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Evaluation of oil extract from *T. catappa* as a sustained release injection base

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ABSTRACT

Terminalia catappa **seed oil with the following parameters: specific gravity-0.9200 ± 0.10, refractive index-0.4640 ± 0.06, viscosity (cps)-36.80 ± 0.94, peroxide value-8.63 ± 0.03, saponification value-157.8 ± 2.88), iodine value-38.63 ± 1.06 was used to formulate haloperidol in injection dosage form of 10 mg/2 ml. The formulation was evaluated for content of active ingredient, stability, toxicity, physicochemical properties and release patterns through dialysis membrane in phosphate buffer at physiologic pH of 7.4. The results were compared with those of a standard haloperidol injection B.P. Active ingredient content was found to be 100.61 ± 0.38 and 101.29 ± 0.64 percent (%) for the oil injection and the B.P formulation, respectively. They all showed requisite sterility as no organisms were isolated after incubation for seven (7) days in appropriate media and temperature. However, there was a significant delay in the release of haloperidol from the oil injection as against the standard B.P injection (P<0.05). The haloperidol oil injection exhibited sustained release behavior and had a predicted shelf life of 2.66 years compared to the 1.65 years evaluated for the haloperidol injection B.P**

Key words: *Terminalia catappa:* haloperidol; injection; formulation, sustained release base

INTRODUCTION

Vegetable oils have been employed successfully in the formulation of diverse excellent injections either in solution or suspension from [1] and other pharmaceutical dosage forms such as ointments [2], emulsions and creams [3]. These products containing vegetable oils possess unique characteristics that make them suitable for different applications.

Moreover, vegetable oils are readily available and cheap when compared to other natural and synthetic or semi-synthetic bases used in the same formulation. Their stability in such formulations can be greatly enhanced by different methods including the use of antioxidants [4] and by micro encapsulation [5]. The extraction procedure, stability study, autoxidation and complete characterization of

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the vegetable oil from *Terminalia catappa* have previously been established [6, 7]. Extensive pre-formulation studies with the oil showed its potential sustained release property. An oily vehicle for injections may be necessary first, if the medicament is insoluble or only slightly soluble in water and secondly, when an oily medium is more suitable for diagnostic procedure [8]. However, these injections can only be given intramuscularly. In an oil injection of the solution type, release rate of a drug is determined by the partitioning of the drug out of the oil into surrounding aqueous medium. The success of an oil solution in achieving sustained release depends on the magnitude of the partition coefficient, which is a function of the drug involved and the oil selected. Only those drugs, which are appreciably oil-soluble and have the desired partition characteristics, are suitable [1]. Haloperidol, a selective central nervous system depressant of the butyrophenone group and mainly used as an antipsychotic in humans was employed in this study because it exhibited the desired characteristics on preliminary evaluation. The objective of this work to prepare an injection of haloperidol in *T. catappa* oil and evaluate the formulation using a haloperidol injection B.P. Our major desire amongst many others is to generate an oil injection of haloperidol, which will circumvent the current frequent dosing with the standard B.P injection in psychotic patients.

MATERIALS AND METHODS

Plant Materials:
The fruits of *T.catappa* were collected from University of Nigeria in 2000, Nsukka, Nigeria and sun-dried for some time so as to obtain fairly whole seeds when cracked. The seeds were further processed according to the method described by Uzoho and Eze [6] to obtain the crude *T.catappa* oil. The crude oil was further refined according to the methods proposed by Ong [9] and Dudrow [10].

Animal Used:
Adult male albino mice (19-21 g) were purchased from the veterinary laboratory of the Faculty of Veterinary Medicine, University of Nigeria, Nsukka. They were kept in steel cages and fed with commercial pellets and clean tap water from domestic supply.

Reagents and Solutions:
Petroleum ether (40-60 ^{0}C), chloroform, methanol, methyl ethyl ketone (MEK) potassium iodide, sodium thiosulphate were all of Merck brand and sourced commercially. Every reagent and solvent was of analytical grade and used as freshly prepared solutions.

Solubility determination of haloperidol powder in *T.catappa* oil at 4, 25, 37 and 42 ^{0}C:

The determination of the solubility parameter was done at two degrees of freedom following the method described by Madder and Grady [11]. Briefly, the above designated temperatures were established in a refrigerator, room temperature or in a thermostat-regulated water bath. Haloperidol powder in increasing quantities were accurately weighed out in analytical balance (Metler-Toledo P51-0.001g max) and introduced into clean dry test tubes containing measured volumes of the oil. Solubility was established on observation for clarity of resulting solution.

Acute toxicological studies on the crude and refined oil extract:
Acute toxicological studies were carried out using the animal described above and in

accordance with the official method described by Shah et al [12].

Formulation of haloperidol *T.catappa* oil injection and haloperidol injection B.P:

The haloperidol *T.catappa* oil injection was formulated according to a modification of the method described by Avis [13] and Nails [14]. Injection containing 10mg of hatoperiodl per 2ml of oil were prepared in aseptic glass ampoules, stabilized to pH 7.4, filtered and sealed hermetically. The oil injections were sterilized by dry heat (Bunsen flame; 160 ^{0}C for 65 min) and stored away from light. The same process was repeated or haloperidol injection B.P. In this case, however, lactic acid with water (3:2) was sued as the vehicle instead of the oil and the injection was sterilized by heating in an autoclave at 121 C for 15 minutes.

Sterility test on the injection formulations:
The sterility and physical characteristics were evaluated following the B.P. 1988 method [15]. Contents of an ampoule, after heating the neck in a flame and swabbing with methylated spirit were emptied and plated on nutrient agar, thioglycollate and cooked meat broth media. They were then incubated for 7 days at 37^{0}C and evidence/absence of growth was noted.

Content uniformity test on the injections:
The content uniformity of both injections was evaluated according to a modification of the B.P. 1980 method [16]. Five (5) injection ampoules were selected at random from each batch. Contents of each batch were emptied carefully into two separate, clean, dry beakers. Into the beaker containing the oil injection was added 5 ml of anhydrous acetic acid and 35 ml of MEK and properly mixed. Then 1.5 ml of chloroform was added and the entire mixture titrated with 0.1 M perchloric acid using 3 drops of crystal violet (0.5% w/v in acetic acid) as indicator. Average content of active ingredient was calculated noting 1ml of 0.1 M perchloric acid is equivalent to 37.59 mg of haloperidol. To the second beaker containing haloperidol injection B.P., 40 ml of water and 50 ml of 0.1 M hydrochloric acid was added. Extraction was done with successive quantities of 75, 75, 50 and 50ml of ether. The ether layer in the extract was removed using a rotary evaporator. Subsequently, the residue was dissolved in anhydrous acetic acid and MEK (1:7), 1.5 ml of chloroform added and titrated as above. The content uniformity test was performed on ten (10) ampoules from each batch selected at random and following the same titration technique.

Release studies on injection formulation:
The release study was a modified official assay technique for haloperidol injection B.P. [17]. Each ampoule was completely emptied into a dialysis membrane bag immersed in a 100ml phosphate buffer, (pH 7.4) solution in a 250ml beaker and held firmly with the aid of a thin rubber band. The unit was clamped and assembled. The phosphate buffer in the beaker was stirred with a magnetic stirrer at a temperature of 37±0.1^{0}C. Subsequently, 1 ml aliquots of the solution were withdrawn at time intervals of 0, 30, 60, 120, 180 and 240 minutes, diluted with fresh phosphate buffer to 5 ml and the absorbance read with a Pye-Unicam SP6 450 UV-Visible Spectrophotometer at 245 nm against a phosphate buffer blank. The receptor phase was kept constant throughout the release study by replacing the removed sample with an equal volume of phosphate buffer, pH. 7.4, maintained at 37± 0.1^0 C. The same process was performed at a temperature of 42 ± 0.1^{0}C and repeated for haloperidol-T *catappa* oil injection.

Accelerated stability testing on injection formulations:

Accelerated stability testing on the injection formulation was carried out according to a modification of the method described by Rodney [18]. The test was run at temperatures of 37, 42 and 55^{0}C for a period of 28 days and haloperidol content assayed at days 1, 3, 7, 14, 21 and 28. A sample was also kept at room temperature for 6 months and assayed at predetermined intervals. The assay method used was as described above under content uniformity test. However titration was done with 0.01 M perchloric acid. Back titration was done to determine the average volume of 0.01 M perchloric acid used. The content of haloperidol remaining at each time interval and temperature was calculated with 1ml of 0.01 M perchloric acid taken as equivalent to 3.759 mg of haloperidol. For the haloperidol injection B.P., successive extractions were carried out as described under content uniformity and subsequently, titration carried out as above and corrections made for measurement at a higher temperature using an established mathematical formular [19].

RESULTS AND DISCUSSION

Drug research and development requires that the industrial Pharmacist be able to determine the rate and course of drug degradation as this information would assist in the design of very stable products [20-22]. The results of all preliminary studies on *T.catappa* (tropical almond) seed oil showed that it could be used as suitable and safe pharmaceutical oil [7]. In addition, the appreciable yield of oil from the seed (about 40 % or more) [6] indicates that *T. catappa* could become a veritable source of this novel raw material. Interestingly, the tropical almond grows well in Eastern Nigeria soil and so attention could be geared towards creating plantations of the plant. This will hitherto enhance on the much happed "greening" of the environment apart from oil production. The result of the autoxidation studies [7] and other relevant pre-formulation studies informed the formulation of an injection with haloperidol, an antipsychotic agent, which had a high solubility of 10mg/ml in the refined oil at room temperature. The unusual reduced solubility of haloperidol at temperatures above 30^{0}C could be explained in terms of molecular aggregation, which tends to increase with temperature. The haloperidol *T. catappa* injection compared favourbale with standard haloperidol injection B.P in terms of content uniformity. The average content uniformity were found to be 101.29 ± 0.64 and 100.61 ± 0.38 for the haloperidol injection B.P and haloperidol oil injection respectively. There was no significant difference between the two batches in terms of content uniformity. This is an indication that the formulations were reasonably properly prepared. The result of the acute toxicological studies proved that the oil is safe with an LD_{50} of 267.2 mg/kg. Moreover, the seeds of T. Catappa are eating by humans without any evidence of poisoning till date.

Interestingly, the release studies on the respective batches of injections showed that *T.catappa* oil is a putative sustained release injection base especially at lower temperature of 32 °C as evidenced from Figure 1. The release at 42 °C did not exhibit appreciable sustained release properties. Oily injections, especially in suspension form, possess longer duration of action as a result of sustained release of the medicament from the formulation [1]. In addition a single daily dose of an injection will be preferable because of better compliance. The release profile of the haloperidol *T.catappa* oil injection and haloperidol injection B.P. were statistically different ($P<0.05$) as could be seen in Figures 2 and 3. The degradation of the drug in the oil

injection and in the standard injection was of first order. The determined shelf life of the oil injection was found to be 2.66 years confirming the higher stability of this product compared to standard haloperidol injection B.P (1.65 years) (see Figure 4). Further studies to enhance the stability of the formulation and improve on its release properties through micellar formation or self emulsifying drug delivery systems (SEDDS) are desirable. Furthermore, we recommend *in vivo* release studies in order to determine the extent of correlation with *in vitro* data.

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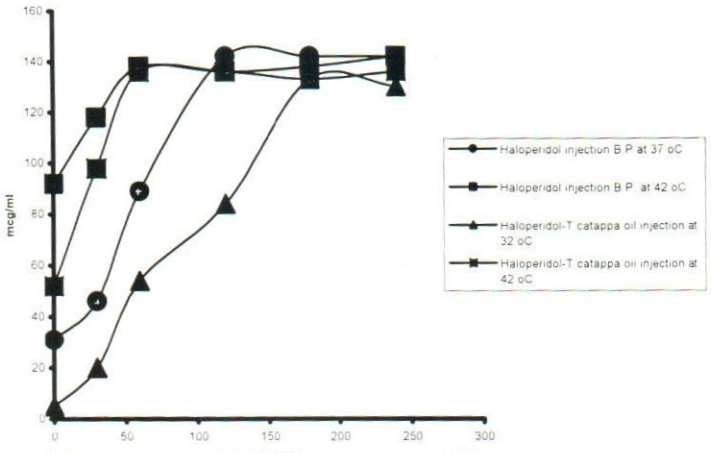

Figure 1:Haloperidol release versus time at 37 and 42 oC

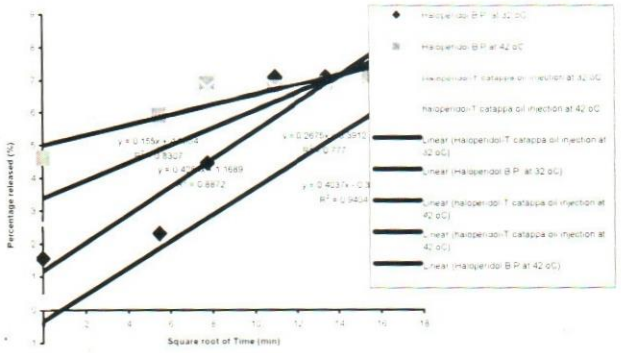

Figure 2: percentage drug released versus square root of time

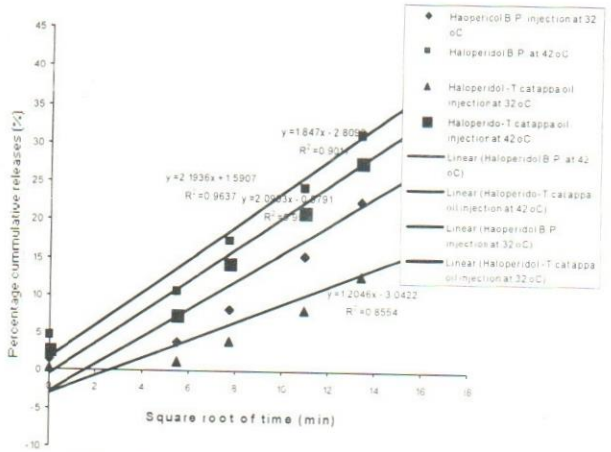

Figure 3: Graph of cummulative haloperidol release versus square root of time

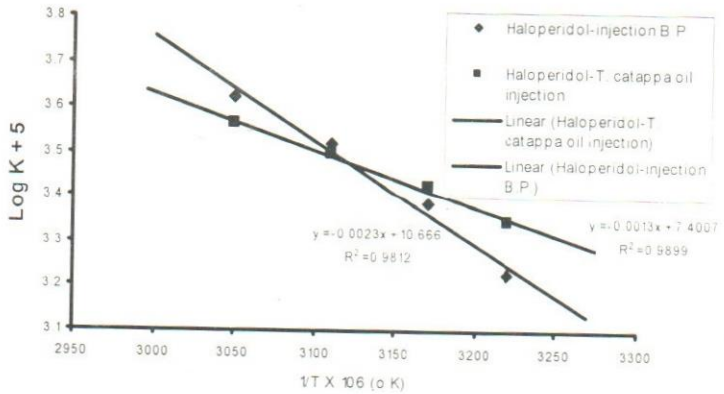

Figure 4: Arrhenius plots of Haloperidol Injection BP and Haloperidol-T.catappa oil injection (10 mg/2 ml)

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