Estimation of Monoamines concentration in rat brain after administration of Fluoxetine Hydrochloride Microsphere

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ABSTRACT: Fluoxetine Hydrochloride a potential antidepressant drug with high affinity of serotonin (5-hydroxytryptamine), dopamine (DA) and noradrenaline (NA) in selected regions of the rat brain.Fluoxetine administration enhances extracellular serotonin (5-HT) in the frontal cortex and no parallel changes in the concentration of dopamine Depression is a debilitating psychiatric condition that remains the second most common cause of disability worldwide. Hence the purpose of present study is to determine effect of Fluoxetine hydrochloride microsphere prepared by using combination of Eudragit RS and HPMC K4polymer on monoamine concentration in rat brain. Our main aim is to study relationship such as serotonin (5HT), dopamine (DA) and nonadrenaline (NA)in the brain of rat It showed that significant reduction occur in monoamine formation in brain of rat. Thus study suggest that prepared Olanzepine microsphere showed decreased the monoamine on rat brain

KEYWORDS: Antidepressant Activity, Microsphere, Monoamines, NA, DA and 5HT.

INTRODUCTION: According to the W.H.O. report depression is a common psychiatric disorder affecting millions of people worldwide and statistics clearly identify it as a major public health problem. Depression is the most prevalent mental disorder and recognized to be symptomatically, psychologically and biologically heterogeneous. Depression, as a mood disorder, is considered a serious problem to human health because of its relatively high prevalence associated with a significant disability [1-2]. Monoamine hypothesis suggests that disturbances in the cerebral level of noradrenaline (NA), dopamine or serotonin play a key role in depression. It occurs from

abnormalities in the interactions between neurotransmitters and hormones in the brain. Depression having three different kinds of pathogenesis such as norepinephrine (NE) hypothesis, 5-hydroxytryptanmine (5-HT) hypothesis and the function of dopamine (DA). Due to the hypothesis of 5-HT, the depletion in synaptic cleft contributing to the reduction of norepinephrine was responsible to the depression. [3- 5].

Fluoxetine Hydrochloride. It is a selective inhibitor of serotonin reuptake type of drug used in the treatment of depression. It is practically soluble in water belongs to BCS class I with a bioavailability approximately 72%. Fluoxetine HCl mucoadhesive microspheres were prepared by using chitosan polymer and emulsion solvent evaporation

Fluoxetine hydrochloride a second generation atypical antipsychotic which is selective inhibitor of serotonin reuptake type drug used in treatment of major depression, obsessive compulsive disorder, bulimia nervosa and panic disorder. Fluoxetine is soluble in water belongs to BCS class I having only 72% oral bioavailability, peak plasma concentrations 15 to 55ng/ml and plasma proteins binding (94.5%) and undergoes extensive hepatic metabolism.

Hence our main focus of this research is to prepare sustain microspheres of Fluoxetine which provides slow release in gastrointestinal tract and assures the presence of dosage form at the site of absorption. Fluoxetine has been shown to selectively bind to central dopamine D2 and serotonin (5-HT) receptors and is effective against the negative symptoms of schizophrenia with a lower incidence of extra pyramidal symptoms.

MATERIALS AND METHODS:

Chemicals: Fluoxetine was obtained from Enaltec Lab Private Ltd, Mumbai, India, Carbapol 974P, HPMC K4and Span 20 was purchased from S.B. Fine chemicals Ltd, Mumbai. Eudragit RS, from Loba chemical, Reagents used for animal study: 1. HCl – Butanol sol: (0.85ml of 37% hydrochloric acid in one-litre n-butanol) 2. Heptane 3. 0.1 M Hcl: (0.85 ml conc. HCl upto 100ml H20 Fluoxetine hydrochloride Microsphere Prepared by Emulsion Solvent Evaporation

2.2. Interaction studies of the drug and polymer:

Presence of any undesirable interaction between the drug and the polymers was assessed using DSC studies. An accurate amount of API and polymer physical mixture kept under stress conditions of 40°C and 75% RH for 2 months, was weighed and transferred to the aluminum pans and the rate of heating was 10°C per minute from 35°C to 300°C. Any changes in the DSC thermograms were recorded.

Method of Preparation of microsphere:

The microspheres were prepared using emulsion solvent evaporation method. To the solution of Eudragit RS in acetone (4-8% w/v), Fluoxetine (200 mg) was added. To this Carbopol 974P and HPMC K4M were added in 1:1 ratio and the solution was cooled at 5ºC for 1 hour. Formed solution

poured in liquid paraffin (300 ml) containing 0.75% span 80 previously cooled at 5 ºC for 1 hour. The emulsion was stirred for 40 min (500-1000 RPM) filtered and washed with n-hexane and dried.

Animal used: Protocol of the present study was approved by IAEC (proposal number RDCOP/ IAEC/ Approval/2016 – 17/ 02 dated 8.8.2016).

Experimental design: Healthy Wistar albino mice divided in three groups as control, pure drug treated and microsphere formulation treated. The animals were provided with housing in polypropylene artificial lighting to stimulate day night cycle and access to food and water ad libitum.

Following test performed to accessing antidepressant efficacy.

Bioanlytical Test: Estimation of Dopamine, Adrenaline and Serotonin: Preparation of tissue extracts by method SchlumfjfM Reagents used:

1. HCl – Butanol sol: (0.85ml of 37% hydrochloric acid in one-litre n-butanol)

2. Heptanes 3. 0.1 M HCL: (0.85ml conc. HCL upto 100ml H20

Procedure: On the day of experiment mice were sacrificed, whole brain was dissected out and the sub cortical region (including the striatum) was separated. Weight tissue was weight and was homogenized in 5ml HCl–butanol for about 1 min. The sample was then centrifuged for 10 min at 2000rpm. An aliquot supernatant phase (1ml) was removed and added to centrifuge tube containing 2.5ml heptane and 0.3ml HCL of 0.1 M. After 10 min of vigorous shaking, the tube was centrifuged under the same conditions as above in order to separate the two phases, and the overlaying organic phase was discarded. The aqueous phase (0.2 ml) was then taken either for 5-HT or NA and DA assay. All steps were carried out at 00C. (N.B: It taken in between 50-75mg of tissue for homogenate with 5ml of Hcl-Butanol in correlation of same tissue concentration 1.5-5mg/0.1ml of HCL -butanol used in Schlumfjf M et al, 1974. This is done to get adequate amount of supernatant liquid for analysis).

***In-vivo* study:**

In-vivo efficiency of the prepared batch was performed by measuring the antidepressant effect produced after oral administration. The Fluoxetine Hydrochloride weighing 72 mg (equivalent to 5 mg) drug was administered and used for the study.

Estimation of Noradrenaline and dopamine:

Reagents: 1.

0.4M Hcl 2. Sodium acetate buffer (pH 6.9), Glacial acetic acid, sodium acetate, 5M NaOH, 0.1 M Iodine solution, Na2SO3 sol., 10M Acetic acid.

Procedure: To the 0.2 ml of aqueous phase, 0.05ml 0.4 M HCl and 0.1ml of EDTA/Sodium acetate buffer (PH6.9) were added, followed by 0.1ml iodine solution (0.1M in ethanol) for

oxidation. The reaction was stopped after 2 min by addition of 0.1ml Na2SO3 solution. 0.1ml Acetic acid is added after 1.5 min. The solution was then heated to 100°C for 6 min when the sample again reached room temperature, excitation and emission spectra were read from the spectrofluorimeter. The readings were taken at 330-375nm for dopamine and 395-485nm for noradrenaline.

Estimation of Serotonin: Reagents 1. O-phthaldialdehyde (OPT) reagent: (20mg in 100ml conc. HCL) Procedure: To 0.2ml aqueous extract 0.25ml of OPT reagent was added. The fluorophore was developed by heating to 100°C for 10 min. After the samples reached equilibrium with the ambient temperature, readings were taken at 360-470nm in the spectrofluorimeter. Tissue blanks for Dopamine and nor-adrenaline were prepared by adding the reagents of the oxidation step in reversed order (sodium sulphite before iodine). For serotonin tissue blank, 0.25ml cont. HCI without OPT was added. Internal Standard: (500µg/ml each of noradrenaline, dopamine and serotonin are prepared in distilled water: HCl-butanol in 1:2 ratio.

Experiment Procedure: On the day of experiment mice were sacrificed, whole brain was dissected out and the sub cortical region (including the striatum) was separated. Tissue was weighed and homogenized in5ml HCL–butanol for about 1 min. The sample was then centrifuged for 10 min at 2000rpm. An aliquot supernatant phase (1ml) wasremoved and added to centrifuge tube containing 2.5ml heptane and 0.31ml HCL of 0.1M. After 10 min of vigorous shaking, the tube was centrifuged under the same conditions as above in order to separate the two phases, and the overlaying organic phase was discarded. The aqueous phase (0.2ml) was then taken either for 5-HT or NA and DA assay. All steps were carried out at 00C. (N.B: It taken in between 50-75mg of tissue for homogenate with 5 ml of HCl-Butanol in correlation of same tissue concentration 1.5-5mg/0.1ml of HCl-butanol used in Schlumpf M et al, 1974. This is done to get adequate amount of supernatant liquid for analysis). [10- 11]

Statistical Analysis:

The data were expressed as mean ± standard error mean (S.E.M). The Significance of differences among the group was assessed using one way analyses of variance. The test followed byTurkey's test p values less than 0.001 were considered as significance.

RESULT AND DISCUSSION:

Table 1: Formulation code Ingredient Formulation Code

Ingredient	Formulation Code								
	F1	F 2	F 3	F 4	F 5	F 6	F 7	F 8	F 9
Drug(Fluoxetine)	1	1	1	1	1	1	1	1	1
Polymer (Eudragit)	1	1	1	3	3	3	5	5	5
Speed (r.p.m.)	500	1000	500	500	1000	750	750	750	1000

Liquid paraffin	300	300	300	300	300	300	300	300	300

1**. Estimation of dopamine, adrenaline and serotonin**:

Effect of Fluoxetine Hydrochloride treatment on brain dopamine, serotonin and noradrenalin levels: A low levels of dopamine, serotonin and noradrenalin had been observed in nontreated depression control mice as compared to normal ($P< 0.001$), serotonin ($P < 0.001$) and noradrenalin ($P < 0.001$). Hence no significant difference observed in the Fluoxetine Hydrochloride treatment indicating effective in restoring the levels of neurotransmitter in the brain.

Table 2: Dopamine, serotonin and noradrenalin levels

	Dopamine (ug/mg of Pr.)	Serotonin (ug/mg of Pr.)	Nordrenaline (ug/mg of Pr.)
Control	50.80 ± 1.18	18.68 ± 0.87	144.80 ± 2.62
D. Control	33.21 ± 1.06*	9.92 ± 0.39*	85.50 ± 3.51*
Fluoxetine	44.90 ± 1.38$^{@}$	16.51 ± 0.96$^{\#}$	118.30 ± 3.02$^{@}$

Figure 1: Estimation of Dopamine

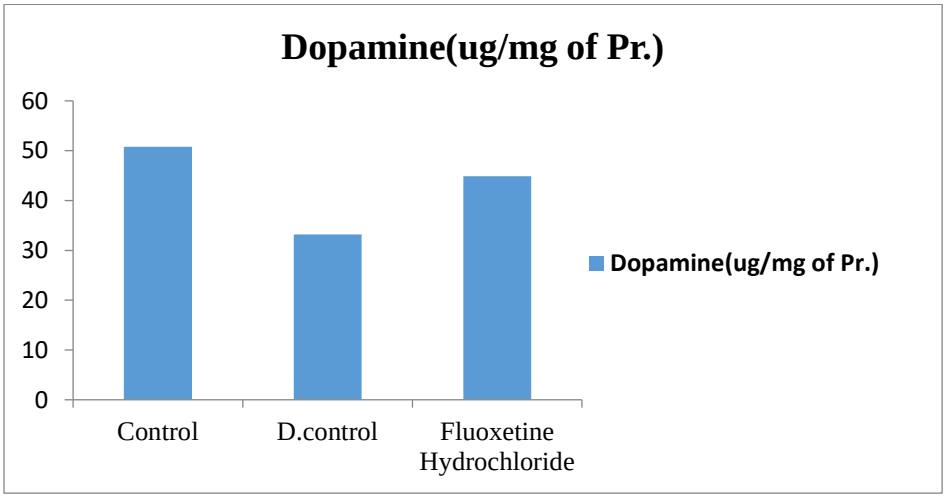


Figure 2: Estimation of Serotonine

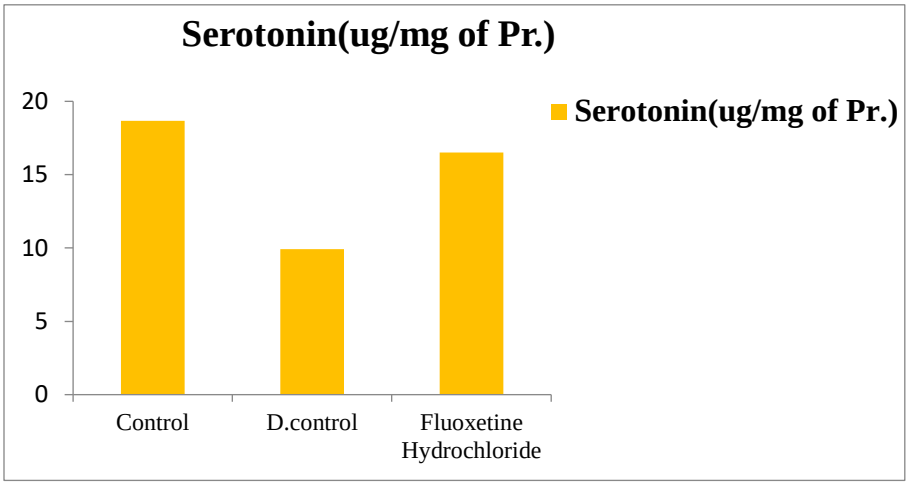
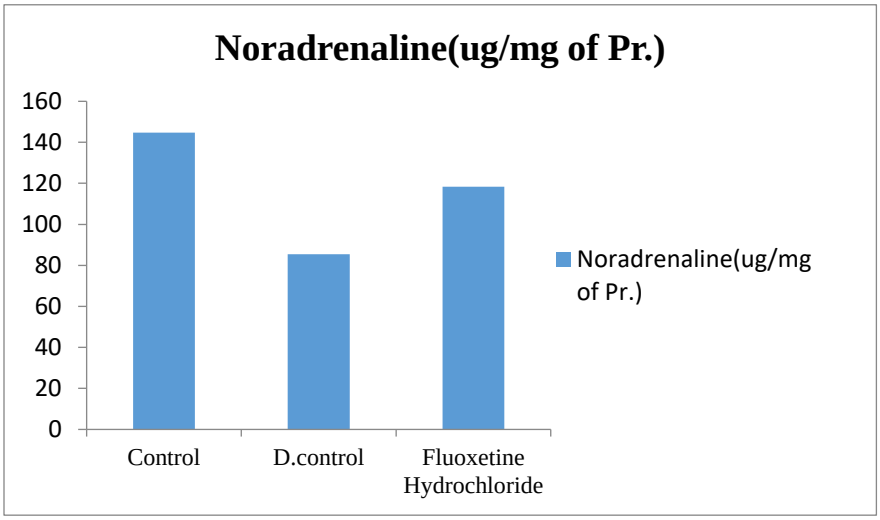


Figure 3: Estimation of Noradrenaline:



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