# PHARMACOLOGICAL APPROACH OF PLUMBAGIN, PIPERINE FOR IMPROVED BIOAVAILABILITY OF SOME ANTI-PSYCHOTIC DRUGS

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***Abstract*:** *The current study standardizes the following models for schizophrenia-induced psychosis (Amphetamine/Dexamethasone/Apomorphine). To investigate the impact of Olanzapine alone and in combination with Plumbagin in Schizophrenia, using at least two dosages of each group and assess behavioral parameters (pharmacodynamics estimation). Using docking software, conduct pharmacokinetic experiments for bioavailability enhancement estimates and ADME studies for pharmacokinetics estimation. The objective of this study is the improvement in the bioavailability of this drug by using the agents which are the promising inhibitors of these sub enzymes of CYP namely plumbagin and piperine which ultimately may lead to an increase in bioavailability and increased in the duration of action of selected drug. In present study procurement of Drugs- Olanzapine, Dexamethasone, Plumbagin, Standardization of Models of Psychosis- Dexamethasone induced psychosis, Pharmacological Evaluation- Olanzapine alone and in combination with plumbagin by following models-Behavioural Models- Hyperactivity, Anhedonia (Splash Test), Stereotypy, Social Interaction Test are studied to enhance the bioavailability and activity of some anti- psychotic drugs. And also pharmacokinetic study for bioavailability estimation and In Silico studies for determination of ADME profiles of drugs are carried out.*

Index Terms: Schizophrenia, Dexamethasone, Plumbagin, piperine, Behavioural Models.

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## I. INTRODUCTION

Schizophrenia or psychosis is a severe brain disorder in which people interpret reality abnormally. Schizophrenia may result in combination of hallucinations, delusions, and extremely disordered thinking and behavior. [1] Contrary to popular belief, schizophrenia is not a split personality or multiple personality disorder. The word "schizophrenia" does mean split mind, but it refers to the disruption of usual balance of emotions and thinking. Schizophrenia is a chronic condition requiring a lifetime treatment. [2] It is found that the atypical second generation antipsychotic drug like Olanzapine was effective in the treatment of acute psychosis associated schizophrenia. But major problem with this agent is that it is rapidly metabolized by the Sub enzymes of CYP family namely CYP1A2 and CYP2D6 and hence its bioavailability is very low because of this enzyme metabolism. Nearly 40% of drug is metabolized before reaching to the systemic circulation. [2]

**Possible Bioavailability Enhancers:** Piperine- It is an alkaloid present in the black pepper (Piper nigrum and Piper longum) belongs to family "Piperaceae." Piperine inhibits human P-glycoprotein and cytochrome P450 3A4 (CYP3A4). Both the proteins contribute to a major extent to first-pass elimination of many drugs. Some of the metabolizing enzymes inhibited or induced by piperine include CYP1A1, CYP1B1, CYP1B2, CYP2E1, CYP3A4 etc. This will alter the metabolism of particular drugs which are metabolized by those enzymes. [3] **Plumbagin-** It is the natural compound which is obtained from *Plumbago zeylanica linn.*

Belonging to family "*Plumbaginaceae*." It inhibits the CYP 450 enzyme particularly CYP1A2, CYP2B1/6, CYP2C9/11, CYP2D1/6, CYP2E1, CYP3A2/4 and hence will be used to enhance the activity of other drugs which are rapidly metabolized by these enzymes. Generally, Plumbagin is also known for its universal inhibitory potential on various enzymes of human body. [4]

**Drug profile:** Olanzapine- It is the atypical antipsychotic agent which is commonly used to treat the psychosis and schizophrenia. It is approved for the medicinal use in 1996. [5]Plumbagin – It is an alkaloid which is obtained in the form of yellow dye extracted from "Plumbago Zylanica Linn" belonging to the family 'Plumbagoginaceae'. It is commonly used in china and in other countries for the treatment of cancer, arthritis and some other diseases like dysmenorrhea. [6]

## II. MATERIALS AND METHODS:

**1. Animals:** Healthy adults Male Wistar rats (200-250g) were obtained from the Vidyabharti College of Pharmacy, Amravati (Reg no. 1504/PO/RE/S/11/CPCSEA) The animals were housed separately in solid bottom polypropylene cage and maintained at (24±1) $^{o}$C. [12,13]

**2. Drugs and Chemicals Procurement:** Olanzapine tablets 10mg and dexamethasone vial was purchased from vasant medical store, Nagpur. Plumbagin was purchased from PcChem.

### 3. Preparation of Drugs and Doses:

**Olanzapine-** The maximum solubility of olanzapine is in the DMSO solution i.e. 16mg/ml. Hence, the whole tablet was dissolved in the DMSO solution and the filtered by using wattman filter paper. (Cayman chemicals). Maximum tolerable oral dose was 30mg/kg. The doses are administered according to the standard experimental protocol. [7, 20]

**Plumbagin –** The maximum solubility is in DMSO solution i.e. 10mg/ml. Hence, a solution of 2 mg / ml in DMSO was made. (Cayman Chemicals). Max tolerable dose for rodent was 6mg/kg ip. Doses are administered according to standard protocol. [8]

**DMSO-** 10ml/kg daily dose was tolerable for 4 weeks of study. (Periodicum biologorum).

**Dexamethasone-** 30ml Vial with capacity 4mg/ml is was used. [14, 17]

## III. EXPERIMENTAL WORK (PHARMACODYNAMICS):

**1. Experimental Group for Dexamethasone Induced Psychosis Model**

Group I- Control group (Received only 0.9% Saline Solution)

Group II- Induction group (Received Dexamethasone 10mg/kg i.p. for 15 days)

**2. Experimental Group to Determine the Effect of Olanzapine on Dexamethasone Induced Psychosis**

Group I- Control Group- (Received only 0.9% Saline Solution)

Group II- Induced Group- (Received Dexamethasone 10mg/kg i.p. for 15 days)

Group III- Treatment Group 1- (Received 5mg/kg Olanzapine in DMSO by oral route for 3 days)

Group IV- Treatment Group 2- (Received 10mg/kg Olanzapine in DMSO by oral route for 3 days)

**3. Experimental Group to Determine the Effect of Combined Olanzapine and Plumbagin on Dexamethasone Induced Psychosis**

Group I- Control Group- (Received only 0.9% Saline Solution)

Group II- Induced Group- (Received Dexamethasone 10mg/kg i.p. for 15 days)

Group III- Treatment Group 1- (Received 5mg/kg Olanzapine oral after 1 hr of 2mg/kg Plumbagin i.p. in DMSO for 3 days).

Group IV- Treatment Group 2- (Received 10mg/kg Olanzapine oral after 1 hr of 2mg/kg Plumbagin i.p. in DMSO for 3 days).

Group V- Treatment Group 3- (Received 5mg/kg Olanzapine oral after 1 hr of 4mg/kg Plumbagin i.p. in DMSO for 3 days).

Group VI- Treatment Group 4- (Received 10mg/kg Olanzapine Oral after 1hr of 4mg/kg Plumbagin i.p. in DMSO for 3 days).

## IV. EXPERIMENTAL WORK (PHARMACOKINETICS)

**1. Method Development of Bioavailability Determination by UV Spectrophotometry:** The present study was conducted using Wistar rats (200-250g). Animals were divided into respective groups with three animals each. [9]

**2. Experimental Group to Determine the Bioavailability of Olanzapine Alone**

Group I- Control Group (Received only 0.9% Saline Solution).

Group II- Treatment Group 1- (Received Olanzapine 10mg/kg 1 hr before blood withdrawal).

Group III- Treatment Group 2- (Received Olanzapine 5mg/kg 1 hr before blood withdrawal).

**3. Experimental Group to determine the Bioavailability of Olanzapine in Combination with Plumbagin**

Group I- (Received 0.9% Saline Solution)

Group II- (Received 5mg/kg Olanzapine oral after 1 hr of 2 mg/kg plumbagin i.p. in DMSO before 1 hr of blood withdrawal)

Group III- (Received 10mg/kg Olanzapine oral after 1 hr of 2 mg/kg plumbagin i.p. in DMSO before 1 hr of blood withdrawal)

Group IV- (Received 5mg/kg Olanzapine oral after 1 hr of 4mg/kg plumbagin i.p. in DMSO before 1 hr of blood withdrawal)

Group V- (Received 10mg/kg Olanzapine oral after 1 hr of 4mg/kg plumbagin i.p. in DMSO before 1 hr of blood withdrawal)

**V. INDUCTION PROTOCOL OF DEXAMETHASONE INDUCED PSYCHOSIS:** A steroidal agent namely Dexamethasone was used to induce psychosis in healthy experimental Wistar rats (200-225g). 10mg/kg of single dose (0.5ml) was given daily by ip route for 15 days. The change in the behavioral parameters was evaluated daily. [10]

**VI. TREATMENT WITH OLANZAPINE:** 5mg/kg and 10mg/kg dose of olanzapine (1ml) and (2ml) was given by oral route using oral gavage needle once a day for 3 days. [11]

**VII. COMBINED TREATMENT WITH BOTH OLANZAPINE AND PLUMBAGIN:** A single dose of plumbagin 2mg/kg and 4mg/kg (0.2ml) and (0.4ml) was given by ip route 1hr before oral administration of olanzapine (1ml) and (2ml). This was followed for 3 days with behavioral analysis. After third day the treatment was stopped and effects were evaluated for 4 days after withdrawal to check the difference. All the parameters are evaluated and results are interpreted. [11,15,16]

**VIII. STATISTICAL ANALYSIS OF EXPERIMENTAL DATA:**

All quantitative data were analyzed using Graph Pad Prism software (version 9.1.2). The data obtained was expressed in Mean ± SEM the data was analyzed using two-way ANOVA followedby Bonferroni's Post-Hoc test.

## RESULT:

### 1. PHARMACODYNAMIC:

**1.1. Statistical Analysis of Experimental Data:** The statistical significance was measured between treated and induced group and P values were calculated (*P ≤ 0.05, **P ≤ 0.01, ***P ≤ 0.001).

**1.Effect of Dexamethasone Induction on Hyperlocomotor Activity (upto 5min): [18]**

| Sr. No. | Days | Control | Induced |
|---|---|---|---|
| 1 | Day 0 | 252.12 ± 8.320 | 254.33 ± 10.220 |
| 2 | Day 1 | 254.745 ± 7.200 | 257.167 ± 11.007 |
| 3 | Day 3 | 252.624 ± 6.800 | 275.667 ± 6.627 |
| 4 | Day 6 | 253.121 ± 7.251 | 308.500 ± 3.222 |
| 5 | Day 9 | 255.110 ± 7.180 | 425.833 ± 15.422 |
| 6 | Day 12 | 252.110 ± 7.147 | 550.167 ± 9.985 |
| 7 | Day 15 | 253.422 ± 6.148 | 553.500 ± 9.566*** |

**Table 1– Effect of Induction on Locomotor Scores**

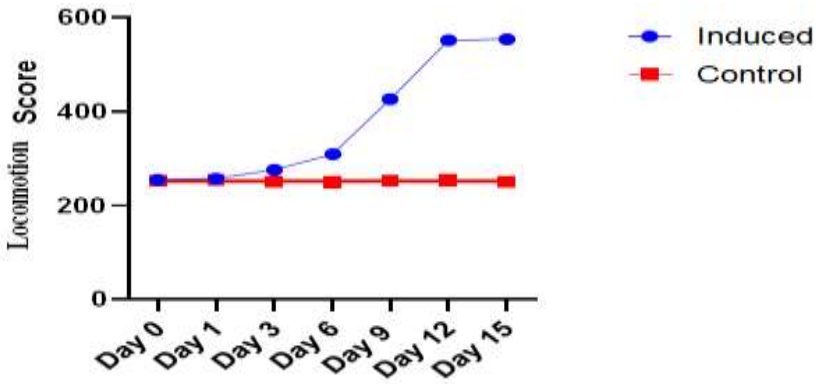



**Fig 1- Effect of Induction on Locomotor Scores**

Here, the effect of dexamethasone induction on hyper locomotion was determined by calculating the number of crossings in actophotometer. The values are expressed in terms of Mean ± SEM. The control group compared with induction group and values are analyzed using graph pad prism 9.0.1 software followed by two way ANOVA and ***p≤0.001 was considered to be statistically significant. In Induction session the locomotor activity was increased significantly ($p \leq 0.001$) as compared to control group from day 9 maximum upto day 15.

**1.3. Effect of Induction on Stereotypy Behavior (Upto 5min):**

| Sr. No | Days | Control | Induced |
|---|---|---|---|
| 1 | Day 0 | 1.167 ± 0.401 | 1.167 ± 0.401 |
| 2 | Day 1 | 1.166 ± 0.411 | 0.833 ± 0.307 |
| 3 | Day 3 | 1.220 ± 0.412 | 2.167 ± 0.703 |
| 4 | Day 6 | 1.154 ± 0.402 | 8.333 ± 1.453 |
| 5 | Day 9 | 1.167 ± 0.399 | 22.833 ± 2.833 |
| 6 | Day 12 | 1.170 ± 0.411 | 25.667 ± 1.626** |
| 7 | Day 15 | 1.168 ± 0.410 | 26.167 ± 2.136** |

**Table 2 – Effect of Induction on Sniffling Scores**

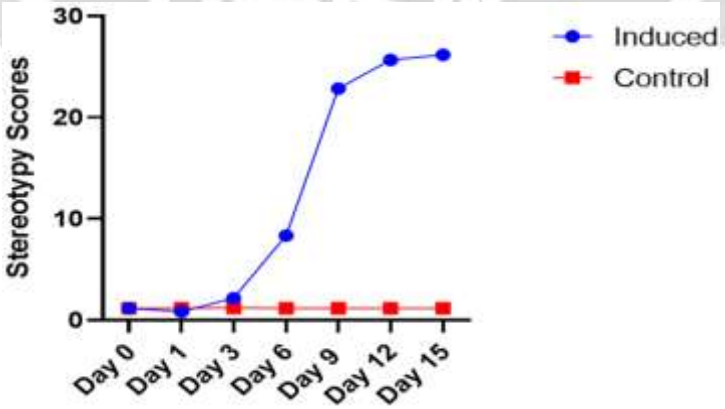



**Fig. 2- Effect of Induction on Sniffling**

Here, the effect of dexamethasone induction on Stereotypy behavior like sniffling, rearing and licking was determined. The values are expressed in terms of Mean ± SEM. The control group compared with induction group and values are analyzed using graph pad prism 9.0.1 software followed by two way ANOVA and ***p≤0.001 was considered to be statistically significant. During the Induction session the stereotyped activity was increased significantly ($p \leq 0.001$) as compared to control group.

**1.4. Effect of Induction on Anhedonia Splash Test (upto 5min):**

| Sr. No | Days | Control (Sec) | Induced (Sec) |
|---|---|---|---|
| 1 | Day 0 | 1.000 ± 0.001 | 1.000 ± 0.000 |
| 2 | Day 1 | 1.000 ± 0.000 | 1.000 ± 0.000 |
| 3 | Day 3 | 1.100 ± 0.002 | 1.167 ± 0.167 |
| 4 | Day 6 | 1.100 ± 0.001 | 32.000 ± 3.396 |
| 5 | Day 9 | 1.100 ± 0.000 | 164.833 ± 9.867 |
| 6 | Day 12 | 1.100 ± 0.002 | 294.167 ± 3.745*** |
| 7 | Day 15 | 1.100 ± 0.003 | 300.000 ± 0.000*** |

**Table 3- Effect of Induction on Time to Induce Grooming**

| Sr. No | Days | Control (Sec) | Induced (Sec) |
|---|---|---|---|
| 1 | Day 0 | 60.121 ± 1.252 | 59.247 ± 1.144 |
| 2 | Day 1 | 58.141 ± 1.200 | 59.211 ± 1.123 |
| 3 | Day 3 | 59.254 ± 1.147 | 51.214 ± 1.139 |
| 4 | Day 6 | 57.111 ± 1.147 | 39.251 ± 1.111 |
| 5 | Day 9 | 58.125 ± 1.255 | 20.111 ± 1.002 |
| 6 | Day 12 | 55.141 ± 1.200 | 11.100 ± 1.002** |
| 7 | Day 15 | 58.141 ± 1.241 | 9.099 ± 0.999** |

**Table 4 – Effect of Induction on Grooming Scores**

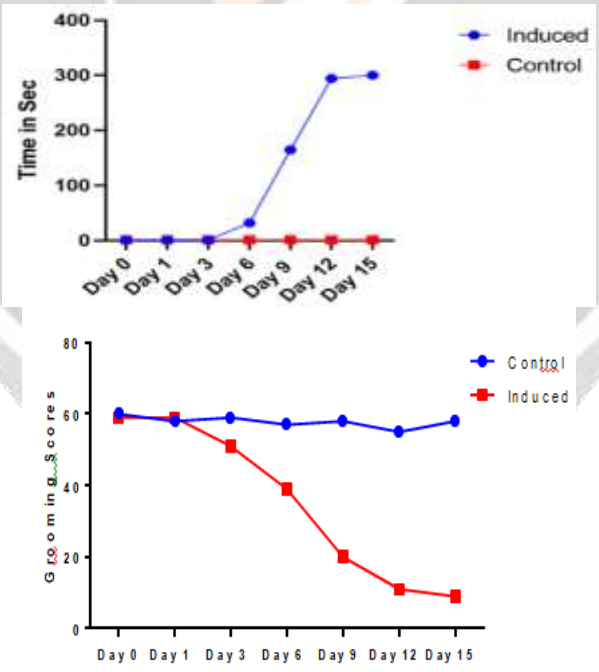



**Fig 3- Effect of Induction on Time to Induce Grooming and Grooming Scores**

Here, the effect of dexamethasone induction on Time to induce grooming and on grooming time was determined. The values are expressed in terms of Mean ± SEM. The control group compared with induction group and values are analyzed using graph pad prism 9.0.1 software followed by two way ANOVA and **p≤0.01 was considered to be statistically significant. During the

Induction session the time to induce grooming was increased significantly ($p \leq 0.001$) and grooming scores are decreased as compared to control group. ($p \leq 0.01$).

## PHARMACODYNAMIC STUDY OF BIOAVAILABILITY ENHANCEMENT:

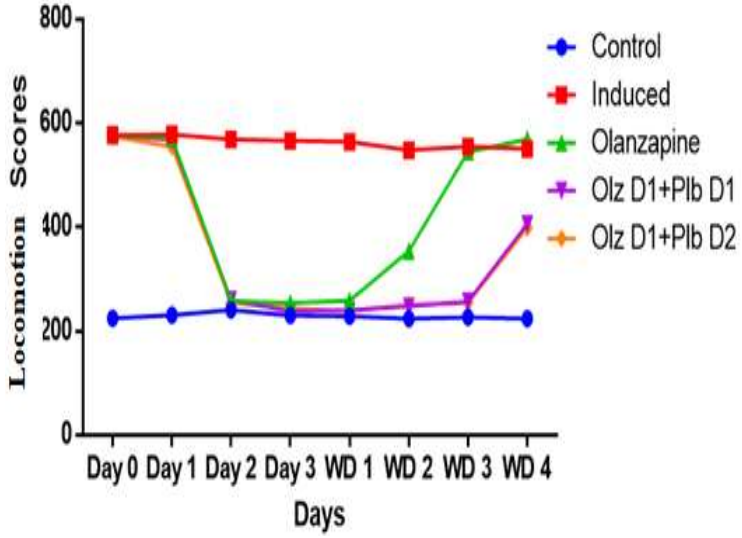
### 1. Effect of Treatment on Hyperlocomotion: [19]

| Sr no | Days | Control | Induced | Olz D1 | Olz D1+PlbD1 | Olz D1 + PlbD2 |
|---|---|---|---|---|---|---|
| 1 | Day 0 | 225.01±1.122 | 577.01±1.121 | 576.02±1.114 | 577.05±1.121 | 574.05±1.111 |
| 2 | Day 1 | 231.14±1.255 | 578.04±1.124 | 572.01±1.117 | 568.45±1.111 | 555.02±1.100 |
| 3 | Day 2 | 241.05±1.244 | 569.02±1.124 | 258.02±1.155 | 260.12±1.121 | 254.02±1.100 |
| 4 | Day 3 | 230.08±1.241 | 566.04±1.111 | 254.02±1.541 | 238.02±1.121 | 244.02±1.147 |
| 5 | WD 1 | 229.08±1.211 | 564.08±1.122 | 259.01±1.126 | 240.02±1.100 | 239.02±1.155 |
| 6 | WD 2 | 224.10±1.245 | 548.05±1.151 | 354.08±1.157 | 248.02±1.099 | 251.02±1.255 |
| 7 | WD 3 | 227.07±1.111 | 555.05±1.141 | 545.05±1.455 | 257.02±1.122 | 255.01±1.200* |
| 8 | WD 4 | 224.09±1.112 | 551.08±1.166 | 569.05±1.147 | 406.02±1.128 | 399.05±1.166* |

**Table 5– Effect of Olz D1 vs OlzD1+PlbD1 and OlzD1+PlbD2 on Hyperlocomotion**

| Sr no | Days | Control | Induced | Olz D2 | Olz D2+PlbD1 | Olz D2 + PlbD2 |
|---|---|---|---|---|---|---|
| 1 | Day 0 | 225.01±1.122 | 577.01±1.121 | 575.02±1.114 | 577.12±1.121 | 574.05±1.111 |
| 2 | Day 1 | 231.14±1.255 | 578.04±1.124 | 569.05±1.117 | 565.16±1.111 | 553.12±1.100 |
| 3 | Day 2 | 241.05±1.244 | 569.02±1.124 | 259.05±1.155 | 258.13±1.121 | 253.15±1.100 |
| 4 | Day 3 | 230.08±1.241 | 566.04±1.111 | 248.05±1.541 | 242.14±1.121 | 244.13±1.147 |
| 5 | WD 1 | 229.08±1.211 | 564.08±1.122 | 249.05±1.126 | 241.12±1.100 | 239.03±1.155 |
| 6 | WD 2 | 224.10±1.245 | 548.05±1.151 | 354.02±1.157 | 248.12±1.099 | 251.15±1.255 |
| 7 | WD 3 | 227.07±1.111 | 555.05±1.141 | 545.03±1.455 | 257.12±1.122 | 250.15±1.200* |
| 8 | WD 4 | 224.09±1.112 | 551.08±1.166 | 569.12±1.147 | 408.13±1.128 | 398.12±1.166* |

**Table 6– Effect of OlzD2 vs OlzD2+PlbD1 and OlzD2+PlbD2**



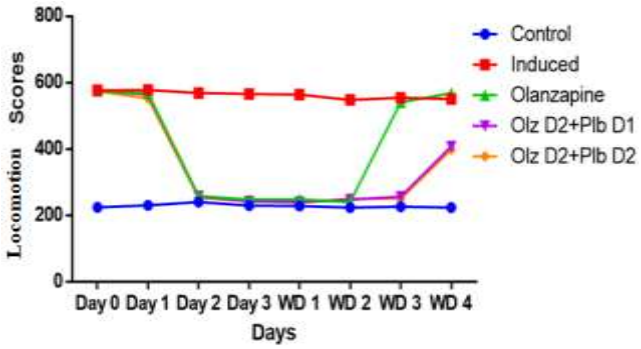



**Fig 4- Effect of Treatment on Locomotor Scores**

Here, the effect of Treatment on hyper locomotion was determined by calculating the no of crossings in actophotometer. The values are expressed in terms of Mean ± SEM. The OlzD1 group compared with olzD1+plbD1 and OlzD1+plbD2 and values are analyzed using graph pad prism 9.0.1 software followed by two way ANOVA and *p≤0.05 was considered to be statistically significant. During treatment both combined olz+plb group’ s shows better effects after withdrawal of therapy as compared to olz alone group.

## 2. Effect of Treatment on Stereotypy:

| Sr no | Days | Control | Induced | Olz D1 | Olz D1+PlbD1 | Olz D1 + PlbD2 |
|---|---|---|---|---|---|---|
| 1 | Day 0 | 1.01±1.001 | 25.01±1.200 | 27.10±1.000 | 29.10±1.100 | 27.10±1.200 |
| 2 | Day 1 | 2.02±1.001 | 26.14±1.211 | 22.11±1.099 | 24.09±1.100 | 26.11±1.199 |
| 3 | Day 2 | 1.12±1.021 | 24.14±1.254 | 4.12±1.111 | 5.08±1.100 | 8.12±1.141 |
| 4 | Day 3 | 1.25±1.022 | 21.12±1.222 | 3.14±1.222 | 3.14±1.120 | 4.13±1.122 |
| 5 | WD 1 | 1.14±1.002 | 23.15±1.220 | 5.14±1.200 | 4.17±1.255 | 3.14±1.122 |
| 6 | WD 2 | 3.01±1.111 | 22.16±1.200 | 23.12±1.144 | 5.05±1.222 | 4.12±1.222 |
| 7 | WD 3 | 1.01±1.200 | 25.16±1.111 | 24.14±1.141 | 6.08±1.121 | 5.08±1.141* |
| 8 | WD 4 | 1.25±1.025 | 24.14±1.110 | 23.11±1.151 | 19.09±1.100 | 22.08±1.122* |

**Table 7- Effect of Olz D1 vs OlzD1+PlbD1 and OlzD1+PlbD2 on Sniffling**

| Sr no | Days | Control | Induced | Olz D2 | Olz D2+PlbD1 | Olz D2 + PlbD2 |
|---|---|---|---|---|---|---|
| 1 | Day 0 | 1.01±1.001 | 25.01±1.200 | 28.11±1.222 | 30.12±1.147 | 28.11±1.258 |
| 2 | Day 1 | 2.02±1.001 | 26.14±1.211 | 25.12±1.200 | 23.12±1.144 | 29.12±1.136 |
| 3 | Day 2 | 1.12±1.021 | 24.14±1.254 | 5.14±1.222 | 6.14±1.244 | 7.14±1.144 |
| 4 | Day 3 | 1.25±1.022 | 21.12±1.222 | 4.12±.1.247 | 3.11±1.258 | 4.12±1.258 |
| 5 | WD 1 | 1.14±1.002 | 23.15±1.220 | 6.14±1.200 | 5.10±1.365 | 3.15±1.255 |
| 6 | WD 2 | 3.01±1.111 | 22.16±1.200 | 22.17±1.254 | 5.11±1.147 | 3.12±1.247* |
| 7 | WD 3 | 1.01±1.200 | 25.16±1.111 | 26.16±1.255 | 6.10±1.1781 | 4.12±1.269* |
| 8 | WD 4 | 1.25±1.025 | 24.14±1.110 | 27.11±1.147 | 19.12±1.258 | 20.12±1.258* |

**Table 8- Effect of OlzD2 vs olzD2+PlbD1 and OlzD2+PlbD2 on Sniffling**

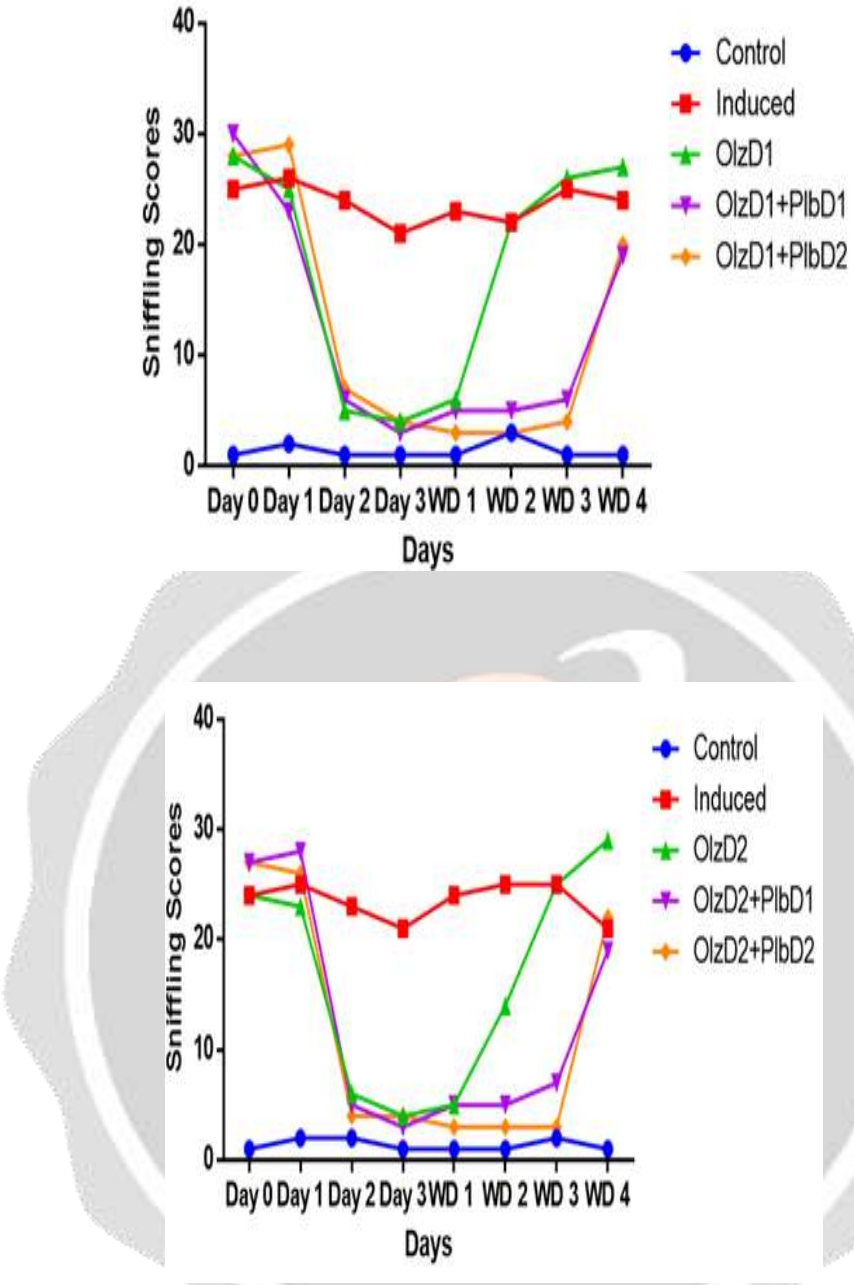



**Fig. 5- Effect of Treatment on Sniffling Scores**

### 3. Effect of Treatment on Anhedonia Splash Test:

| Sr no | Days | Control (Sec) | Induced(Sec) | Olz D1 (Sec) | Olz D1+PlbD1 (Sec) | Olz D1 + Plb D2 (Sec) |
|---|---|---|---|---|---|---|
| **1** | Day 0 | 1.01 ± 0.001 | 299.01±1.002 | 299.01±1.111 | 298.12±1.122 | 289.12±1.121 |
| **2** | Day 1 | 1.02 ± 0.000 | 298.12±1.005 | 185.16±1.112 | 199.01±1.166 | 188.60±1.122 |
| **3** | Day 2 | 1.10 ± 0.002 | 299.13±1.002 | 3.02±1.120 | 5.01±1.154 | 4.02±1.100 |
| **4** | Day 3 | 1.18 ± 0.001 | 289.10±1.005 | 2.06±1.110 | 2.03±1.114 | 3.05±1.120 |
| **5** | WD1 | 1.15 ± 0.000 | 287.10±1.020 | 3.01±1.008 | 3.02±1.147 | 3.05±1.147 |

| | | | | | | |
|---|---|---|---|---|---|---|
| **6** | WD 2 | 1.11 ± 0.002 | 288.12±1.010 | 225.12±1.007 | 5.04±1.147 | 7.11±1.158 |
| **7** | WD 3 | 1.12 ± 0.003 | 291.13±1.111 | 278.66±1.111 | 11.55±1.159 | 10.09±1.144* |
| **8** | WD 4 | 1.02 ± 0.001 | 289.12±1.100 | 277.65±1.121 | 288.12±1.122 | 268.11±1.146* |

**Table 9- Effect of OlzD1 vs OlzD1+PlbD1 and OlzD1 and PlbD2 on Time to InduceGrooming**

| Sr no | Days | Control (Sec) | Induced (Sec) | Olz D2 (Sec) | Olz D2+PlbD1 (Sec) | Olz D2 + PlbD2 (Sec) |
|---|---|---|---|---|---|---|
| **1** | Day 0 | 1.01 ± 0.001 | 299.01±1.002 | 299.02±1.122 | 296.12±1.200 | 288.16±1.100 |
| **2** | Day 1 | 1.02 ± 0.000 | 298.12±1.005 | 185.12±1.111 | 196.15±1.478 | 174.14±1.102 |
| **3** | Day 2 | 1.10 ± 0.002 | 299.13±1.002 | 3.11±1.120 | 4.14±1.471 | 5.01±1.104 |
| **4** | Day 3 | 1.18 ± 0.001 | 289.10±1.005 | 2.09±1.166 | 3.17±1.200 | 4.03±1.145 |
| **5** | WD 1 | 1.15 ± 0.000 | 287.10±1.020 | 3.01±1.147 | 4.02±1.121 | 5.05±1.171 |
| **6** | WD 2 | 1.11 ± 0.002 | 288.12±1.010 | 225.02±1.125 | 4.05±1.235 | 7.05±1.120 |
| **7** | WD 3 | 1.12 ± 0.003 | 291.13±1.111 | 278.12±1.147 | 12.05±1.147 | 10.01±1.120* |
| **8** | WD 4 | 1.02 ± 0.001 | 289.12±1.100 | 277.11±1.258 | 279.04±1.155 | 266.17±1.122* |

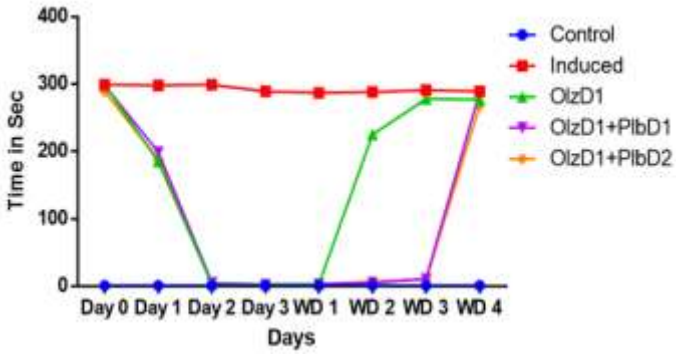
**Table 10- Effect of OlzD2 vs OlzD2+PlbD1 and OlzD2+PlbD2 on Time to Induce Grooming**

| Sr no | Days | Control | Induced | Olz D1 | Olz D1+PlbD1 | Olz D1 + PlbD2 |
|---|---|---|---|---|---|---|
| **1** | Day 0 | 60.01±2.000 | 8.01±1.003 | 5.00±1.002 | 7.10±2.000 | 7.14±2.369 |
| **2** | Day 1 | 58.10±2.123 | 7.02±1.111 | 6.10±1.003 | 9.14±2.132 | 9.12±2.458 |
| **3** | Day 2 | 60.11±2.144 | 9.01±1.112 | 46.12±1.005 | 54.12±2.121 | 54.16±2.18 |
| **4** | Day 3 | 51.12±2.005 | 8.02±1.009 | 56.13±1.005 | 58.15±2.555 | 53.18±2.394 |
| **5** | WD 1 | 55.14±2.004 | 7.05±1.008 | 57.14±1.007 | 54.17±2.248 | 55.19±2.147 |
| **6** | WD 2 | 52.15±2.007 | 9.03±1.005 | 10.14±1.101 | 56.15±2.365 | 55.12±2.354 |
| **7** | WD 3 | 59.14±2.008 | 8.04±1.111 | 9.15±1.006 | 49.17±2.148 | 48.18±2.147* |
| **8** | WD 4 | 60.11±2.001 | 7.02±1.008 | 6.16±1.111 | 9.13±2.589 | 9.16±2.158* |

**Table 11- Effect of OlzD1 vs OlzD1+PlbD1 and OlzD1+PlbD2 on Grooming Scores**

| Sr no | Days | Control | Induced | Olz D2 | Olz D2+PlbD1 | Olz D2 + PlbD2 |
|---|---|---|---|---|---|---|
| **1** | Day 0 | 60.01±2.000 | 8.01±1.003 | 6.10±1.000 | 9.12±1.025 | 8.11±1.002 |
| **2** | Day 1 | 58.10±2.123 | 7.02±1.111 | 6.12±1.100 | 8.13±1.024 | 7.12±1.036 |
| **3** | Day 2 | 60.11±2.144 | 9.01±1.112 | 45.15±1.121 | 53.15±1.258 | 54.18±1.258 |
| **4** | Day 3 | 51.12±2.005 | 8.02±1.009 | 55.18±1.258 | 58.09±1.036 | 53.19±1.354 |
| **5** | WD 1 | 55.14±2.004 | 7.05±1.008 | 57.16±1.100 | 52.08±1.025 | 55.17±1.247 |
| **6** | WD 2 | 52.15±2.007 | 9.03±1.005 | 10.13±1.111 | 56.10±1.369 | 54.17±1.255 |
| **7** | WD 3 | 59.14±2.008 | 8.04±1.111 | 8.13±1.123 | 48.11±1.487 | 49.15±1.002* |
| **8** | WD 4 | 60.11±2.001 | 7.02±1.008 | 7.10±1.354 | 7.15±1.259 | 9.12±1.003* |

**Table 12- Effect of OlzD2 vs OlzD2+PlbD1 and OlzD2+PlbD2 on Grooming Scores**



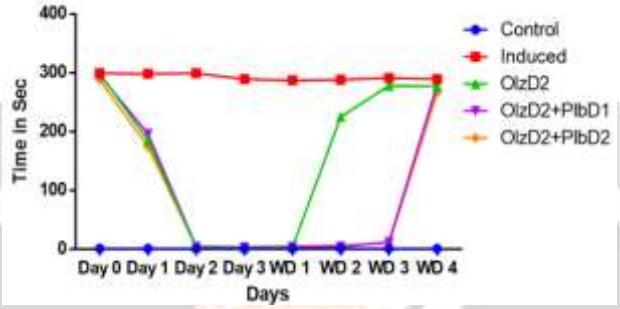
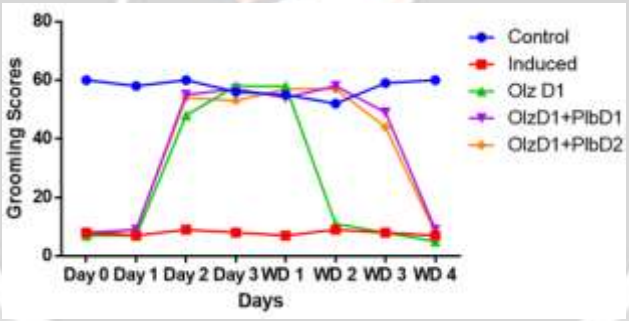




**Fig 6- Effect of Treatment on Time to Induce Grooming in Sec**



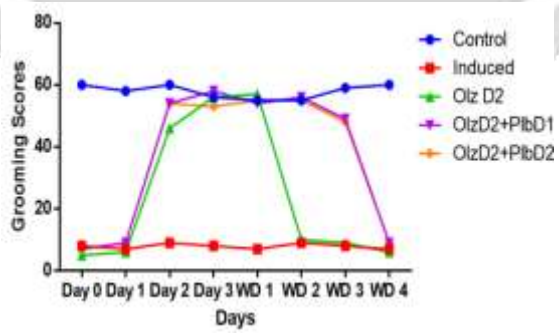




**Fig 7- Effect of Treatment on grooming Scores**

## 2. PHARMACOKINETICS:

### 2.1 Standard Calibration Curve of Olanzapine Stock Solution (Linearity Curve):

| Conc. | Absorbance |
|---|---|
| 1 | 0.1 ± 0.011 |
| 2 | 0.114 ± 0.014 |
| 3 | 0.128 ± 0.017 |
| 4 | 0.139 ± 0.019 |
| 5 | 0.147 ± 0.024 |
| 6 | 0.155 ± 0.011 |
| 7 | 0.168 ± 0.012 |
| 8 | 0.179 ± 0.014 |
| 9 | 0.191 ± 0.025*** |
| 10 | 0.206 ± 0.022*** |

**Table 13- Respective Absorbances of Specific Concentrations of Olanzapine Stock Solutions**

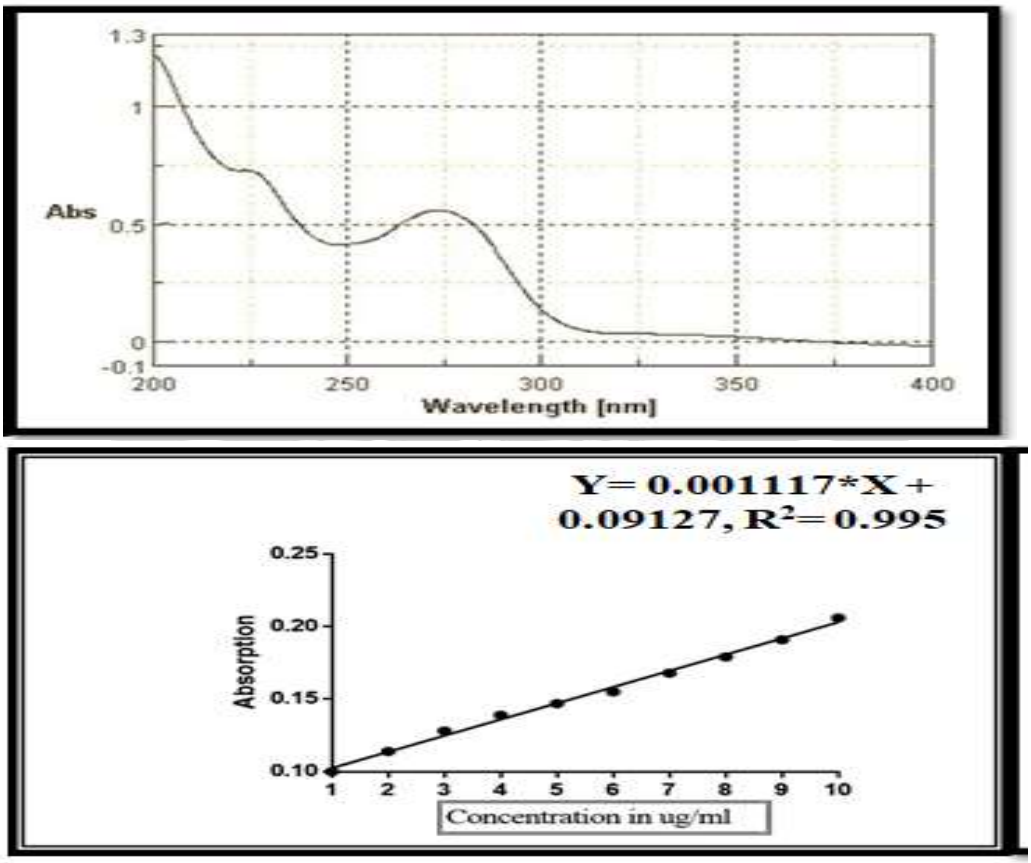



**Fig 8 - Graphs Indicating the Standard linearity cum Calibration Curve of Olanzapine Stock Solution (left) and the Other Graph Showing Absorption Spectrum Indicating 266nm Absorption Maximum.**

**2.2. Bioavailability Curve of 5 mg/kg Olanzapine vs 5 mg/kg Olanzapine + 2mg/kgPlumbagin:**

| Concentration | Absorbance |
|---|---|
| Unknown 1 | 0.011 ± 0.021 |
| Unknown 2 | 0.096 ± 0.023 |
| Unknown 3 | 0.122 ± 0.014 |
| Unknown 4 | 0.158 ± 0.021 |
| Unknown 5 | 0.168 ± 0.014 |
| Unknown 6 | 0.172 ± 0.018 |
| Unknown 7 | 0.168 ± 0.019 |
| Unknown 8 | 0.09 ± 0.020 |
| Unknown 9 | 0.088 ± 0.016* |
| Unknown 10 | 0.008 ± 0.014* |

| Concentration | Absorbance |
|---|---|
| Unknown 1 | 0.006 ± 0.000 |
| Unknown 2 | 0.018 ± 0.001* |
| Unknown 3 | 0.111 ± 0.009* |
| Unknown 4 | 0.399 ± 0.010 |
| Unknown 5 | 0.503 ± 0.014 |
| Unknown 6 | 0.519 ± 0.010 |
| Unknown 7 | 0.522 ± 0.009 |
| Unknown 8 | 0.451 ± 0.010 |
| Unknown 9 | 0.077 ±0.004* |
| Unknown 10 | 0.088 ±0.001* |

**Table 14.a, b.- a) Serum Absorption of Unknown Concentrations of Olanzapine after Oral Dose of5mg/kg. b) Serum Absorptions of Unknown Concentrations of Combined 5mg/kg Olanzapine + 2mg/kg Plumbagin**

**Table 15– Concentration Ranges Obtained After Interpolation of Unknown Absorptions onStandard Curve**

| Time in hrs | Olanzapine Conc. in Ug/ml | Olz + Plb Conc in ug/ml |
|---|---|---|
| 1.00 | 0.983 ± 0.001 | 0.414 ± 0.001 |
| 1.30 | 1.440 ± 0.001 | 1.250 ±0.002 |
| 3.00 | 2.330 ± 0.001 | 3.330 ± 0.025 |
| 6.00 | 4.790 ± 0.012 | 4.870 ± 0.036 |
| 12.00 | 6.090 ± 0.014 | 6.570 ± 0.012 |
| 24.00 | 6.760 ± 0.012 | 6.980 ± 0.147 |
| 36.00 | 6.090 ± 0.001 | 7.060 ± 0.001 |
| 48.00 | 0.521 ± 0.025 | 5.390 ± 0.002 |
| 60.00 | 0.120 ± 0.025 | 2.480 ± 0.005* |

| | | |
|---|---|---|
| 72.00 | 0.113 ± 0.036 | 0.654 ± 0.001* |

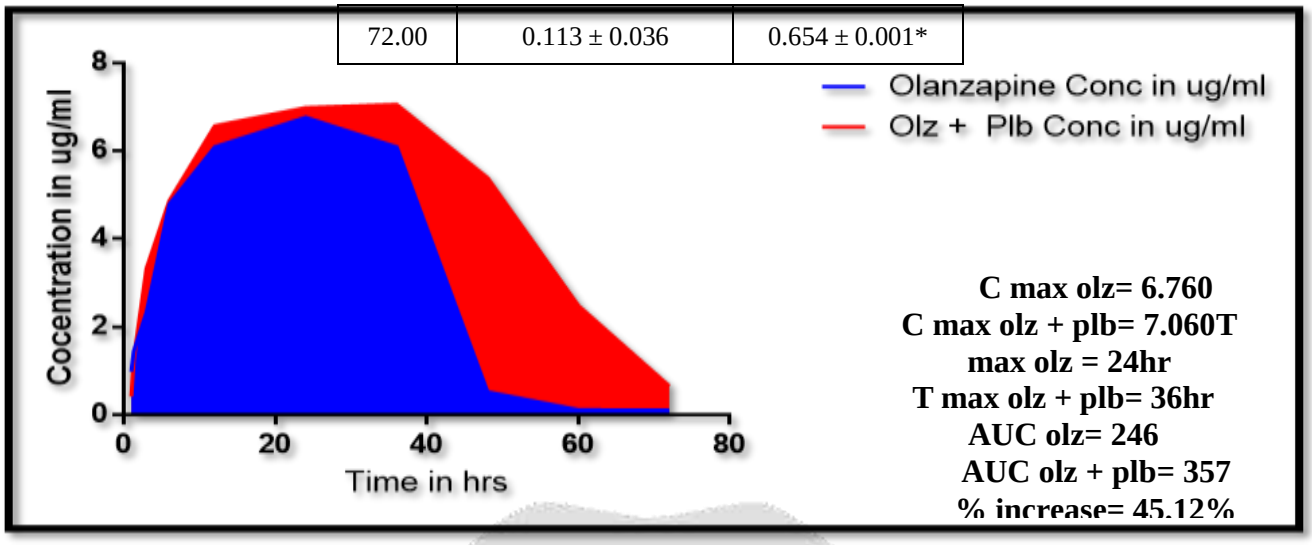



**Fig 9- Bioavailability Curve of Olanzapine 5mg/kg vs Olanzapine 5mg/kg + Plumbagin2mg/kg**

Here, The UV absorbance of separated serum samples which was diluted with Dimethyl Sulfoxide to makeup the volume of upto 4 ml was determined. These absorbance ranges are interpolated on the standard linearity curve of Olanzapine stock solutions to determine the respective concentration ranges. These ranges then plotted against the time at which those samples are collected to make a comparative graph of bioavailability curves. The values are expressed in terms of Mean ± SEM. The Olanzapine alone group compared with Olanzapine + Plumbagin group and values are analyzed using graph pad prism 9.0.1 software followed by two way ANOVA and ***p≤0.001 was considered to be statistically significant. During this process the bioavailability of olanzapine was found to be more in presence of plumbagin. The value p≤0.01 *** and p≤0.05* was considered to be significant.

### 2.3. Bioavailability Curve of 5 mg/kg Olanzapine vs 5 mg/kg Olanzapine + 4mg/kg Plumbagin:

| Concentration | Absorbance |
|---|---|
| Unknown 1 | 0.007 ± 0.001 |
| Unknown 2 | 0.017 ± 0.001 |
| Unknown 3 | 0.110 ± 0.008 |
| Unknown 4 | 0.400 ± 0.010 |
| Unknown 5 | 0.503 ± 0.011 |
| Unknown 6 | 0.517 ± 0.014 |
| Unknown 7 | 0.525 ± 0.007 |
| Unknown 8 | 0.452 ± 0.009 |
| Unknown 9 | 0.078 ± 0.004* |
| Unknown 10 | 0.089 ± 0.002* |

| Time in hrs | Olanzapine Conc. in Ug/ml | Olz + Plb Conc in ug/ml |
|---|---|---|
| 1.00 | 0.983 ± 0.001 | 0.417 ± 0.001 |
| 1.30 | 1.440 ± 0.001 | 1.257 ± 0.006 |
| 3.00 | 2.330 ± 0.001 | 3.220 ± 0.002 |
| 6.00 | 4.790 ± 0.012 | 4.870 ± 0.002 |
| 12.00 | 6.090 ± 0.014 | 6.500 ± 0.001 |
| 24.00 | 6.760 ± 0.012 | 7.000 ± 0.004 |
| 36.00 | 6.090 ± 0.001 | 7.067 ± 0.003 |
| 48.00 | 0.521 ± 0.025 | 5.410 ± 0.002 |
| 60.00 | 0.120 ± 0.025 | 2.487 ± 0.001*** |
| 72.00 | 0.113 ± 0.036 | 0.664 ± 0.001*** |

**Table 16. a, b- a) Serum Absorptions of Unknown Concentrations of Combined 5mg/kg Olanzapine +4mg/kg Plumbagin. b) Concentration Ranges Obtained After Interpolation of Unknown Absorptions on Standard Curve**

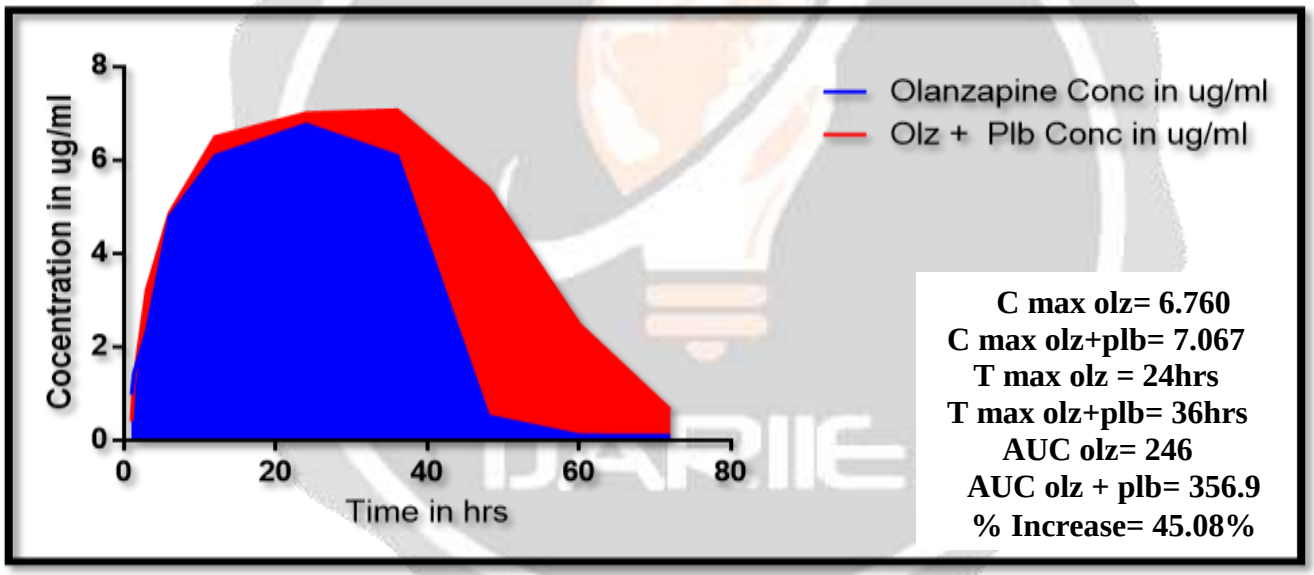



**Fig 10- Bioavailability Curve of Olanzapine 5mg/kg vs Olanzapine 5mg/kg +Plumbagin 4mg/kg**

Here, The UV absorbance of separated serum samples which was diluted with Dimethyl Sulfoxide to makeup the volume of upto 4 ml was determined. These absorbance ranges are interpolated on the standard linearity curve of Olanzapine stock solutions to determine the respective concentration ranges. These ranges then plotted against the time at which those samples are collected to make a comparative graph of bioavailability curves. The values are expressed in terms of Mean ± SEM. The Olanzapine alone group compared with Olanzapine + Plumbagin group and values are analyzed using graph pad prism 9.0.1 software followed by two way ANOVA and ***$p \leq 0.001$ was considered to be statistically significant. During this process the bioavailability of olanzapine was found to be more in presence of plumbagin. The value $p \leq 0.01$ *** and $p \leq 0.05$* was considered to be significant.

**2.3. Bioavailability Curve of 10 mg/kg Olanzapine vs 10 mg/kg Olanzapine + 2mg/kg Plumbagin:**

| Concentration | Absorbance |
|---|---|
| Unknown 1 | 0.010 ± 0.021 |
| Unknown 2 | 0.097 ± 0.023 |
| Unknown 3 | 0.132 ± 0.014 |
| Unknown 4 | 0.157 ± 0.021 |
| Unknown 5 | 0.162 ± 0.014 |
| Unknown 6 | 0.169 ± 0.018 |
| Unknown 7 | 0.170 ± 0.019 |
| Unknown 8 | 0.097± 0.020 |
| Unknown 9 | 0.086 ± 0.016* |
| Unknown 10 | 0.009 ± 0.014* |

| Concentration | Absorbance |
|---|---|
| Unknown 1 | 0.004 ± 0.000 |
| Unknown 2 | 0.016 ± 0.001* |
| Unknown 3 | 0.109 ± 0.009* |
| Unknown 4 | 0.401 ± 0.010 |
| Unknown 5 | 0.501 ± 0.014 |
| Unknown 6 | 0.521 ± 0.010 |
| Unknown 7 | 0.522 ± 0.009 |
| Unknown 8 | 0.450 ± 0.010 |
| Unknown 9 | 0.074 ± 0.004* |
| Unknown 10 | 0.090 ± 0.001* |

**Table 17.a, b. a) Serum Absorption of Unknown Concentrations of Olanzapine after Oral Dose of 10mg/kg. b) Serum Absorptions of Unknown Concentrations of Combined 10mg/kg Olanzapine + 2mg/kg Plumbagin**

| Time in hrs | Olanzapine Conc. in Ug/ml | Olz + Plb Conc in ug/ml |
|---|---|---|
| 1.00 | 0.981 ± 0.001 | 0.914 ± 0.001 |
| 1.30 | 1.442 ± 0.001 | 1.550 ± 0.002 |
| 3.00 | 2.335 ± 0.001 | 3.332 ± 0.025 |
| 6.00 | 4.790 ± 0.012 | 4.870 ± 0.036 |
| 12.00 | 6.089 ± 0.014 | 6.570 ± 0.012 |
| 24.00 | 6.764 ± 0.012 | 6.980 ± 0.147 |
| 36.00 | 6.091 ± 0.001 | 7.060 ± 0.001 |
| 48.00 | 0.521 ± 0.025 | 5.421 ± 0.002 |
| 60.00 | 0.120 ± 0.025 | 2.479 ± 0.005* |
| 72.00 | 0.110 ± 0.036 | 0.154 ± 0.001* |

**Table 18-Concentration Ranges Obtained After Interpolation of Unknown Absorptions on Standard Curve**

Here, The UV absorbance of separated serum samples which was diluted with Dimethyl Sulfoxide to make up the volume of upto 4 ml was determined. These absorbance ranges are interpolated on the standard linearity curve of Olanzapine stock solutions to determine the respective concentration ranges. These ranges then plotted against the time at which those samples are collected to make a comparative graph of bioavailability curves. The values are expressed in terms of Mean ± SEM. The Olanzapine alone group compared with Olanzapine + Plumbagin group and values are analyzed using graph pad prism 9.0.1 software followed by two way ANOVA and ***p≤0.001 was considered to be statistically significant. During this process the bioavailability of olanzapine was found to be more in presence of plumbagin. The value p≤0.01 ** and p≤0.05* was considered to be significant.

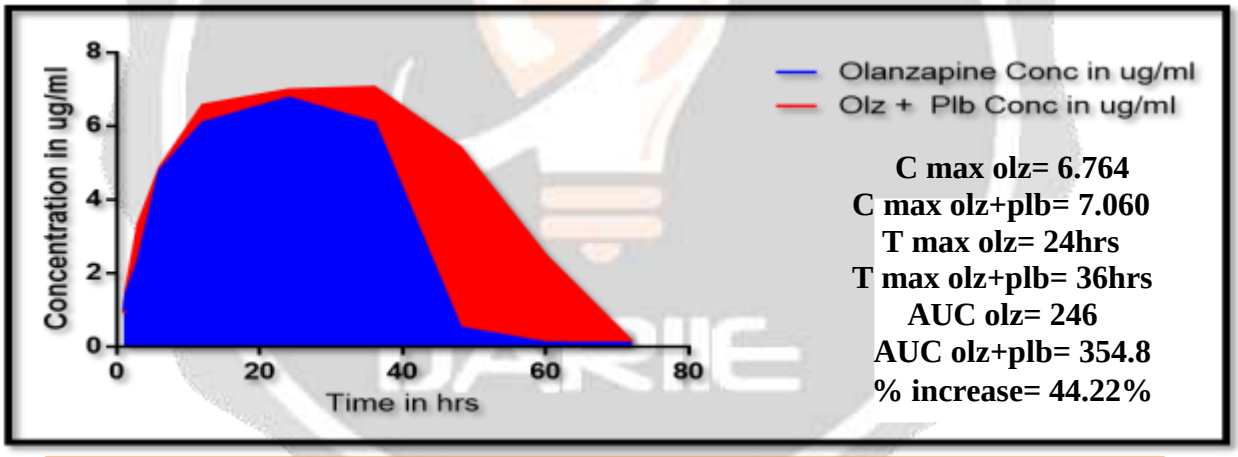



**Fig 11- Bioavailability Curve of Olanzapine 10mg/kg vs Olanzapine 10mg/kg +Plumbagin 2 mg/kg**

## CONCLUSION:

The findings of this investigation proved the therapeutic potential of enzyme inhibitor plumbagin in increasing the drug bioavailability of olanzapine. Our study also identified that the long term exposure to the steroidal agent dexamethasone leads to the development of schizophrenia like psychosis and mania in experimental animals. In our study we identified that the enzyme inhibitors like plumbagin increases the bioavailabilityof olanzapine by inhibition of its metabolizing enzyme CYP1A2. The overall pharmacodynamic and pharmacokinetic investigation showed the increased bioavailability of antipsychotic drug olanzapine due to combination with plumbagin. This was also supported by Swiss ADME molecular docking studies. Hence, it was concluded that our hypothesis was proven to be true

## FUTURE SCOPE:

There are large number of medicinal agents which are too effective for treating the disorder but cannot prescribed due to low bioavailability. So, the further studies need to be conducted in future to determine the exact reason why their bioavailability is less. If it was due to the enzymatic metabolism then our study is helpful in increasing the bioavailability. The enzyme inhibitors are the key players in increasing the bioavailability of drugs which are metabolized by enzymatic action. Further studies need to be conducted in future to determine the role of bioavailability enhancers in increasing bioavailability of other drugs.

Instead of using the costly and time consuming methods of bioavailability estimation the new approaches need to be discovered by using a simple methods like UV spectroscopy. In such case our study was very helpful. Our study also open the doors of further research to explore the roles of UV spectroscopy inspired methods in biological estimations. Future study need to conducted to explore the more obvious mechanisms of development of schizophrenia and psychosis as well as the future discovery of more effective antipsychotic agents needs to be conducted. Some more drugs whose bioavailability is less need to be tested in combination with bioavailability enhancers like plumbagin, piperine etc. The same study need to be performed in future but with different combination like by using other enhancers like piperine. Possible interaction and side effects of metabolic and enzymatic inhibition therapy need to be explored.

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