SENRA Academic Publishers, British Columbia
Vol. 8, No. 3, pp. 2985-2992, October 2014
Online ISSN: 1920-3853; Print ISSN: 1715-9997

Canadian Journal of pure&applied sciences

PHARMACOKINETIC AND PHARMACOLOGICAL EVALUATION OF DOV 21947 AND VENLAFAXINE BY ORAL AND INTRANASAL ADMINISTRATION IN MICE

Joga Singh[1], Sudhir Sharma[2], Paramdeep Singh[2], Gurjeet Singh[2], Ravinder Dang[3] and *Neena Bedi[1]
[1]Department of Pharmaceutical Sciences, Guru Nanak Dev University, Amritsar-143005
[2]Department of Discovery Biology, Drug Discovery Research, Panacea Biotec Ltd., SAS Nagar (Mohali)-160055
[3]Department of Pharmacy, GGN Khalsa College, Civil Lines, Ludhiana-141001, India

ABSTRACT

Intranasal administration is a non-invasive method of drug delivery which may bypass the BBB to allow therapeutic substances direct access to the central nervous system. Recently direct delivery of drugs from nasal cavity to the CNS via the olfactory pathway is attracting increasing attention in order target the drugs directly to the CNS for the treatment of diseases like Schizophrenia, Meningitis, Parkinson's disease, Alzheimer's disease as well as depression also. This alternative approach can also lead to reduction in systemic side effects. The study investigated the plasma pharmacokinetics and brain uptake of DOV 21947 (Triple Reuptake Inhibitor) and venlafaxine a serotonin-norepinephrine reuptake inhibitor in Swiss albino mice after oral and intranasal administration. Enhanced pharmacological effect was observed which was evaluated by means of forced swimming test, suggesting that intranasal administration of drug produced higher direct delivery to CNS as well as to systemic circulation. Thus, offering a promising route of administration and an alternative to per-oral administration.

Keywords: Intranasal, per-oral, antidepressant, forced swim test, venlafaxine.

INTRODUCTION

A syndrome that reflects a sad and/or irritable mood exceeding normal sadness or grief is known as depression. According to the DSM-IV (Diagnostic and Statistical Manual of Mental Disorders) 'depression' and 'depressed mood' is a clinical syndrome, or cluster of symptoms, covering changes in effect, cognition and behaviour, and which meet the diagnostic criteria for a Major Depressive Disorder (Aaron *et al*., 2004; APA, 1996). Treatment of central nervous system (CNS) diseases is very difficult due to the blood–brain barrier's (BBB) ability to severely restrict entry of almost all the drugs except small, non-polar compounds (Berardi *et al*., 2002). Intranasal administration is a non-invasive method of drug delivery which may bypass the BBB to allow direct access of therapeutic substances to the CNS (Mumford *et al*., 1997; Charlton *et al*., 2007). The nasal route could provide an attractive needle-free alternative for currently injectable drugs which may improve patient compliance and allow extended use of self-medication for many chronic diseases/ acute conditions (Scranton *et al*., 2011; Frey *et al*., 1997).

In the present study, the possibility of using an intranasal delivery system for antidepressant drug therapy by evaluating the transport to the systemic circulation and brain and the pharmacological effect after intranasal (*i.n.*) administration were investigated. Two drugs namely venlafaxine (a serotonin-norepinephrine reuptake inhibitor i.e. SNRI) and DOV 21947 (a triple re-uptake inhibitor i.e. TUI or SNDRI) were selected. Chemically, venlafaxine is (R/S)-1-[2-(dimethylamino)-1-(4-methoxyphenyl) ethyl] cyclohexanol hydrochloride. It is used primarily for the treatment of depression, general anxiety disorder, social phobia, panic disorder and vasomotor symptoms whereas DOV 21947 is [(1R, 5S) -1-(3, 4-dichlorophenyl)-3-azabicyclo [3.1.0] hexane hydrochloride]. DOV 21,947 or EB-1010, also known as Amitifadine, is an antidepressant drug being developed by Euthymics Bioscience Inc., which is currently in clinical trials. Based upon preclinical studies, this triple reuptake inhibitor has the potential utility in treating a wide variety of central nervous system disorders including depression and obesity (Skolnick *et al*., 2003; Tizzano *et al*., 2008). Intranasal administration of antidepressant drugs (DOV 21947 and venlafaxine) was found to produce a higher direct delivery to the CNS as well as to the systemic circulation.

MATERIALS AND METHODS

Materials

Quality grade anti-depressant drug: venlafaxine hydrochloride and DOV 21947 were obtained from Panacea Biotec Ltd, Mohali (Punjab, India). Polyethylene glycol (PEG 400) and Propylene glycol (PG) were procured from Spectrochem Private Ltd, Mumbai, India.

*Corresponding author email: neenagndu@yahoo.com

Carboxy methyl cellulose sodium and sodium chloride GR were purchased from Loba Chemie Private Ltd, Mumbai and Merck Specialities Private Ltd, Mumbai, respectively. Other chemicals like Ethylene diamine tetraacetic acid (EDTA) tri potassium salt dehydrate and Tween® 80 were purchased from Sigma Life Science (Sigma-Aldrich Co.), USA and Halothane from Raman and Weil Private Ltd, Mumbai, India. All the chemicals acquired were of high quality and of analytical grade.

Animals

Animals used in this study were post weaned (4-weeks old) either sex SAM provided by Small Animal Facility for Experimentation and Breeding (SAFEB), Drug Discovery Research, Panacea Biotec Ltd, Mohali (Punjab, India) and maintained as per the guidance laid by Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA). Controlled conditions in animal house were light (12 hr duration light: dark), temperature and relative humidity at 21±2°C and 50-55%, respectively.

Formulation Development

To study the effect of different vehicles, drug was either solubilised in (1) R.O.Water (2) 10% PEG + 50% PG + 40% R.O.Water (3) 20% PEG + 50% PG + 30% R.O.Water. Weighed quantity of drug was taken in mortar. Two to three drops of Tween 80 (as a surfactant) was added and slowly triturated to form a homogenous paste. Drug was solubilised in the calculated amount of vehicle, added in small volumes with continuous trituration. Solution was mixed thoroughly by using vortex mixer (IKA® Werke) for about one minute so that drug gets evenly dispersed in the vehicle. Drug solutions were sonicated (Elmasonic) for two to three minutes to dissolve any remaining drug particle.

Peroral Administration of Drugs in Test Animals

Drug suspended in suitable vehicles was administered via a feeding cannula attached to a syringe at 10 mg/kg or 30 mg/kg dosage.The drug was administered to eight batches (eight time points) of group of four either sex Swiss albino mice housed in a ventilated room at ambient temperature and were fed with standard pellet diet and water *ad libitum*. The mice were fasted overnight before dosing. The animals used were between weight ranges of 25g-30g. DOV 21947 was prepared as a suspension and administrated orally at a dose of 30 mg/kg and 10 mg/kg in case of intranasal administration. The formulations were prepared on the day of dose administration and were administered within 15 minutes of preparation by oral gavage in different vehicles and proportions as indicated in table 1.

Blood samples (100 µl) were drawn by capillary bleeding from retro-orbital plexus and brain samples were also extracted. Samples were collected at pre-dose (0 h), and then 0.25, 0.5, 1, 2, 4, 8 and 24 hours after administration. Animals were sacrificed by cervical dislocation. The protocol was approved by the Institutional Animal Ethics Committee (IAEC).

Intranasal Administration of Drugs in Test Animals

Drug was administered in drop wise manner on the nostril of animal using a 50µl Hamilton syringe. Prior to administration, animals were anesthetized using an inhalational anaesthetic (Halothane) in the desiccators (Schaefer *et al*., 2002; Guerrero *et al*., 2011).

Swiss albino mice (25 to 30g) were anesthetized with halothane. Throughout the entire experiment, all mice were kept in supine position so that drug solutions can contact a larger area of nasal olfactory mucosa compared to the prone position. DOV 21947 nasal drops were administered at a dose of 10 mg/kg to each mouse. A volume of drug solution (10 µl per 30g of body weight) was administered into both nostrils carefully using a Hamilton syringe.

Before (0 minute) and after drug administration at 15, 30, 60, 120, 240, 480 and 1440 minutes, blood samples of 100 µl were withdrawn from retro-orbital plexus and then centrifuged for 6 minutes at 6000 rpm to obtain plasma. After the blood sampling, the mouse was sacrificed by cervical dislocation and brain was excised carefully. Brain sampling was completed within 5 minutes following the blood sampling. All plasma and brain samples were stored at -20 and -80°C, respectively, until analysis.

Collection of Plasma

Blood was collected by inserting the capillary into the retro-orbital sinus in labelled microfuge tubes containing EDTA at predetermined times after administration and plasma samples were obtained by centrifugation (Eppendorf Research).

Table 1.Various formulations used in the study along their composition

Formulation Code		Composition
Venlafaxine	DOV 21947	
Formulation V_1	Formulation D_1	R.O. Water + DRUG
Formulation V_2	Formulation D_2	10% PEG + 50% PG + 40% Water + DRUG
Formulation V_3	Formulation D_3	20% PEG + 50% PG + 30% Water + DRUG
Formulation V_4	Formulation D_4	30% PEG + 50% PG + 20% Water + DRUG

Extraction of Brain Samples

Animals were sacrificed by cervical dislocation. Bone cutter was used to make circumferential incision from behind the eye sockets (avoiding injury to the brain tissue) from extending to the opening for the ears in the skull to all round the cranium. Brain samples were placed in 15 ml tube having adequate amount of 0.85% NaCl and kept in ice until it was further processed.

Homogenisation of Brain Samples

Extracted brain samples were weighed individually to make 10% *w/v* brain homogenate in 0.85% NaCl solution and homogenized. Homogenizer probe was washed thoroughly first with R.O water and then with absolute ethanol in between the two samples. Finally, 1 ml from each tube was transferred to fresh, properly labelled, clean and dry micro centrifuge tubes and kept at -80°C until analysis.

Drug Quantification

The concentration of drug was determined by an appropriate HPLC method. A Symmetry C_{18} column (250 mm×4.6 mm, 5 µm, cartridge) was used for chromatographic separation. The injection volume and run time was 20 µl and 15 minutes, respectively.

A LC-MS analysis was performed using a Discovery 5µm column (5x4mm). Plasma and brain drug samples were analyzed using the above technique. Detection was performed using a 4000 Q Trap instrument. The spectrophotometer was used in MS/MS mode with MRM of fragmentation reactions selected for each drug.

Assessment of anti-depressant like activity of DOV 21947 by the most frequently used behavioural model, Forced Swim Test:

Swiss albino mice (22–28g) from the Small Animal Facility for Experimentation and Breeding (SAFEB), Drug Discovery Research, Panacea Biotec Ltd., Mohali, were used. Fifteen mice were housed per cage. The cages were placed in the experimental room 48 hour before the test for acclimatization. The animals were fed a standard laboratory diet and water *ad libitum* under standard environmental conditions (Woode *et al*., 2010; Porsolt *et al*., 1997).

Clear plastic cylinders (diameter 12 cm, height 25 cm) were filled to a depth of 10 cm with water (25ºC). Briefly, mice were dropped individually into glass cylinders and left there for 6 minutes. Behaviour was recorded on video. The duration of immobility was recorded during the last 4 minutes of the 6-minutes test using Forced swim scan™ 2.0 software by Clever Sys, Inc. The test was performed 15 minutes after administration of drug.

Animals were randomized based on body weight. At time T_{-30}, vehicle to control group, standard compounds (suitable dose mg/kg) in vehicle to standard group, test compounds (suitable dose mg/kg) in vehicle to test group were administered. Animals were individually forced to swim inside a glass jar containing 10 cm of water maintained at 23-25°C. After the initial 1-2 minute of vigorous activity the animals showed periods of immobility by floating with minimum movements. The immobility time is the time spent by the mouse floating in the water without struggling and making only those movements necessary to keep their heads above the water. Animal is considered to be immobile whenever it remained floating passively in the water in a slightly hunched but upright position, its nose above the water surface. The total immobility time for the period of 6 minutes was recorded.

RESULTS AND DISCUSSION

(A_1) Transfer of DOV 21947 to the Brain and Systemic Circulation after *p.o.* Administration

Plasma profile

The maximum plasma concentration of DOV 21947 was observed after oral administration of Formulation D_4 followed by D_3. The AUC values were calculated by trapezoidal rule. Data suggested (Plasma $t_{1/2}$ values) that in spite of low peak plasma concentration, formulation D_2 was cleared slowly from plasma (Fig. 1). For all formulations administered, highest and lowest values of tmax were observed in Formulation D_4 and Formulation D_2, respectively.

Brain profile

The drug release pattern of DOV 21947 in Formulation D_3 showed better results in brain profiling but time required to achieve the maximum concentration was minimum with Formulation D_2 out of all the tested formulations.

After analysis it was decided that formulation D_2 can be further carried for intranasal administration in mice. Comparison was done with Formulation D_1.

(A_2) Transfer of DOV 21947 to the Brain and Systemic Circulation after *i.n.* Administration

Plasma Profile

At several time points, significant differences were observed between the DOV 21947 levels in plasma and brain tissue of the intranasal and oral routes. Following oral administration, DOV 21947 in Formulation D_1 attained a peak concentration at 15 minutes, and then followed by an exponential decline with the passage of time (Fig. 2). Nasally administered DOV 21947 in Formulation D_1 displayed slightly increased levels. Comparatively, nasally administered DOV in Formulation D_3 displayed a slow and poor absorption of active drug across the nasal mucosa into the systemic circulation. The AUC and half life values of concentration time curves in

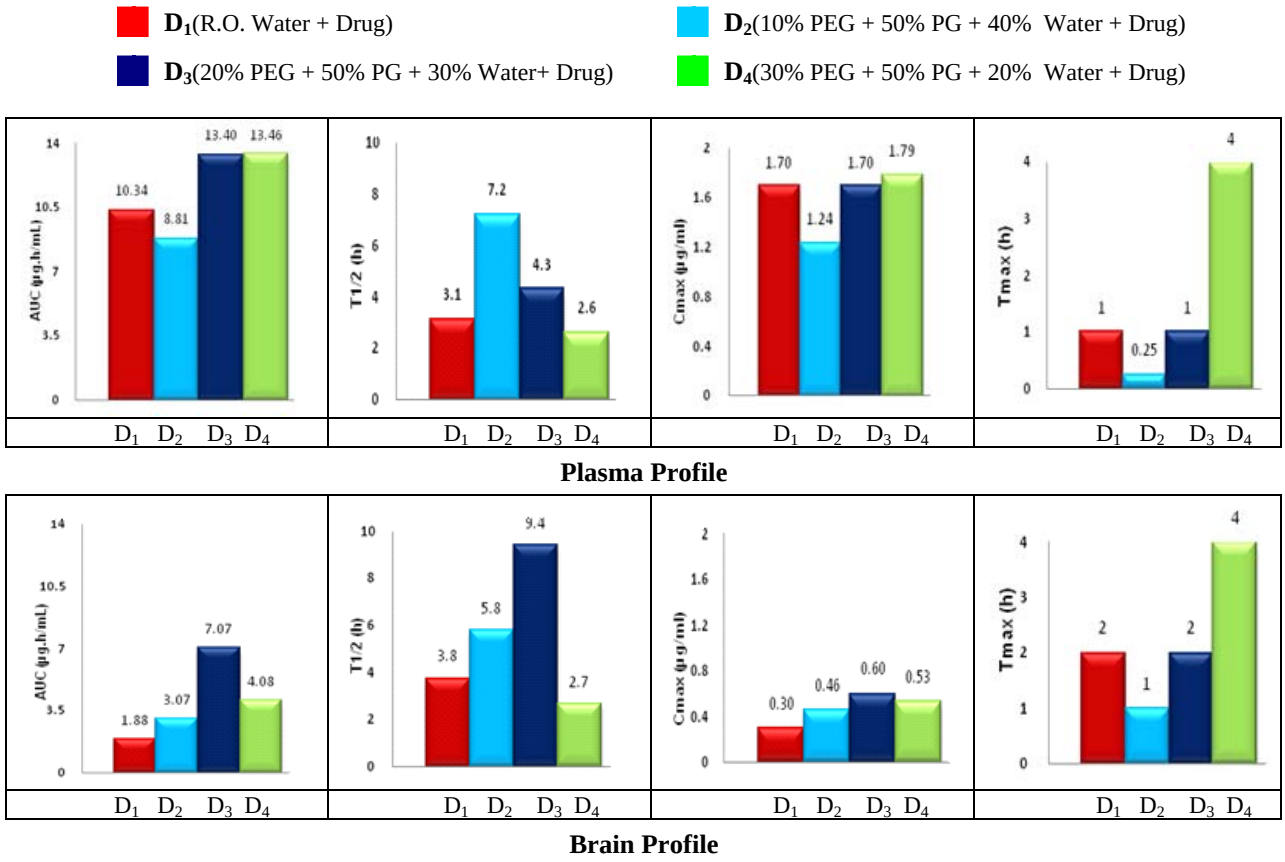

Fig. 1. Pharmocokinetic parameters of DOV 21947 in different vehicles administered orally.

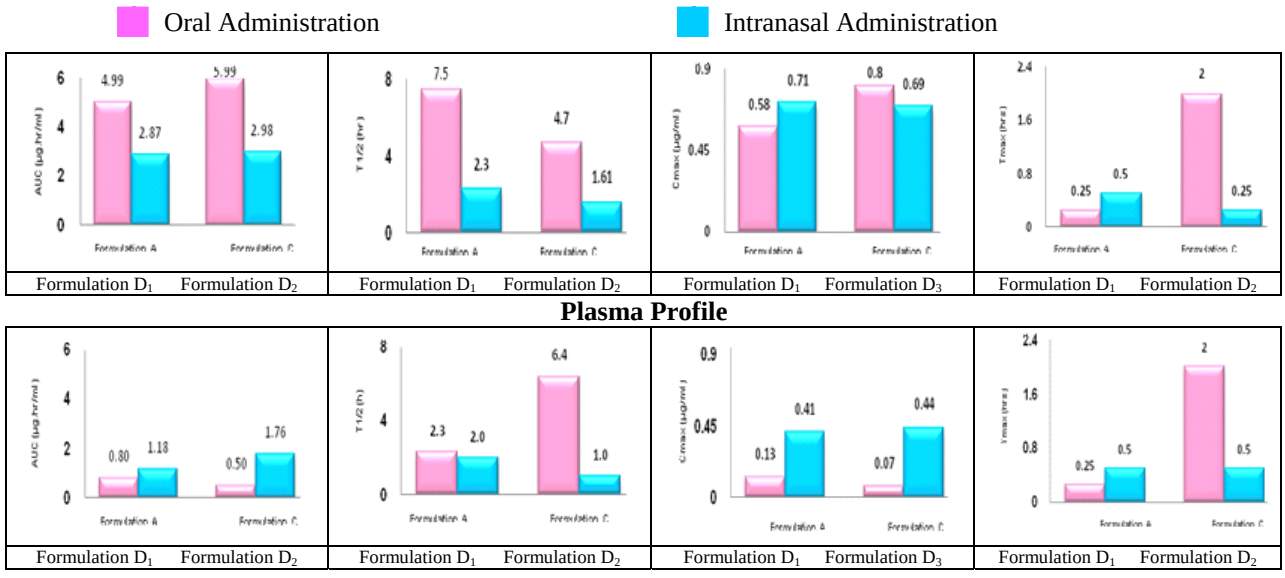

Fig. 2. Comparative pharmocokinetic parameters of DOV 21947 administered by oral and intranasal administration.

plasma after nasal administration were significantly lower than those in post oral administration in Formulations D_1 and D_3.

Brain Profile

Following DOV 21947 (in either formulation) intranasal administration, the drug levels in brain samples were all significantly higher than those in post oral administration brain samples. At 30 minutes post-nasal dose, DOV 21947 concentrations in brain reached peak values of 0.41 µg/g and 0.44 µg/g from Formulation D_1 and Formulation D_3, respectively.

(A_3) Pharmacological Effect 15 min after *i.n.* Administration of DOV 21947

Figure 3 shows the effects of treatment with DOV 21947 on the immobility time in the forced swimming test in mice. In the case of *p.o.* and *i.n.* administration of DOV 21947, the immobility time of mice was reduced significantly compared with controls, in both the formulations. Moreover, the pharmacological effect after *i.n.* administration was higher than that after *p.o.* administration and significant differences were observed for both the routes between the immobility times.

The possible CNS antidepressant effect of DOV 21947 after oral and intranasal administration was studied by the forced swim test. In this test, animals treated with both the DOV 21947 formulations (i.e. D_1 & D_2) showed decrease in their immobility times, which was significant (77.8 ± 23.1 and 139.6 ± 28.6, respectively after oral administration and 27.2 ± 7.7 and 6.3 ± 3.1, respectively after Intranasal administration) when compared with the control group (191.7 ± 10.9) (Fig. 3). Similarly, animals treated with the drug (DOV 21947 in water 32 mg/kg, oral), as expected showed a significant decrease in their immobility time (2.2 ± 0.9). $E.D_{50}$ (dose responsible for 50% of linear regression equation on percent decrease in immobility time data for both the delivery routes (oral route 15.52 and intranas maximal effect) for DOV 21947 was determined by applying al route 10.21) and significant difference was observed as depicted in figure 4.

(B_1) Transfer of Venlafaxine to the Brain and Systemic Circulation after *p.o.* Administration.

Venlafaxine was prepared as a suspension (in different vehicles and proportions as given in Table 1) and administrated orally at a dose of 30 mg/kg.

Plasma profile

Due to high solubility of drug in water, it showed better results in Formulation V_1. Out of peglated formulations, Formulation V_2 showed maximum plasma concentration as evident from figure 5.

Brain profile

In brain profiling, Formulation V_2 achieved maximum concentration and also remained there for significant duration of time.

As is relevant from the data, Formulation V_2 outshined other formulations so it was taken further for pharmacological investigations.

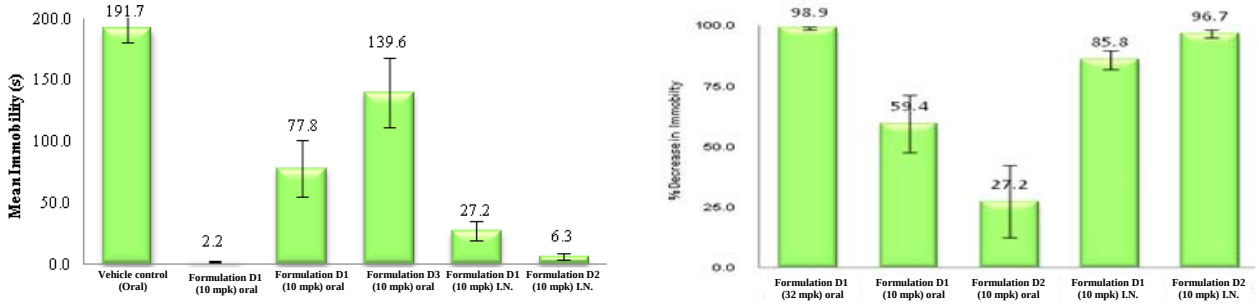

Fig. 3. Effects of DOV 21947 on the duration of immobility in the forced swim test.

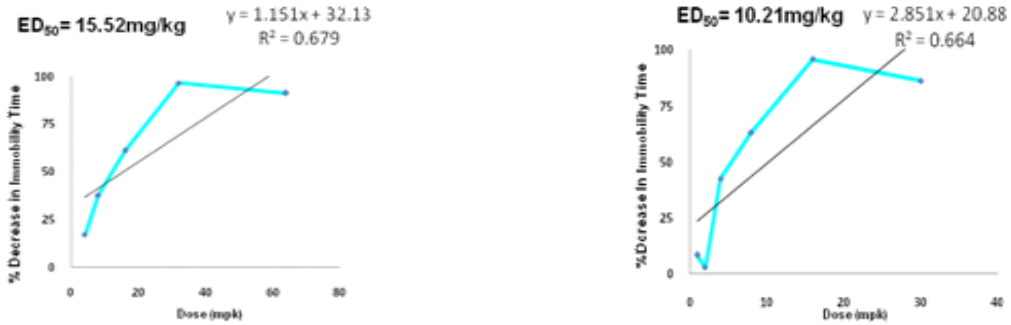

Fig. 4. ED_{50} values of DOV 21947 in the forced swim test after Oral administration (left) and after Nasal administration (right).

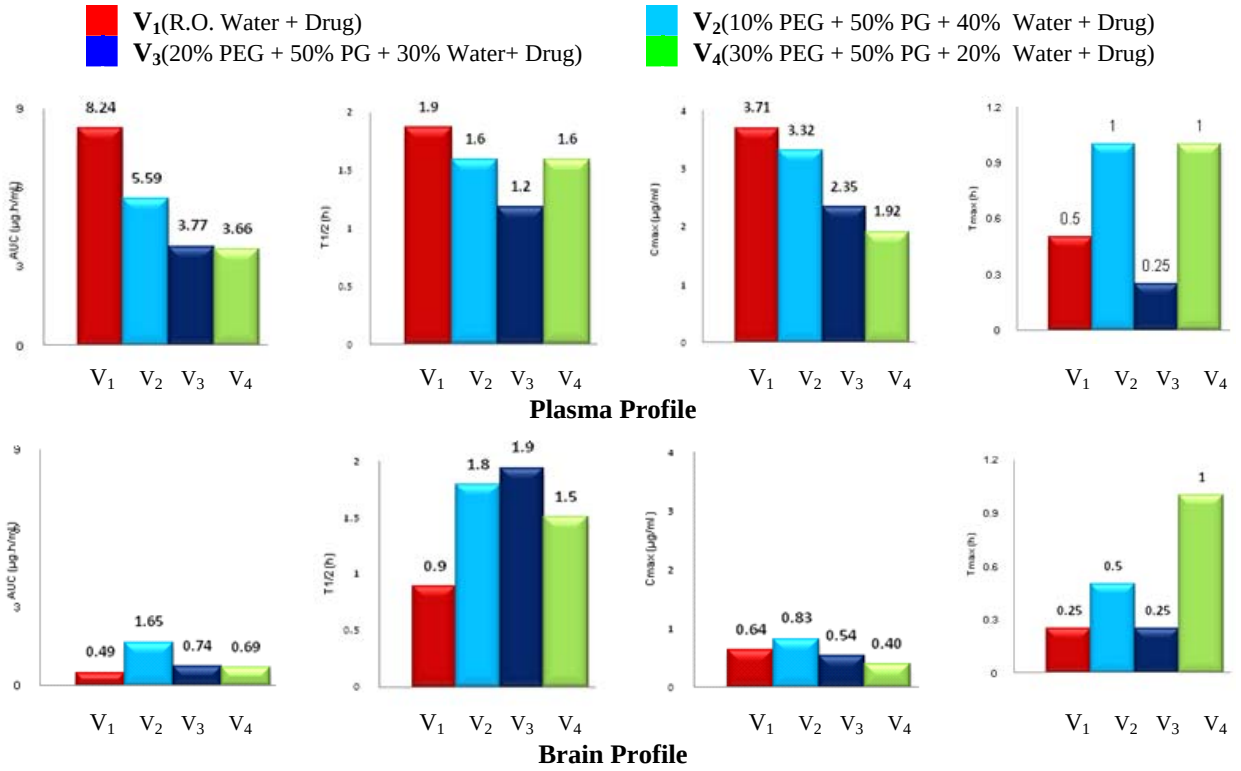

Fig. 5. Pharmocokinetic parameters of Venlafaxine in different vehicles administered orally.

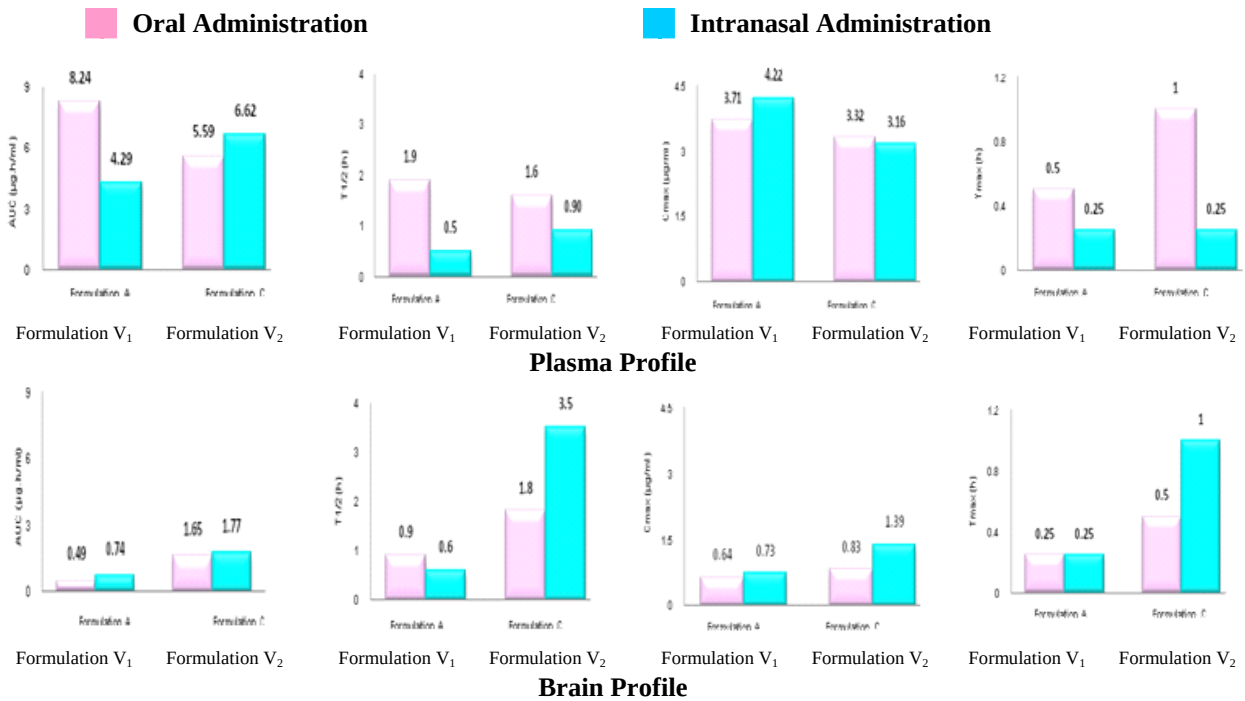

Fig. 6. Comparative pharmocokinetic parameters of Venlafaxine administered by oral and intranasal administration.

(B_2) Transfer of Venlafaxine to the Brain and Systemic Circulation after *i.n.* Administration.

Procedure and methodology was same while administering venlafaxine via intranasal route as

described in previous drug administration i.e. DOV21947. Venlafaxine nasal drops were administered at a dose of 30 mg/kg to each mouse.

Plasma Profile

At several time points, significant differences could be observed between the venlafaxine levels in plasma and brain tissue of the intranasal and oral routes. Comparatively, nasally administered venlafaxine in Formulation V_1 displayed slightly increased levels (Fig. 6) while oral administration of venlafaxine in Formulation V_2 attained a peak plasma concentration of 3.32µg/ml at 1 hour. Nasally administered venlafaxine in Formulation V_2 displayed no significant difference in peak concentration but faster absorption across the nasal mucosa into the systemic circulation as compared to orally administered venlafaxine. The half life values of concentration time curves in plasma after nasal administration were all significantly lower than those in post oral administration in both Formulation V_1 andV_2.

Brain Profile

Following venlafaxine (in formulation V_2) intranasal administration, the drug levels in brain samples were all significantly higher than those in post oral administration brain samples. At 1 hour post-nasal dose, venlafaxine concentrations in brain reached peak values of 1.39µg/g while for venlafaxine (Formulation V_1) intranasal administration, the peak drug levels in brain were of no significant difference.

(B_3) Pharmacological Effect 15 min after *i.n.* Administration of Venlafaxine

The possible CNS antidepressant effect of venlafaxine after oral and intranasal administration was studied by the forced swimming test. In this test, animals treated with both the venlafaxine formulations (i.e. V_1&V_2) showed decrease in their immobility times, which was significant (83.5 ± 29.4 and 108.3 ± 17.2, respectively after oral administration and 25.7 ± 12.6 and 27.6 ± 11.54, respectively after Intranasal administration) when

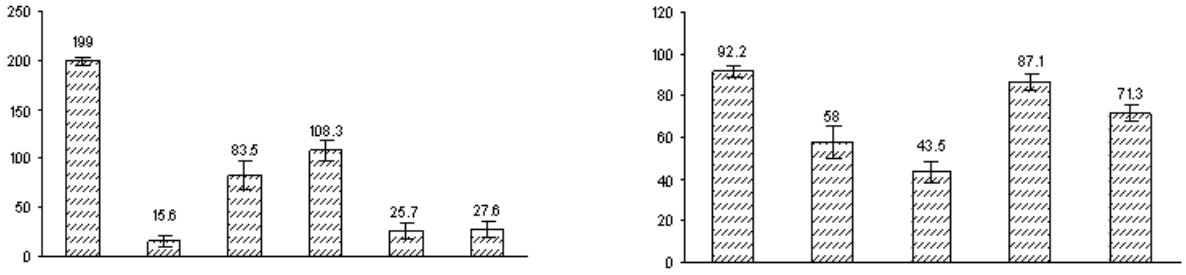

Fig. 7. Effects of Venlafaxine on the duration of immobility in the forced swim test.

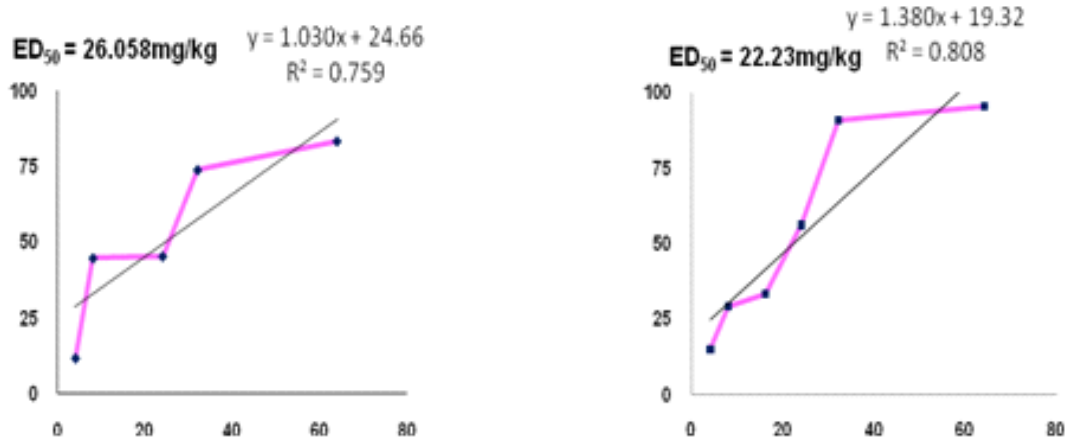

Fig 8. ED_{50} values of Venlafaxine in the forced swim test after Oral administration (left) and after Nasal Administration (right).

compared with the control group (199.0 ± 4.9). Similarly, animals treated with the drug (DOV 21947 in water 32 mg/kg, oral), as expected showed a significant decrease in their immobility time (15.6±6.5) (Fig. 7). $E.D_{50}$ (dose responsible for 50% of maximal effect) for venlafaxine was determined by applying linear regression equation onpercentage decrease in immobility time data for both the delivery routes and slight difference was observed (Fig. 8).

CONCLUSION

The present study evaluated an alternative route for direct delivery to brain of antidepressant drugs. The intra nasal administration of antidepressant drugs produces a higher direct delivery to brain suggesting that it is a promising route of administration and an alternative to per oral administration. The direct transport to brain, results in higher antidepressant effect compared to that with per oral administration. Our overall data suggest that the nasal route could be exploited to increase the availability of antidepressants inside the brain.

ACKNOWLEDGEMENT

The authors are thankful to Dr. Sanjay Trehan, President, Panacea Biotec Ltd. for granting permission and providing all facilities to conduct my project work in this prestigious institute and also express sincere gratitude to project coordinator Dr. Ajay Singh, Sr. Scientist, Biological Research Department, Panacea Biotec Ltd. for his kind and encouraging attitudes.

REFERENCES

Aaron, R., Joseph, A., Abraham, S., Muliyil, J., George, K. and Prasad, J. 2004. Suicides in young people in rural southern India. Lancet. 363:1117-1118.

American Psychiatric Association. (APA). 1996. American psychiatric association practice guidelines. Washington, DC, USA. Available Source <http://dev.summacare.com/Libraries/Documents/Depression_Guidelines.sflb.ashx>.

Berardi, D., Leggieri, G., Ceroni, GB., Rucci, P. and Pezzoli, A. 2002. Depression in primary care-a nationwide epidemiological survey. Journal of Family Practice. 19:397-400.

Charlton, S., Jones, NS., Davis, SS. and Illum, L. 2007. Distribution and clearance of bioadhesive formulations from the olfactory region in man: Effect of polymer type and nasal delivery device. European Journal of Pharmaceutical Sciences. 30:295-302.

Frey, WH. II, Liu, J., Chan, X., Thorne, RG., Fawcett, JR., Ala, TA. and Rahman, YE. 1997. Delivery of 125I-NGF to the brain via the olfactory route. Drug Delivery. 4:87-92.

Guerrero-Cazares, H., Gonzalez-Perez, O., Soriano-Navarro, M., Zamora-Berridi, G., Garcia-Verdugo, JM. and Quinones-Hinojosa, A. 2011. Cytoarchitecture of the lateral ganglionic eminence and rostral extension of the lateral ventricle in the human fetal brain. Journal of Comparative Neurology. 519:1165-1180.

Mumford, DB., Saeed, K., Ahmad, I., Latif, S. and Mubbashar, MH. 1997. Stress and psychiatric disorder in rural Punjab: a community survey. British Journal of Psychiatry. 170:473-478.

Porsolt, RD., Le-Pichon, M. and Jalfre, M. 1977. Depression: A new animal model-sensitive antidepressant treatments. Nature. 266:730-732.

Schaefer, ML., Bottger, B., Silver, WL. and Finger, TE. 2002. Trigeminalcollaterals in the nasal epithelium and olfactorybulb: a potentialroute for directmodulation of olfactory information by trigeminal stimuli. Journal of Comparative Neurology. 444(3):221-226.

Scranton, RA., Fletcher, L., Sprague, S., Jimenez, DF. and Digicaylioglu, M. 2011. The rostralmigratory stream plays a keyrole in intranasal delivery of drugs into the CNS. University of Texas Health Science Centre San Antonio, USA, America PLoS One. 6(4):e18711.

Skolnick, P., Popik, P., Janowsky, A., Beer, B. and Lippa, AS. 2003. Antidepressant like actions of DOV 21947: a "triple" reuptake inhibitor. European Journal of Pharmacology. 461:99-104.

Tizzano, JP., Stribling, DS., Pereztilve, D., Strack, A., Frassetto, A., Chen, RZ., Fong, TM., Shearman, L., Krieter, PA., Tschöp, MH., Skolnick, P. and Basile, AS. 2008. The triple uptake inhibitor (1R,5S)-(+)-1-(3,4-dichlorophenyl)-3-azabicyclo [3.1.0]hexanehydrochloride (DOV 21947) reduces body weight and plasma triglycerides in rodent models of diet-induced obesity. Journal of Pharmacology and Experimental Therapeutics. 324(3):1111-1126.

Woode, E., Boakye-Gyasi, E., Amidu, N., Ansah, C. and Duwiejua, M. 2010. Anxiolytic and Antidepressant Effects of a Leaf Extract of *Palisota hirsuta* K. Schum. (Commelinaceae) in Mice. International Journal of Pharmacology. 6(1):1-17.

Received: Feb 6, 2014; Accepted June 26, 2014