Research Article

POTENTIALS OF *DETARIUM MICROCARPUM* (GUILL AND SPERR) SEED OIL AS A MATRIX FOR THE FORMULATION OF HALOPERIDOL INJECTION

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ABSTRACT

Air-dried *Detarium microcarpum* seeds were pulverized to a coarse powder and the fixed oil extracted exhaustively (8 hours) in a soxhlet apparatus at 40-60 ℃ using petroleum ether (40-60℃) as solvent. Oil yield was estimated gravimetrically, and some physicochemical properties of the seed oil were determined. Depot injection formulations of haloperidol in *D. microcarpum* seed oil (*DMO*), arachis oil and aqueous medium, respectively, stored at different temperatures (28, 60 and 1℃) for 180 days were assayed for drug content via non-aqueous titration; to ascertain product stability. Results were compared, using univariate analysis of variance (ANOVA). The free fatty acid, saponification, peroxide, iodine and ester values of *DMO* were: 0.11 *mgKOH/g*, 198.80 *mgKOH/g*, 1.62 *milliEqui./kg*, 121.60 *g iodine/100 g* and 198.69 *mgKOH/g*, respectively. Other physicochemical parameters of *DMO* were: melting point (2.50 ℃), relative density (0.92), and refractive index (1.46). *DMO* has low deterioration rate and good edibility; thereby being adequate for consumption, industrial uses and pharmaceutical applications. The statistical analysis indicated that the results obtained with *DMO*, in the accelerated stability study, were comparable to those of arachis oil ($p < 0.05$). Thus *DMO* is recommended for use, in place of arachis oil, in the formulation of haloperidol injection and other oil based medicinal injections.

Keywords: *Detarium microcarpum* Seed oil, Physicochemical properties, Haloperidol, Depot injection, Antipsychotics.

INTRODUCTION

The development of depot injections in the 1960s gave rise to their extensive use as a means of long term maintenance treatment; and haloperidol decanoate, as depot injection, is in clinical use. Depot injections showed no significant difference in global impression behaviour, mental state, side effects, or outcomes of death. While patients, receiving depot injection of haloperidol decanoate needed less additional antipsychotic medication. This suggested that, haloperidol decanoate may have a substantial effect in improving the symptoms and behaviour associated with schizophrenia. Thus, the need to formulate and standardize antipsychotic drug dosage forms that will guarantee an excellent sustained delivery system became imperative.

Haloperidol, an amorphous or microcrystalline powder, is practically insoluble in water[1] and has a selective central nervous system (CNS) depressant effect[2]. It is primarily used for the management of psychotic disorders such as schizophrenia, brain damage, mania, intractable hiccup[3], hence the need to formulate it in a manner that will reduce frequency of administration.

Detarium microcarpum belongs to the family *Caesalpinaeceae* and it is found mostly in savannah forest of the drier type[4]. The fruit is edible and rich in vitamin C, the kernels are deep purple brown and more or less oily and also edible[5]. The defatted seed yields gum, which have been utilized as a bio-adhesive agent in the formulation of mucoadhesives and sustained release tablets[6]. While the seed oil, may be important in the formulation of emulsions, creams and ointments. In Nigeria, the *D. microcarpum* seed is used, traditionally, as a soup thickening delicacy.

Various parts of *D. microcarpum* are valuable in ethnomedicine as phytotherapeutics for the treatment and management of tuberculosis, meningitis, puriety and diarrhea. Its foliage are avoided by animals and the roots used in perfume[7]. A number of bioactive diterpenes have been isolated from the fruits of *D. microcarpum*[8]. The clerodane diterpenes showed inhibition of the growth of the plant pathogenic *Cladosporium cucumerinum* and of the enzyme acetyl cholinesterase, implicated in Alzheimer's disease. Specifically, the antifungal and enzyme inhibition properties of 3,4-epoxycleroda-13E-en-15-oic acid, 5a, 8 a-(2-oxokolavenic acid) and 3,4-dihydroxyclerodan-13Z-en-15-oic acid were very prominent; while 2-oxokolavenic acid showed a slight inhibition of the enzyme[8]. The other diterpenes (3,4-dihydroxyclerodan-13E-en-15-oic acid and copalic acid) had no significant antifungal and acetyl cholinesterase inhibitory activities. The present study, report the physicochemical properties of *D. microcarpum* seed oil

and the stability profiles of haloperidol injections formulated with the seed oil.

MATERIALS AND METHODS

Plant material

Detarium microcarpum seeds were sourced from Ogige Community, Nsukka-Nigeria, in July, 2004. They were identified and authenticated by Mr. Alfred Ozioko, Bioresources Development and Conservation Programme (BDCP), University of Nigeria, Nsukka-Nigeria. A voucher specimen was deposited at institute's herbarium. The seeds were sorted, air-dried and pulverized to a coarse powder, and then placed in an air-tight container and stored away from heat and light.

Oil extraction

Two hundred gram of the sample was packed into a soxhlet apparatus and the fixed (seed) oil was extracted exhaustively with petroleum spirit (40-60°C) for 8 h. The extract was filtered and the solvent distilled off *in vacuo* at less than 40 °C to obtain the pure seed oil. The weight of the resultant oil was noted and the per cent yield deduced; after which the neutral lipid was stored at 4°C in a refrigerator till when needed for physicochemical analysis and drug formulation.

Purification of *D. microcarpum* seed oil

A 15 % (w/w) mixture of fuller's earth and the seed oil was prepared and transferred to a round bottom flask and immersed in a paraffin bath maintained at 150°C. The system was maintained for 30 min with intermittent shaking every 10 min. Then the flask was cooled and the content filtered through a Buchner funnel. The treatment was repeated, to obtain a completely bleached lipid.

Physicochemical characterization of the seed oil

The oil extracted directly from *D. microcarpum* seeds were analysed for iodine number, acid value, free fatty acid, peroxide value, saponification value, ester value, saponification equivalent, refractive index, relative density, melting point, viscosity and per cent unsaponifiable matter using standard procedures described by AOAC[9], Pearson[10] and Gunstone[11].

Preparation of haloperidol injection

Haloperidol injection was formulated in three batches utilizing 3 different vehicles namely: bleached *D. microcarpum* seed oil, arachis oil and water/acetic acid (7:3) mixture. Batch one consisted of 15 ampoules, each containing 5 mg/ml haloperidol; and another 15 ampoules of 1 mg/ml haloperidol in three different vehicles.

To obtain the 5 mg/ml haloperidol injection, 30 mg of haloperidol powder was triturated in 6.00 ml of bleached *detarium* oil and homogenized. A 1.10 ml portion of the homogenized drug was transferred into each of the 15 ampoules. The same procedure was followed for arachis oil and water/acetic acid medium and for batches 2 and 3. Then 6.00 mg of the active ingredient was triturated in 6.00 ml of *detarium* oil and homogenized, and 1.10 ml of the formulation was aseptically transferred into each of the 15 ampoules. The same process was repeated for arachis oil and the water/acetic acid medium and for batches 2 and 3.

Sealing

The glass ampoules were sealed with a twin jet burner. The leak test or verification method was employed, which involved the boiling of the ampoules at 100 °C for 30 min in a beaker of amaranth dyed water. Any red colouration of the content of any of ampoule indicated leakage or improper sealing.

Sterilization

The injection in sealed ampoules were put in a beaker and placed in an autoclave and heated to 115°C 30 min.

Accelerated stability test

The average drug content of the products was assayed at 28°C (room temperature), immediately, on production. Subsequently, they were stored at 3 different temperatures (1, 28 and 60°C) for 180 days, after which the drug content was evaluated.

Assay of the haloperidol powder

Using non-aqueous titration, 0.30 g of haloperidol powder was dissolved in 50 ml of anhydrous acetic acid/methylketone (1:7) mixture and titrated against 0.10 M perchloric acid with 0.20 ml of naphthol benzene solution as indicator. Each ml of perchloric acid was equivalent to 37.50 mg of haloperidol. The per cent active ingredient content was deduced.

Determination of drug content

The content of each 5 mg/ml ampoules of haloperidol, dissolved in 0.83 ml of the solvent mixture was titrated against 0.10 M perchloric acid with one drop of naphthol benzene end point indicator. Haloperidol content was obtained as stated earlier. Also, the average drug content of each 1 mg/ml haloperidol ampoule was evaluated by the same non-aqueous titration in 0.17 ml of anhydrous acetic acid/methylketone (1:7) mixture. At all times, drug assay (before and after various treatments) were performed thrice and the average drug content and the standard error of the mean (SEM) deduced.

RESULTS AND DISCUSSION

The physicochemical properties of *D. microcarpum* seed oil presented in TABLE 1, indicated that the neutral lipid was liquid at room temperature and its light yellow colour suggested the presence of carotinoids[11].

The iodine value of the oil (121.60 g I_2/100 g) was more than one hundred but less than one hundred and thirty. This implies that the oil is moderately semi-drying. This was supported by its saponification equivalent (282.19); which indicated 1Δ-octadecenoic acid[11] as the dominant

fatty acid. Semi-drying oils are fairly slow to oxidation and remain as liquid foe a long time; this is a useful property required in the soap, cosmetics, lubricants, leather (for dressing) and candle industries.

Table 1: Physicochemical properties of *D. microcarpum* seed oil

Parameter	*D. microcarpum seed oil*
Iodine value *(g iodine/ 100 g)*	121.60 ± 0.22
Acid value *(mgKOH/g)*	0.22 ± 0.04
Free fatty acid value *(mgKOH/g)*	0.11 ± 0.02
Peroxide value *(miliEqui./kg)*	1.62 ± 0.01
Saponification value *(mgKOH/g)*	198.80 ± 0.62
Saponification equivalent *(Approx. molecular mass)*	282.19 ± 0.62
Ester value *(mgKOH/g)*	198.69 ± 0.62
Per cent unsaponifiables	2.81 ± 0.01
Relative density	0.92
Melting point *(°C)*	2.50
Refractive index	1.46
Relative viscosity	38.70
Colour	Light yellow

Values, where applicable, were mean ± SEM; Levels of significance (Student t-Test) $p < 0.05$.

The acid and peroxide values (0.22 mgKOH/g and 1.62 milliEqui./kg, respectively) of *D. microcarpum* seed oil were lower than the respective minimum safe limit (4.00 mgKOH/g and 10.00 milliEqui./kg) meant for consumption[11-13]. These suggest that the oil has low deteriorating rate and therefore can be stored for relatively long period. Both values are the indices of biogenic and oxidative rancidity, respectively, in neutral lipids; and higher values are associated with higher rate of rancidity. The free fatty acid content (0.11 mgKOH/g) was equally low, insinuating that *D. microcarpum* seed oil has low deteriorating rate and high edibility. High free fatty acid value (i.e. value > 2.00 mgKOH/g) is associated with high deteriorating rate and low edibility resulting from the development of objectionable flavour and odour[12]. The level of unsaponifiables in the seed oil (2.81 %), was as expected[11]; while the saponification value (198.80 mgKOH/g) was moderately high. The saponification value agreed with the value (220.60 mgKOH/g) reported by[13] in 2004, at zaria-Nigeria, and compared favourably with saponification values of palm oil (196-205), olive oil (185-196), soya bean oil (193), cotton seed oil (193-195), butter (220-233) and linseed oil (193-195)[14]. The moderately high saponification value of *D. microcarpum* oil suggests that it, relatively, contains high proportion of higher molecular weight fatty acids (~ C_{18} fatty acids). This was confirmed by its average molecular weight (282.19), which compared well with the molecular weight of 1Δ-octadecenoic acid (282). The ester value (198.69 mgKOH/g) was high and indicated very high proportions of intact glycerides and that the oil was of appreciable quality.

The relative density, 0.92 (at room temperature), lies between the expected values for neutral lipids (0.90-0.94)[15]. It suggests that the seed oil of *D. microcarpum* is mainly constituted by fatty acids of high mean molecular weights. This was in agreement with the mean molecular weight of the oil, 282.19 (*cf* saponification equivalent). Relative viscosity of 38.70, opined that the fluidity of and flow properties of the seed oil are adequate at room temperature. High relative viscosity is associated with poor flow properties and fluidity at ordinary temperatures. Very viscous oil is not good for the formulation of pharmaceuticals; as its transport along the product lines is usually met with much resistance. The oil, subsequently, becomes denatured.

Table 2: Drug content (mg Haloperidol) of the various 1 mg/ml ampoules of haloperidol injections

Keeping condition	*Drug content (mg Haloperidol)*		
	Water/acetic acid based ampoule	**Arachis oil based ampoule**	***D. microcarpum* seed oil based ampoule**
28 °C *(at day zero)*	1.13 ± 0.01	1.13 ± 0.01	1.13 ± 0.01
28 °C *(at day 180)*	0.75 ± 0.00	0.75 ± 0.00	1.13 ± 0.00
60 °C *(at day 180)*	0.38 ± 0.00	0.75 ± 0.0	0.75 ± 0.01
1 °C *(at day 180)*	0.38 ± 0.00	0.75 ± 0.01	0.38 ± 0.00

Values were mean ± SEM; Levels of significance (Student t-Test) $p < 0.05$.

Table 3: Drug content (mg Haloperidol) of the various 5 mg/ml ampoules of haloperidol injections

Keeping condition	*Drug content (mg Haloperidol)*		
	Water/acetic acid based ampoule	**Arachis oil based ampoule**	***D. microcarpum* seed oil based ampoule**
28°C *(at day zero)*	4.89 ± 0.01	4.89 ± 0.01	4.89 ± 0.01
28 °C *(at day 180)*	2.63 ± 0.00	2.63 ± 0.00	3.01 ± 0.00
60°C *(at day 180)*	2.26 ± 0.00	2.63 ± 0.0	2.26 ± 0.01
1 °C *(at day 180)*	2.26 ± 0.00	3.01 ± 0.01	1.88 ± 0.00

Values were mean ± SEM; Levels of significance (Student t-Test) $p < 0.05$.

The drug content of the various haloperidol injections (TABLES 2 and 3), showed that the active ingredient was stable in *D. microcarpum* seed oil at the various keeping conditions. This compared favourably with the drug's stability in arachis oil (positive standard). The stability in water/acetic acid matrix was more or less lower than those detected in the positive standard and *detarium* seed oil. Univeriate analysis of variance (ANOVA), at $p < 0.05$, indicated that the drug contents of the 1 and 5 mg/ml ampoules of arachis oil and *D. microcarpum* seed oil based haloperidol injections were not significantly different. This confirmed the suspected equi-stability of haloperidol in both matrices. Hence, *D. microcarpum* seed oil could be used as substitute for arachis oil in the formulation of haloperidol and other oil based injections.

CONCLUSION

This study confirmed that *D. microcarpum* seed oil has low biogenic and oxidative rancidity; a desired property in oils meant for consumption, industrial purposes and pharmaceutical applications. Also, its potential as a matrix in the formulation of haloperidol injections were comparable to those of arachis oil. Thus *D. microcarpum* seed oil is recommended for use as a substitute to arachis oil, in the formulation of oil based medicinal injections. This will save the huge foreign exchange expended, annually, in the importation of arachis oil and, as well, increase the local content of home (Nigeria) made pharmaceuticals.

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