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Evaluation of nootropic activity and formulation of transdermal patches of cod liver oil

Sharadha Srikanth, K. Prathyusha, V. Uma Maheswara Rao and G. Krishna Mohan*

C.M.R. College of Pharmacy, Kandlakoya (v), Medchal-501401, Hyderabad, Telangana, India
** Center for Pharmaceutical Sciences, J.N.T.U (H), Kukatpally-500072, Hyderabad, Telangana, India*

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Abstract

The evaluation of the nootropic activity of cod liver oil was carried out, using standard animal models. Cod liver oil is rich source of essential fatty acids like, ω-3-fatty acids such as docasahexaenoic acid and eicosapentaenoic acid which are antioxidants by nature. They can scavenge free radicals and prevent oxidative damage which is essential to reverse the effects of memory deficit which is a common problem now-a-days, occurring due to many reasons. Our literature survey revealed that the nootropic activity and formulation of cod liver has not been scientifically evaluated. Hence, we decided to evaluate and formulate it. Our preclinical studies revealed that it has significant nootropic activity as evidenced by its ability to reduce the transfer latency in elevated plus maze, step down latency ($p<0.05$), water maze ($p<0.01$) and reduce the acetyl choline esterase activity ($p<0.01$). The transdermal patches of cod liver oil were prepared by solvent evaporation method. Its preformulation studies and patch evaluation were carried out successfully.

Key words : Cod liver oil, nootropic, anticholinesterase, antioxidants, transdermal patch

1. Introduction

Nootropics, also referred as smart drugs, memory enhancers, cognitive enhancers, and intelligence enhancers, are drugs, supplements, nutraceuticals, and functional foods that improve mental functions such as cognition, memory, intelligence, motivation, attention and concentration. Nootropics have been shown to improve many aspects of brain and body function, along with elevating mood and concentration levels (Dwivedi *et al*., 2012).

Nootropics decrease platelet aggregation, increase cerebral blood flow and oxygen consumption. Nootropics increase adenylate cyclase activity and breakdown of ADP to ATP, increase density of frontal cortex ach receptors by 30-40% (Mishu, 2012).

Age, stress and emotional trauma are conditions that may lead to impaired learning, memory loss, amnesia, and dementia or two more ominous threats like Schizophrenia and Alzheimer's disease (Nirmal and Milind, 2003). To overcome this memory deficit, drugs and natural remedies have been prescribed to enhance memory in people (Rai *et al*., 2011).

Many synthetic nootropics like Piracetam and Donepezil are available which have been widely used and are associated with adverse effects such as diarrhoea, insomnia, nausea, bronchitis and muscular cramps. Therefore, plants are used since ancient times for health benefits. Plants also may be used to counter act memory loss in many ways (Lokhart and Lestage, 2003).

Antioxidants are said to suppress neuronal oxidative stress and inhibit acetyl cholinesterase activity. They also positively affect brain signalling to enhance neural communication (Shinoy *et al*., 2009; Sravanthi, 2013; Pandey and Rizvi, 2009).

Cod liver oil is a rich source of essential fatty acids. It has been proved for it antiatherothrombotic potential (Lorenz *et al*., 1983). Benefits of cod liver oil were mainly due to high concentrations of omega-3 polyunsaturated fatty acids, which are potent antioxidant agents. EFAs eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) possess anti-inflammatory properties (Galarraga, 2008).

The use of adhesive skin patches to deliver drugs systemically is a relatively new phenomenon. Transdermal drug delivery uses the skin as an alternative route for the delivery of systemically acting drugs (Priyanka and Biswajit, 2002).

The main objective of the proposed work was to evaluate the beneficial effect of cod liver oil for its nootropic activity in different experimental animal models and to formulate them in to a novel drug delivery system.

2. Materials and Methods

2.1 Materials

2.1.1 *Animals*

Healthy Swiss Albino male mice weighing 20-35 g were procured from Shri Venkateshwara Enterprises, Hyderabad. They were housed in a clean and transparent polypropylene cage in a group of

Author for correspondence: Mrs. Sharadha Srikanth
CMR College of Pharmacy, Medchal-501401, Hyderabad, Telangana, India
E-mail: sharusrik97@gmail.com
Tel.: +91-9885833269

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six per cage and were maintained under 12/12 h natural light-dark cycle at ambient temperature, 45-55% relative humidity. They were allowed to acclimatize one week before the experiment and free access to standard pellet and water *ad libitum* was provided. All the experimental procedures were carried out in accordance with Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA). The study was reviewed and approved by the Institutional Animal Ethics Committee (IAEC), CMR College of Pharmacy, Medchal, Hyderabad.

2.1.2 *Chemicals*

All the drugs and chemicals used in the study were obtained from authorized dealers. Nootropil (Piracetam 150 mg/kg) and Hyoscine (Scopolamine 0.4 mg/kg) were purchased from Yashoda Hospital, Secunderabad. Dithiobisnitrobenzoic acid (DTNB), Acetyl thiocholine iodide (ATCI), and thiobarbituric acid were purchased from Chemicals and Chemicals Stores, Shahpur, Hyderabad. Cod liver oil was purchased from an authorized dealer.

2.1.3 *Acute toxicity studies* (LD_{50})

The acute toxicity studies were performed in mice by giving cod liver oil at doses of 0.5, 1 and 1.5 ml/kg body weight. The animals did not exhibit any toxic symptoms even at 5 ml/kg body weight and the dose was fixed at 0.5 ml/kg body weight based on the OECD guidelines 423.

2.1.4 *Experimental design*

Group I : Male albino mice served as control received distilled water 10ml/kg for 7 days.

Group II : Male albino mice served as negative control received scopolamine (0.4mg/kg) through intraperitoneal route.

Group III : Male albino mice served as standard received piracetam 150mg/kg through intravenous route for 7 days.

Group IV : Male albino mice served as test drug received cod liver oil 0.5ml/kg by oral route for 7days.

2.2 Laboratory models for testing learning and memory

2.2.1 *Elevated plus-maze* (Exteroceptive model)

Procedure: Albino male mice (18-22 g) were divided into 7 groups of 6 mice in each and were fasted overnight prior to the test but water was supplied *ad libitum*. Elevated plus maze (EPM) is used to evaluate learning and memory in mice. Procedure for testing, learning and memory was followed as per the neuropsycho-pharmacological principle. The apparatus consisted of two open arms (16 cm × 5 cm) and two enclosed arms (16 cm × 5 cm). The maze was elevated to the height of 25 cm from the floor. The center platform extended 5 cm × 5 cm from the arms. Transfer latency (TL) is the time taken by the mouse to enter into one of the enclosed arms (Dhingra *et al*., 2004).

Mouse was placed at the end of the open arm, facing away from the centre platform. TL was recorded when the mouse enters with all its four legs into one of the enclosed arms. TL was recorded on the 1st and 8th day. Before the 1st day, the mouse was exposed to plus maze by spending time in it for 10 seconds. Cut-off time is 90 seconds for the model (Dhingra *et al*., 2004).

Table 1: Summary of elevated plus maze results for cod liver oil

S.No.	Treatment	Transfer latency on 1st day MEAN±SEM	Transfer latency on 8th day MEAN±SEM
1	Control	39.50 ± 2.187	36.00 ± 1.065^{ns}
2	Control.N	39.50 ± 2.217	40.33 ± 2.108
3	Standard	34.16 ± 0.600	13.00 ± 0.966**
4	Cod liver oil	33.83 ± 0.945	17.50 ± 0.7638*

Values are Mean ± SEM *, ** and ^{ns} indicates the difference with negative control group at $p<0.05$, $p<0.01$ and n=6 in each group. Cod liver oil showed better results when compared with negative control group.

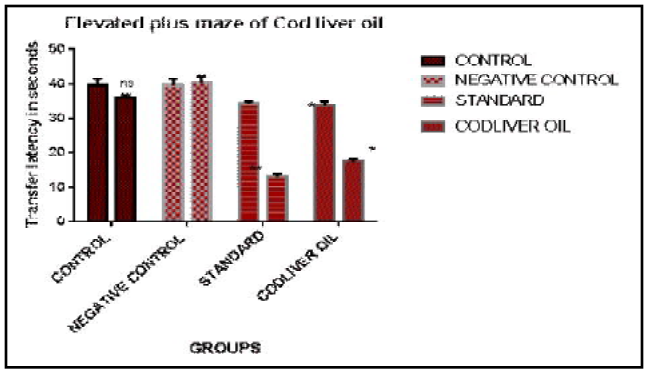

Figure 1: Bar charts showing elevated plus maze results for cod liver oil

2.2.2 *Morris water maze*

Method: Albino male mice (25-35 g) were divided into 7 groups of 6 mice in each and were fasted overnight prior to the test but water was supplied *ad libitum*. Morris water maze consists of a large circular tank with a depth of 30 cm, diameter 50 cm. In the centre, a platform of 15 cm, having dimensions, 5 cm × 5 cm is mounted. The pool was filled with water, added with milk in order to make it opaque. Later animals were allowed for training before the experimental day. On the 1st day, animals were treated with different doses of standard and test samples.

The animal was placed at the corner of the tank and allowed to swim until it identifies the hidden platform. The cut-off time is 90 seconds. The transfer latency was considered as the time taken by the mouse to identify the platform. TL was recorded on 1st day and 8th day (Sunil and Kshirsagar, 2011).

Table 2: Summary of morris water maze results for cod liver oil

S.No.	Treatment	Transfer latency on 1st day MEAN±SEM	Transfer latency on 8th day MEAN±SEM
1	Control	42.00 ± 1.571	37.00 ± 0.7303*
2	Control.N	42.167 ± 1.302	40.66 ± 1.856
3	Standard	38.50 ± 0.223	14.33 ± 1.282**
4	Cod liver oil	39.50± 0.428	20.00 ± 0.365**

Values are Mean ± SEM *, ** indicates the difference with negative control group at $p<0.05$, $p<0.01$ n=6 in each group. Cod liver oil showed better results when compared with negative control group.

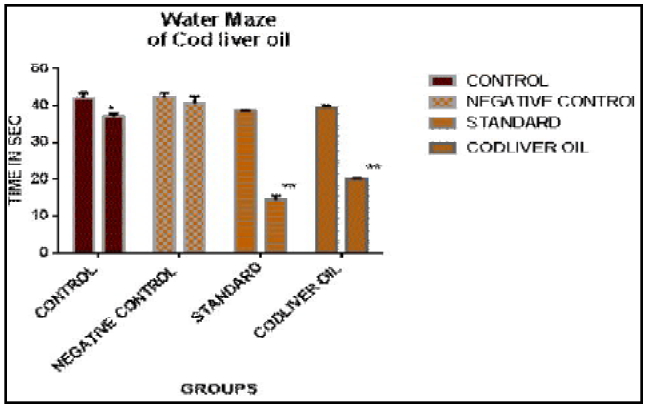

Figure 2: Bar charts showing morris water maze results for cod liver oil

2.2.3 *Step down*

Procedure: Albino male mice (18-22 g) were divided into 7 groups of 6 mice in each and were fasted overnight prior to the test but water was supplied *ad libitum*. Step down type of passive avoidance test was used to examine long term memory. The apparatus consists of transparent acrylic cage (30 × 30 × 40 cm in height) with a grid floor; a platform (4 × 4 × 4 cm) is fixed in the centre of the grid floor. Electric shocks of 1Hz, 500 msec, 40V DC are delivered to the grid floor. The training is carried out before the experimental day. On the experimental day, mouse is placed on platform in the centre of the cage, when the mouse steps down and places all its paws on the grid floor, shock is delivered. Later animal was placed again on the platform after 60-90 minutes and step down latency (SDL) was recorded with an upper cut of time of 300 seconds (Chandrakant *et al.*, 2012).

Table 3: Summary of step down latency results for cod liver oil

S.No.	Treatment	Transfer latency on 1st day MEAN±SEM	Transfer latency on 8th day MEAN±SEM
1	Control	10.33 ± 0.4216	10.83 ± 0.7032*
2	Control N	11.83 ± 0.5426	10.17 ± 1.400
3	Standard	9.167 ± 0.7923	21.67 ± 0.4944**
5	Cod liver oil	10.17 ± 0.7032	20.33 ± 1.085**

Values are Mean ± SEM *, ** indicates the difference with negative control group at $p<0.05$, $p<0.01$ and $p<0.05$ n=6 in each group. Cod liver oil showed better results when compared with negative control group.

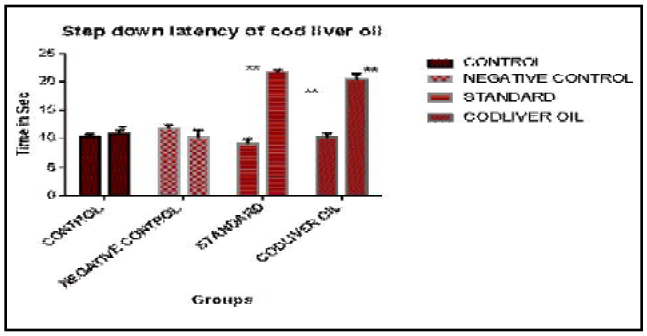

Figure 3: Bar charts showing step down results for cod liver oil

2.2.4 *Estimation of acetyl cholinesterase enzyme activity of whole brain*

On the 8th day, animals were treated with scopolamine and after 90 minutes, the brains were decapitated. The whole brain was taken out in normal saline, later suspended in phosphate buffer pH 8. The brain was homogenized in tissue homogenizer and then 0.4 ml of the homogenate is mixed with 10 microliters of DTNB. The absorbance is recorded in UV-Spectrometer. After few minutes, the sample is mixed the acetyl thiocholine (ATC) and readings were taken, change in the absorbance per minute was noted (Chandrakant *et al.*, 2012).

The rate of moles of substrate hydrolyzed per minute per gram of tissue was later calculated as per the following equation:

$$R = \frac{\delta \text{ O.D} \times \text{Volume of essay}}{\text{E} \times \text{mg of protein}}$$

where R= Rate of enzyme activity in 'n' mole of acetylcholine iodide hydrolyzed/min/mg protein. Δ O.D = Change in absorbance/ min and E = Extinction coefficient = 13600/M/cm.

Table 4: AChE enzyme activity for cod liver oil

S.No	Treatment	Dose	Acetyl cholinesterase enzyme activity
1	Control	10ml/kg	29.5 ± 0.691ns
2	Control N	0.4mg/kg	31.19 ± 0.710
3	Standard	150mg/kg	11.01 ± 0.551**
4	Cod liver oil	0.5ml/kg	15.4 ± 0.6110**

Values are Mean ± SEM ** and ns indicates the difference with negative control group at $p<0.01$ and $p<0.05$ n=6 in each group. Cod liver oil showed better results when compared with negative control group as increasing the acetylcholine esterase enzyme activity.

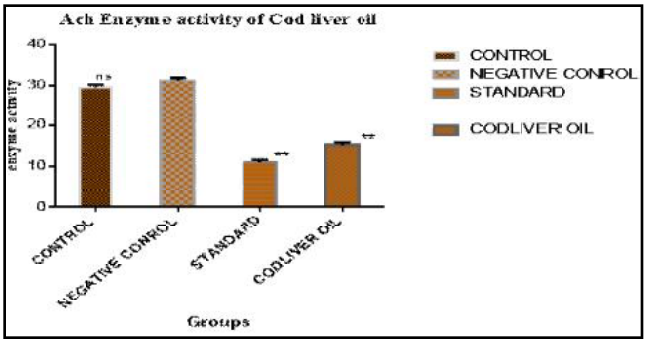

Figure 4: Bar chart showing AChE enzyme activity for cod liver oil

2.2.5 *Statistical Analysis*

The step-down latency and transfer latency were analysed using the Student's Paired't' test. A probability level of $p<0.01$ was considered as significant.

2.3 Transdermal patches

2.3.1 *Preformulation studies*

The following preformulation studies were performed for cod liver oil and polymers:

- **Determination of melting point**

Melting point of the drug was determined by taking small amount of drug in a capillary tube closed at one end. The capillary tube was placed in a melting point apparatus and the temperature at which drug melts was recorded. This was performed thrice and average value was noted.

- **Determination of solubility**

The solubility of cod liver oil was determined by as follows:

An excess amount of drug was taken and dissolved in a measured volume of distilled water in a glass vial to get a saturated solution. The solution was sonicated and kept at room temperature for the attainment of equilibrium. The concentration of sample in the filtrate was determined spectrophotometrically by measuring at 360nm after 24 hrs.

- **Determination of partition coefficient**

The known quantity of cod liver oil was added into 5 ml of 1-octanol and it was mixed with 5 ml of water in a separating funnel. Then two phases were allowed to equilibrate at 37°C for 24 hrs with intermittent shaking. The concentration of the drug in the aqueous phase and organic phase was determined by UV spectroscopic method after necessary dilution. The apparent partition coefficient (Kp) was calculated as the ratio of drug concentration in each phase by the following equation:

$$K_p = \frac{C_{org}}{C_{aq}}$$

where, C_{org} is concentration of drug in organic phase and C_{aq} is the concentration of drug in aqueous phase.

Table 5: Melting point, solubility and partition coefficient of cod liver oil

S.No.	Parameters	Value of Parameters for cod liver oil
1	Melting Point	23° to 26°C
2	Solubility	Insoluble in water, miscible with light petroleum, slightly soluble in ethanol
3	Partition coefficient	11.42

- **Drug-excipient compatibility studies**

The infrared (IR) spectra were recorded, using an FTIR by the KBr pellet method and spectra were recorded in the wavelength region between 4000 and 400 cm–1. The spectra obtained for cod liver oil, polymers and physical mixtures of cod liver oil with polymers were compared. Disappearance of peaks or shifting of peak in any of the spectra was studied.

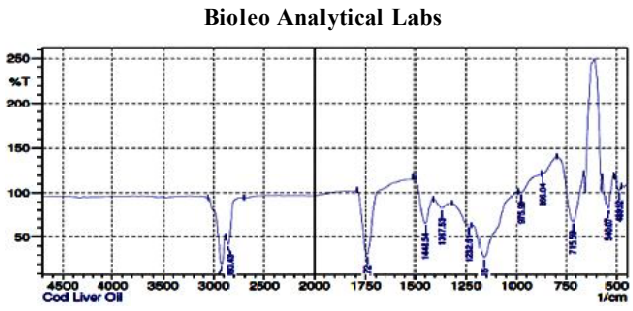

Figure 5: Graph showing FTIR spectrum of cod liver oil

Table 6: FTIR spectrum of drug and polymer mixture

S.No.	IR spectrum of	Groups	Peak (cm-1)
1	Cod liver oil		
		C-H	2860
		C-O	975
		C=C	715
		C=O(aldehyde)	1742
		C-OH	1449
2	Cod liver	C-H	2858
	oil +HPMC	C-O	966
		C=C	717
		C=O(aldehyde)	1748

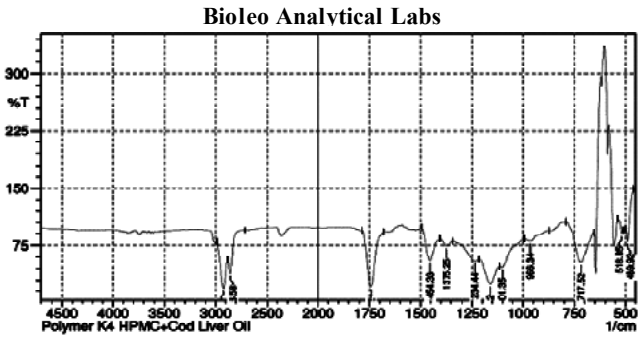

Figure 6: Graph showing FTIR spectrum of cod liver oil with HPLC polymer

2.3.2 *Calibration curve of cod liver oil in phosphate buffer solution (pH 7.4):*

From the cod liver oil standard stock solution (1000 µg/ml), 10 ml solution was diluted to 100 ml, using 7.4 pH phosphate buffer solutions (100 µg/ml). Appropriate aliquots were taken into different volumetric flasks and made up to 10 ml with phosphate buffer solution (pH 7.4), so as to get drug concentrations of 2.0 to 10.0 µg/ml. The absorbance was estimated at λmax 360 nm. This procedure was performed in triplicate to validate the calibration curve (Amjab *et al*., 2011).

Table 7: Results showing calibration curve for cod liver oil in phosphate buffer pH7.4

S.No.	Concentration (µg/ml)	Absorbance(360 nm)
1	0	0
2	2	0.106 ± 0.001
3	4	0.198 ± 0.003
4	6	0.317 ± 0.001
5	8	0.435 ± 0.005
6	10	0.561 ± 0.002

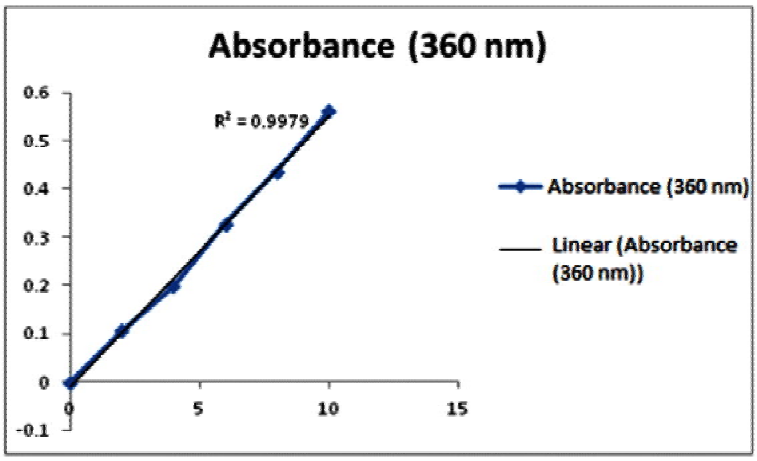

Figure 7: Calibration curve for cod liver oil

Table 8: Composition of different formulations containing cod liver oil

Ingredients	Purpose	F1	F2	F3	F4	F5	F6	F7	F8	F9	F10
Cod liver oil	Test drug	10%	10%	10%	10%	10%	10%	10%	10%	10%	10%
HPMC-K_4	Polymer	10%	15%	20%	30%	30%	30%	30%	30%	30%	30%
Propylene glycol	Solubilizer	20%	20%	20%	20%	20%	20%	20%	20%	20%	20%
Tween 80	Surfactant	20%	20%	20%	20%	5%	10%	15%	15%	15%	15%
Dibutyl phthalate	Plasticizer	10%	10%	10%	10%	10%	10%	10%	5%	10%	15%
Water	Solvent	30%	25%	20%	10%	25%	20%	15%	20%	15%	10%

F1-F4 indicates different concentrations of HPMC-K4; **F5-F7** indicates concentrations of Tween-80; and **F8-F10** indicates concentrations of Dibutyl phthalate. Total volume of the patch is 5 ml which is considered to be 100%.

2.3.3 *Preparation of cod liver oil patch*

Transdermal patch of cod liver oil was prepared with the polymer HPMC-50CPS as dispersion polymer. Weighed quantity of cod liver oil and HPMC with suitable solvent was continuously stirred for 1 hour, then poly ethylene glycol was added as plasticizer and tween 80 in order to solubilize oil and the stirring was continued for another 1 hour on the magnetic stirrer. Glycerol was used as humectant and plasticizer, Propylene glycol as penetration enhancer. Then the solution was allowed to keep in the sonicator to remove the air bubbles. The drug polymer solution was casted in a bangle of area 16cm^2 which was then placed in a petridish. The mould was kept aside for drying at room temperature for 24 hrs. Inverted funnel was placed over the mould to prevent disturbance by air draft. After drying, the patches were peeled from petridish, and preserved in desiccator for further studies (Amit *et al.*, 2012).

2.3.4 *Evaluation of transdermal patches of cod liver oil*

- **Physical appearance**

The prepared patches were physically examined for colour, clarity and surface texture.

- **Thickness uniformity**

The thickness of patches was measured by using electronic caliper, with a least count of 0.01mm. Thickness was measured at three different points on the film and average readings were taken.

- **Uniformity of weight**

The patch of size 1x1 cm^2 was cut and weight of each patch was taken individually, the average weight of the patch was calculated.

- **Folding endurance**

The folding endurance was measured manually for the prepared patches. A strip of patch (2 x 2 cm^2) was cut and repeatedly folded at the same place till it broke. The number of times the film could be folded at the same place without breaking gave the value of folding endurance (Dhiman *et al.*, 2011).

- **Percentage moisture loss**

The patches were weighed individually and kept in a desiccator containing calcium chloride. The final weight was noted when there was no change in the weight of individual patch. The percentage of moisture content was calculated as a difference between initial and final weight with respect to final weight.

- **Percentage moisture uptake**

The patches were weighed accurately and placed in a desiccator where a humidity condition of 80-90% RH was maintained by using saturated solution of potassium chloride. The patches were kept until uniform weight is obtained, then taken out and weighed. The percentage of moisture uptake was calculated as the difference between final and initial weight with respect to initial weight.

Table 9: Showing evaluation parameters of appearance, folding endurance, surface pH, thickness

Formulation code	Appearance	Folding endurance	Surface pH	Thickness	Percentage Moisture content	Percentage Moisture uptake	Weight variation	In-Vitro Drug content
Cod liver oil	Transparent	89 ± 1	5.3 ± 0.3	0.198 ± 0.021	3.24 ± 0.2	1.64 ± 0.02	18.98 ± 1.01	99.95 ± 0.14

Table 10: *In vitro* release of cod liver oil from patch F4 in phosphate buffer

Time	"T	log T	% CDR	Log % CDR
0	0	0	0	0
1 hr	1.000	0.000	12.026 ± 2.321	1.1080 ± 0.0271
2 hr	1.414	0.301	22.256 ± 1.624	1.3474 ± 0.2161
4 hr	2.000	0.602	31.546 ± 1.629	1.4989 ± 0.1172
6 hr	2.449	0.778	43.096 ± 1.112	1.6344 ± 0.0025
8 hr	2.828	0.903	55.936 ± 1.606	1.7476 ± 0.0120
10 hr	3.162	1.000	69.026 ± 0.203	1.8390 ± 0.1100
12 hr	3.464	1.079	79.931 ± 1.002	1.9027 ± 0.0201

The cumulative amount of drug released at 12th hour from F4 was found to be 79.931±1.002%.

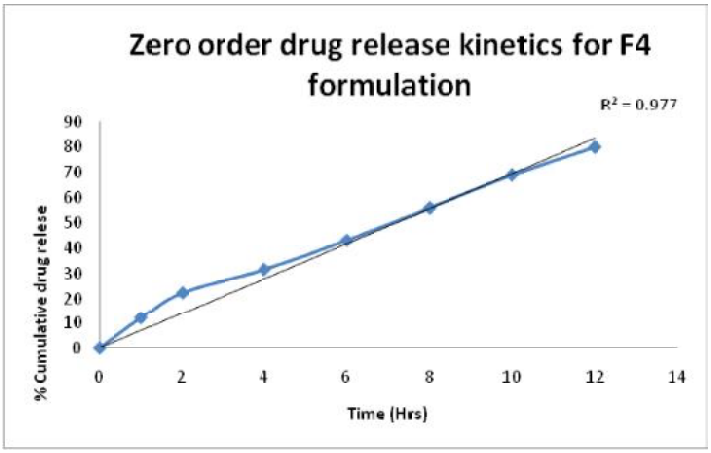

Figure 8: Graph showing zero order release kinetic profile for F4 formulation

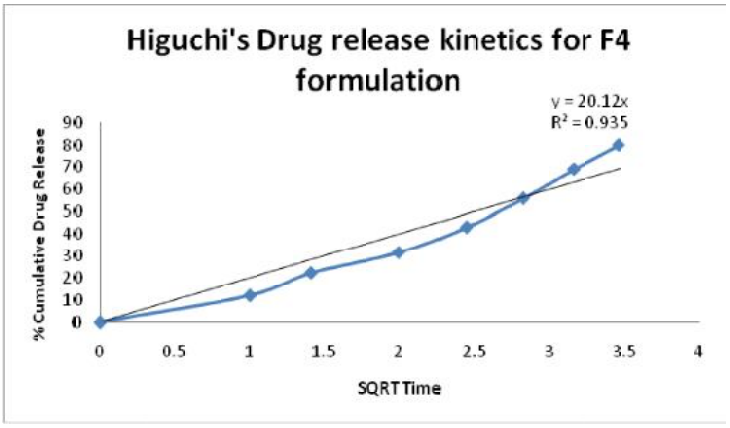

Figure 9: Graph showing Higuchi release kinetic profile of F4 formulation

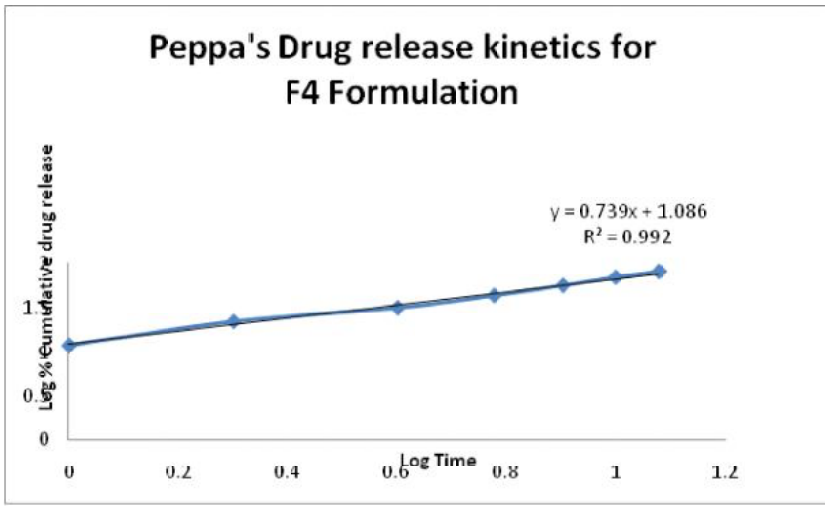

Figure 10: Graph showing Peppas release kinetic profile of F4 formulation

- **Drug content uniformity**

The patches were tested for the content uniformity. The patches of size 1 cm^2 was cut and placed in a 100 ml volumetric flask. The contents were stirred using a magnetic bead for 24hrs to dissolve the patches. Subsequent dilutions were made with phosphate buffer (pH 7.4). The absorbance of the solution was measured against the corresponding blank solution at 360 nm using UV-visible spectrophotometer (Ansari *et al*., 2011).

- ***In vitro* drug release studies**

The fabricated patchs were cut into 1cm^2 and placed on the semi permeable membrane, was attached to the diffusion cell such that the cell's drug releasing surface towards the receptor compartment which was filled with phosphate buffer solution of pH 7.4 at 37 ± 1°C. The elution medium was stirred magnetically. The aliquots (1ml) was withdrawn at predetermined time intervals and replaced with same volume of phosphate buffer of pH 7.4. The samples were analysed for drug content, using UV spectrophotometer at 360nm (Amit *et al*, 2012).

2.5 Drug release kinetics for best formulation

The release kinetics was evaluated by making use of zero order, first order, Higuchi's diffusion and Korsemeyer - Peppas equation. Calculated regression co-efficient values for different formulations are tabulated in Table 11. These values are compared with each other for model and drug equation. Based on the higher regression values (r^2), the best fit model was first order for F4 formulations and the drug release kinetics follows Non Fickian type diffusion mechanism.

Table 11: Showing results of model fitting of cod liver oil patch TDDS *In vitro*

Formulation	Zero order	Higuchi	Peppas kinetics
F4	0.9773	0.9355	0.7191

3. Results and Discussion

Nootropics work by increasing the supply of neurochemicals, improving brain's oxygen supply or by stimulating nerve growth. Inspite of extensive research, the neurochemical basis for learning remains unclear but the role of cholinergic transmission was found to play a vital role in learning and memory processes. Deficiency of cholinergic system was implicated in the memory deficit cases. (Avadesh *et al*., 1992). Besides cholinergic system, large number of other receptor and neurotransmitter (dopamine, serotonin, noradrenaline, glutamate) systems were also found to be involved in the behavioural expression of dementia (Rao *et al*., 2008).

Nootropic activity of cod liver oil was evaluated and from results it was proved that cod liver oil has an ability to reduce the transfer latency of experimental animals in behavioural models like elevated plus maze, water maze ($p<0.01$) (Table 2), step down latency ($p<0.05$) (Table 3), and reduce the acetyl choline esterase activity ($p<0.01$) (Table 4).

Oral route of administration of drugs is the most commonly preferred route of administration. In spite of ease of usage of this type of drug delivery, it shows few disadvantages like first pass metabolism and decreased bioavailability that affects therapeutic efficacy of the drug. Therefore, to improve the bioavailability of the drug, other drug delivery systems that have benefits over oral route are suggested (Prausnitz and Langer, 2009).

Transdermal patches control the delivery of drugs at controlled rates by employing an appropriate combination of hydrophilic and lipophilic polymers. In addition, the transdermal dosage form is user friendly, convenient, painless and offers multiday dosing. TDDS offer pharmacological advantages over the oral route and improved patient acceptability and compliance. The transdermal route for drug delivery avoids first pass metabolism and large variations in plasma drug concentrations (Margetts and Sawyer, 2007).

The transdermal route of administration has been recognized as one of the potential route for the local and systemic delivery of drugs. It provides a steady plasma state, particularly for drugs with short half-lives and reduced systemic side effects with improved patient compliance and avoidance of the first pass metabolism (Daleshwari *et al*., 2011). Transdermal patches deliver the drug at a predetermined and controlled rate (Sandhu *et al*., 2011).

The λ_{max} of cod liver oil in pH 7.4 phosphate buffer solution was found to be 360 nm which is same as that of literature review. The calibration curve of cod liver oil (Figure 7) in pH 7.4 phosphate buffer solution shows linearity with r^2 of 0.997.

The patches were prepared for dose 0.5 ml of cod liver oil in an area of 16 cm^2, using HPMC k-4 individually as a parent polymer with incorporation of propylene glycol and Tween 80 as a permeation enhancer and Dibutyl phthalate as a plasticizer by solvent evaporation method.

The compatibility between drug and polymer was studied by using FTIR absorption spectra, shown in Figures 5 and 6. The preliminary study conducted on compatibility between Drug and HPMC revealed that there is no interaction between the drug and polymer as from FTIR spectra.

The physicochemical characteristics such as thickness of the patch, folding endurance, percentage of moisture absorbed, percentage of moisture lost, and drug content analysis were found to be within the acceptable limits as shown in Table 9. The patches were found to be stable to withstand the stress.

The results of *in vitro* drug release revealed that the drug was released in a controlled manner from all the formulations and F4 showed maximum drug release at the end of 12^{th} hour shown in Table 10.

Formulation F4 was considered to be the best formulation as the drug content, and the percentage cumulative drug release were high (79.93%) for F4. As the drug release kinetics follows Non Fickian type diffusion mechanism, which is suitable for prolonged and controlled drug release.

Transdermal films were prepared as a convenient means to improve learning and memory. Data obtained from the study showed significant neuro-protection and memory enhancement by the sample (cod liver oil) at a dose of 0.5 ml/kg p.o.

Transdermal therapeutic systems are defined as self-contained, discrete dosage forms which, when applied to the intact skin, deliver the drug(s), through the skin, at a controlled rate to the systemic circulation. The transdermal drug delivery system has potential advantages of avoiding hepatic first pass metabolism, maintaining constant blood levels for longer period of time resulting in a reduction of dosing frequency, improved bioavailability, decreased gastrointestinal irritation that occur due to local contact with gastric mucosa, and improved patient compliance.

4. Conclusion

The results on evaluation of cod liver oil patch showed that oils can be incorporated into a transdermal drug delivery system for its efficient and convenient use. The transdermal drug delivery system holds a promising future in effective bioactive agents and opportunities for clinicians to experiment with various drugs to study their systemic and local effects.

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Conflict of interest

We declare that we have no conflict of interest.

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