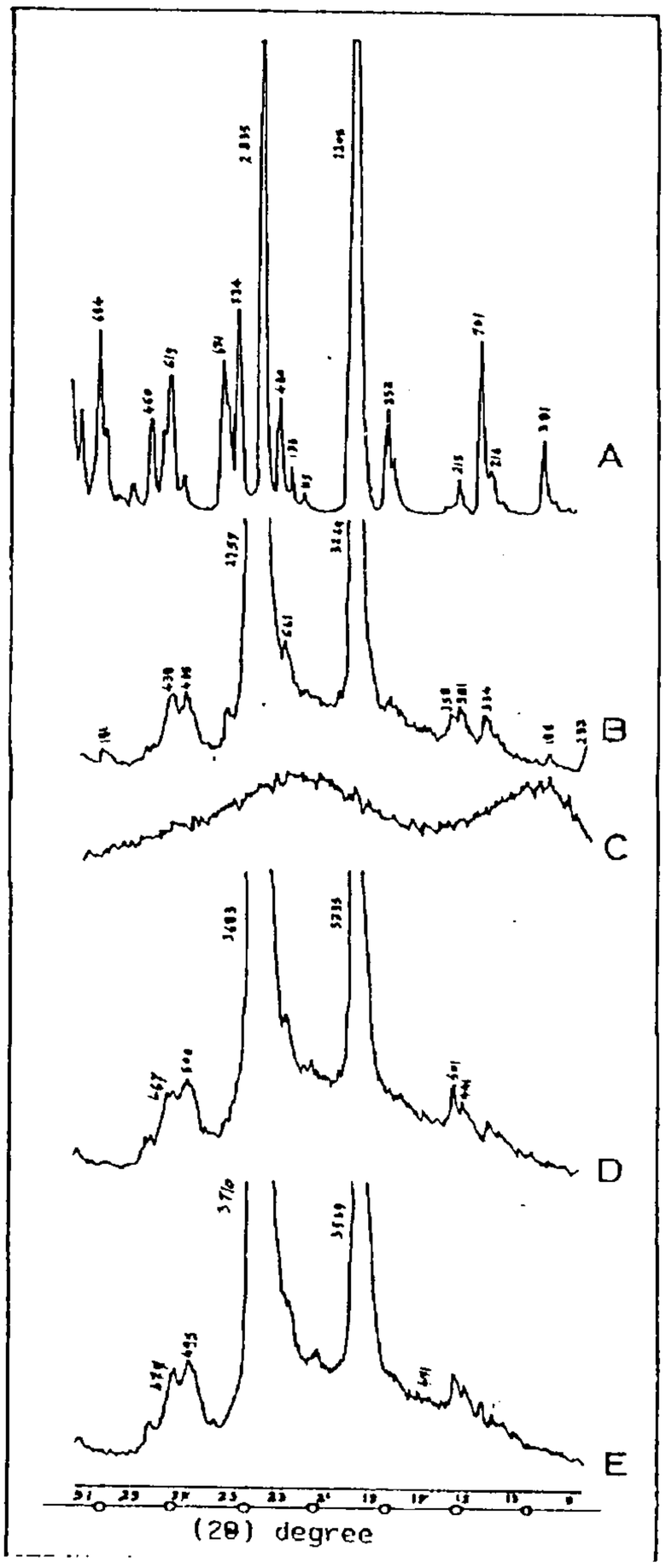

Fig. 2: X-ray diffraction of (A) diazepam, (B) Diazepam-PVP freez-dried sample, (C) PVP 44000, (D) diazepam-PF-127 freez-dried sample and (E) PF-127.

Consequently the IR and X-ray patterns might explain the increase in solubility of the studied drug.

Moreover no change in the IR or X-ray spectra of the tested samples upon aging for 6 months was observed.

Dissolution:

Further use of diazepam freeze-dried powder as solid dosage form necessitates that, all the prepared freeze-dried samples must subjected to dissolution studies.

The results, revealed that the freeze-dried samples required from 1-3 minutes to dissolve compared to 20 minutes in case of untreated drug. Consequently, it is of interest to say that this instantaneous dissolution of diazepam prepared by subliming water from the frozen composition of the drug and an aqueous solution of carrier can met the requirement of zydis[18].

This solid dosage form is practically suitable for children, old patients with frequent vomiting, who frequently have problems swallowing tablets and capsules. Also, zydis is convenient for active, busy people who do not have easy access of water[18].

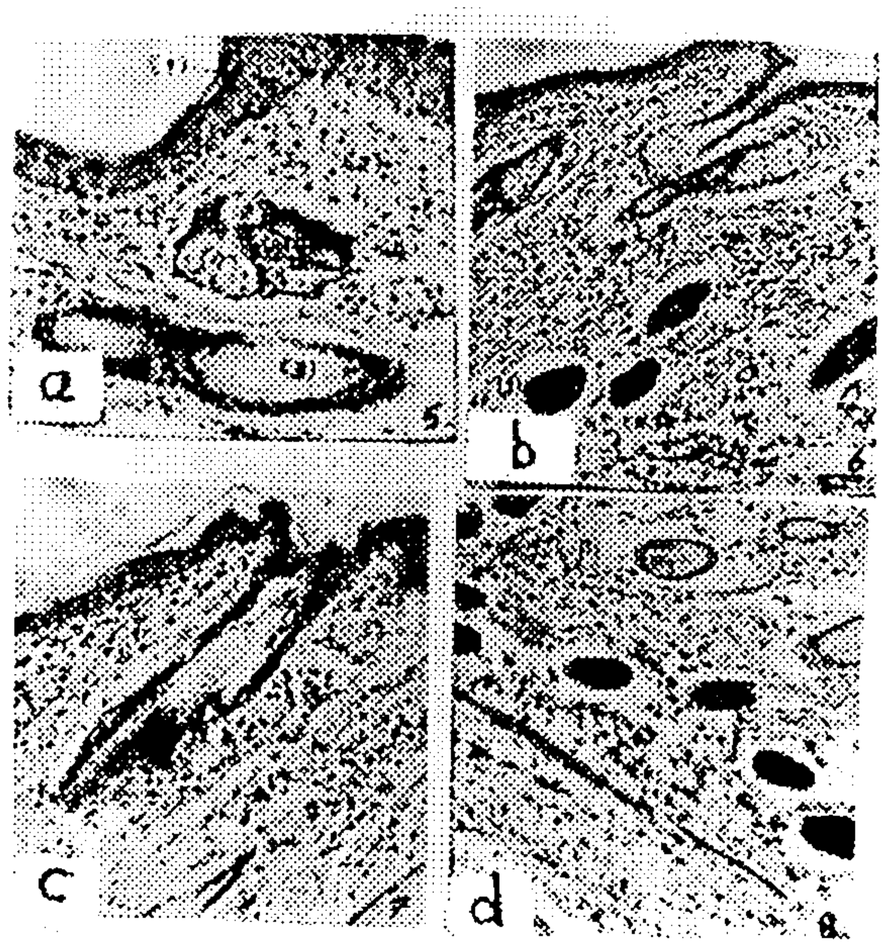

Fig. 3: Section (10x25) in the skin of mice following S.C. injection

a- The normal structure of skin. It consists of superfacial epidermis (1), the dermis (2) containing hair follicles (3) and sebaceous glands(4).

b- Showing that injection of hydroalcoholic preparation lead to some oedema (1) especially around hair follicles. Also, few inflammatory cells in the deep layers of the dermis (2).

c- Diazepam-PVP, notice that the picture is more or less similar to control.

d- Diazepam-PF-127 showing oedematous spaces around hair follicles.

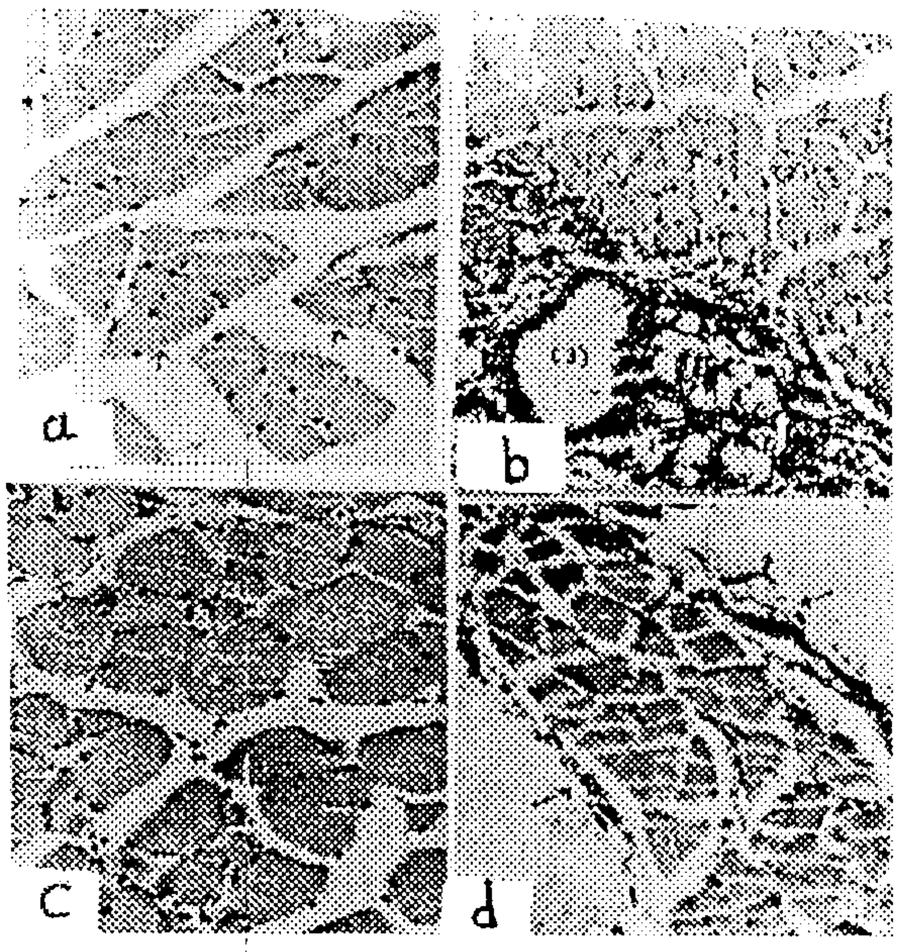

Fig. 4: Section (1x25) in the skeletal muscle of mice following I.M. injection

a- The skeletal muscle fibers of control mice are cylindrical in shape with peripheral multiple nuclli and compact myofibrills filling their cytoplasm. They are arranged in groups.

b- Shows that injection of the hydroalcoholic prepararion leading to inflammatory reaction as indicated by oedema (1) and perivascular cellular infiltration (2) and vascular infiltration (3).

c- Diazepam-PVP freez-dried preparations, notice only slight enlargement of the muscle fibers were observed due to relaxing effect of diazepam.

d- Diazepam-PF-127 preparation showing compression of the muscle bundles, and insignificant increase in the connective tissue cells around them.

Histology:

The histological evaluation after the S.C. and i.m injection of the reported hydroalcoholic preparation of diazepam (9) was not clarified. Hence, comparative study was performed on the reported formula[9] and the prepared freeze-dried samples.

Fig. 3 and 4 representing the different sections taken from either skin or skeletal muscle of mice after injection. There was a marked difference in the effect induced by the S.C. and i.m administration of the hydroalcoholic diazepam solution and the freeze-dried solution. The S.C.injection of the hydroalcoholic preparation resulted in a more prominent inflammatory reaction in the form of oedema and cellular infiltration (Fig. 3b).

After diazepam-PVP injection (Fig. 3C) the only changes observed is slight swelling of skeletal muscle fibers due to relaxant effect of diazepam. Separation of connective tissue fibers, which may be related to the presence of the drug in the vicinity of these tissues[19] occurred.

Injection of diazepam-PF-127 freeze-dried sample showed insignificant non injurious changes in both skin and skeletal muscle in the form of slight Oedematous changes and spaces around hair follicles (Fig. 3C and 4C).

Injection of the two blank solutions (PVP and PF-127) in equivalent amount present in the freeze-dried samples showed nearly similar appearance as the control.

Conclusion:

1- It can be concluded that the samples prepared by the freeze-drying method offers ease of reconstitution of the freeze-dried drug with buffer pH 7.2, compatibility of the soluble drug with body fluid as well as lack of tissue irritation at the site of injection.

2- The freeze-dried samples can met the requirement for zydis. This conclusion can be further used for the appropriate design of this work.

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