Chapter 7: Pharmacodynamic studies
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7. PHARMACODYNAMIC STUDIES

One can select different animal models for dynamic study but due to practical feasibility most widely used model are rat and mice. Although rat model is usually selected over mice model due to ease of administration of formulation, as rat nostrils are quite large compared to the mice nasal cavity. Different dynamic responses are studied after dosing of the formulation in appropriate animal model. All experiments conducted on animals were approved by the Committee for the purpose of control and supervision of experiments on animals, Ministry of Social Justice and Empowerment, Government of India, New-Delhi, India.

7.1 EVALUATION OF ANTI-OBESITY EFFECT OF SIBUTRAMINE

7.1.1 Animals
The most widely used animal model to study anti-obesity effect is rat model. Sprag dawley rats of either sex having weight between 350-400g, 12-14 months old were provided by biochemistry department, The Maharaja Sayajirao University of Baroda. The rats were housed as 3-4 rats per cage. The beddings were raised regularly otherwise the animals are expected to eat whatever they find for example feces (coprophagy) and even tail or skin of other animals. All experiments were conducted between 10:30 to 11:30 am (Bol et al 2002).

7.1.2 Methods
7.1.2.1 Induction of obesity in animals
The animals were feed with high fat diet whose composition is given in table 7.1, for a period of 10 weeks, to induce obesity. The body weight and serum lipid levels (estimated in 10-12 hr fasted animals) of the animals (in comparison to control group fed with only normal rodent pellet diet without cholesterol supplement) were carefully estimated both at the initiation and at the end of 8 weeks, and recorded in table 7.2.

7.1.2.2 Evaluation of anti-obesity activity
At the end of 10 weeks the animals were divided into three groups ‘the control group (I)’ and ‘the test group (II & III)’. The control group received 10 μL saline intranasally while the treated group received 10 μL of the formulation intranasally or orally containing sibutramine (thrice a week) equivalent to $0.1125 \pm 0.02$ mg/kg.
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The intranasal formulation was administered using micropipette (10 to 100 
µL) attached with low-density polyethylene (LDPE) tubing, having 0.1 mm internal 
diameter at the delivery site (Vyas et al. 2005). While for oral delivery, formulation 
was administered using oral feeding cannula attached to 1mL syringe. The dosing was 
continued for a period of 45 days while the animals were kept at high fat diet. At the 
end of dosing the animals were weighed and sacrificed humanely after collecting 
blood by cardiac puncture. The serum was separated and analyzed for leptin and 
serum lipids. The mass of two abdominal white adipose tissue depots - infrarenal 
white adipose tissue and gonadal white adipose tissue (either periovarian fat in female 
mice or epididymal fat in male mice) were also determined for sacrificed animals 
(Thorburn et al. 2000). The results were recorded in table 7.3.

**Group I:** Control – not receiving any drug but received saline intranasally.

**Group II:** Treated – received PE-mAb-Tfr-SNp intranasally.

**Group III:** Treated – received PE-mAb-Tfr-SNp orally.

*Collection of Serum*

The blood samples were withdrawn from the retro-orbital plexus under light ether 
anesthesia without any anticoagulant and allowed to clot for 10 minutes at room 
temperature and centrifuged at 3000 rpm for 20 minutes. The serum obtained was 
kept for 4°C until use.

7.1.2.2.1 *Estimation of white adipose tissue (WAT) mass*:- The two abdominal white 
adipose tissue depots-infrarenal white adipose tissue and gonadal white adipose tissue 
(either periovarian fat in female mice or epididymal fat in male mice) were removed 
from sacrificed animals, washed with saline, quickly blotted on filter paper and 
weighed. The mass of WAT was expressed as %w/w of total animal body weight.

7.1.2.2.2 *Estimation of serum leptin*:- The serum leptin was estimated as per the 
procedure given in radioimmunoassay kit of Linco, US.

7.1.2.2.3 *Estimation of serum lipid profile*:-

1) Monozyme Liquichem Cholesterol (Total cholesterol and HDL cholesterol 
estimation kit), Monozyme India Ltd, Secunderabad, India.
*Estimation of Serum Total cholesterol (CHOD/POD-Phosphotungstate Method)*

In vitro quantitative determination of total cholesterol in serum was done using Monozyme Liquichem Cholesterol kit (Monozyme India Ltd).

**Principle**

\[
\text{CHE} + \text{H}_2\text{O} \\
\text{Cholesterol ester} \quad \rightarrow \quad \text{Cholesterol} + \text{Free fatty acids}
\]

\[
\text{CHOD} + \text{O}_2 \\
\text{Cholesterol} \quad \rightarrow \quad \text{Cholestene 4 ene 3 one} + \text{H}_2\text{O}_2
\]

\[
\text{H}_2\text{O}_2 + \text{Phenol} \quad \text{POD} \\
+ \quad \rightarrow \quad \text{Red Quinonemine complex} + \text{H}_2\text{O}
\]

4- Aminoantipyrine

**Specimen**

Serum/ heparinised or EDTA plasma.

**Procedure**

Pipette into a centrifuge tube:

- Serum/Plasma \hspace{1cm} 0.2 ml
- Precipitating Reagent \hspace{1cm} 0.3 ml

Mix well and allow to stand at room temperature for 5 minutes. Centrifuge at 3000 rpm for 10 minutes to get a clear supernatant.
### Calculation

\[
\text{Total cholesterol (mg/dL)} = \frac{\text{Absorbance of } T_C}{\text{Absorbance of } S} \times 200
\]

### *Estimation of Serum Triglycerides (GPO Method)*

In vitro quantitative determination of Triglycerides in serum was done using kit (ENZOPAK, Reckon diagnostic Pvt. Ltd.)

**Principle**

Lipase hydrolyses triglycerides sequentially to Di and Monoglycerides and finally to glycerol. Glycerol kinase (GK) using ATP as PO4 source converts glycerol liberated to Glycerol-3-phosphate (G-3- phosphate). G-3-phosphateoxygenase (GPO) oxidizes, G-3-phosphate formed to Dihydroxy acetone phosphate and hydrogen peroxide is formed. Peroxidase (POD) uses the hydrogen peroxide formed, to oxidize 4-Aminoantipyrine ADPS to a purple colored complex, measured at 546 nm or with green filter which is proportional to Triglycerides concentration.

\[
\text{Lipase} \\
\text{Triglycerides} + \text{H}_2\text{O} \rightarrow \text{Glycerol} + \text{fatty acids} \\
\text{GK} \\
\text{Glycerol} \rightarrow \text{Glycerol-3-phosphate} + \text{ADP}
\]
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Fig. 7.1.2. Reaction mechanism of glycerol-3-phosphate oxidase (GPO) and peroxidase (POD)

\[ \text{Glycerol-3-phosphate (GPO)} \rightarrow \text{Dihydroxy acetone phosphate} + \text{H}_2\text{O}_2 \]

\[ \text{H}_2\text{O}_2 + 4\text{-Aminoantipyrine} \quad \text{POD} \]

\[ + \quad \rightarrow \text{Quinoneimine} + \text{H}_2\text{O} \]

\[ \text{ADPS} \]

Procedure

2.5 ml procedure

<table>
<thead>
<tr>
<th></th>
<th>B</th>
<th>S</th>
<th>Test</th>
</tr>
</thead>
<tbody>
<tr>
<td>Working reagent</td>
<td>1.0 ml</td>
<td>1.0 ml</td>
<td>1.0 ml</td>
</tr>
<tr>
<td>Standard</td>
<td>-</td>
<td>20 µl</td>
<td>-</td>
</tr>
<tr>
<td>Sample</td>
<td>-</td>
<td>20 µl</td>
<td></td>
</tr>
</tbody>
</table>

a) Mix well and incubate at 37°C for 15 minutes

Distilled water | 1.5 ml | 1.5 ml | 1.5 ml |

Mix and read the absorbances of standard (S) and Test (T) against reagent Blank (B) at 546 nm.

Calculation

\[ \text{Triglycerides (mg/dL)} = \frac{\text{Absorbance of Test}}{\text{Absorbance of Std}} \times 200 \]

7.1.2.3 Statistical analysis

All data are reported as mean ± SEM, and the difference between the groups were tested using Student’s t test at the level of p<0.05, and differences greater at p<0.05 were considered insignificant.

7.2 EVALUATION OF ANTI-ISCHEMIC EFFECT OF NICERGOLINE

7.2.1 Animals

The most widely used animal models to study neuroprotective effect of drugs in cerebral ischemia are the mice and rat model. Male wistar rats weighing 200-250g were selected for the study on the basis of randomization technique. The animals were housed three per cage. The bedding were changed regularly otherwise the animal are
expected to eat whatever they find for example feces (coprophagy) and even tail and skin of other animals. Animals were fed standard laboratory rat chow and provided water *ad libitum*. 12 hrs before surgery the food was withdrawn but water was allowed *ad libitum*.

### 7.2.2 Methods

#### 7.2.2.1 Induction of transient global ischemia in brain

In accordance to the CPCSEA guidelines, animals were anesthetized with administrations of ketamine (100 mg/kg, I.P.) and diazepam (5 mg/kg, I.M.) respectively, and maintained with additional I.P. doses of ketamine (25 mg/kg) administered as necessary.

Several animal models have been developed in rats, mice and gerbils for the study of ischemic, hypoxic and hypoxic-ischemic brain injury. (Hossmann 1998; Vannucci et al 1999). Global cerebral ischemia in mice was induced by two vessel carotid artery occlusion model. Ligation of bilateral common carotid arteries by silk thread/occlusion of both the arteries by aneurysm clips induce delayed neuronal death in animals (Durukan et al 2007; Candelario-Jalil 2003; Hwang 2006; Gillingwater 2004).

Nicergoline and hydergine have almost similar mechanism of action with nicergoline having added neuroprotectives properties and therefore the neuroprotectives effect was evaluated for nicergoline (Vairetti et al; 2002; Nishio et al 1998). The animals were divided into four groups each consisting three animals. Sham operated/control group animals were treated with saline. The animals were anaesthetized and the rectal temperature monitored with a digital thermometer inserted into the anus, was maintained at 37°C to 38°C throughout the operation by placing the animals under a heat lamp and warming them with a blanket. A midline cervical incision was made and the right common carotid artery was surgically exposed (Yu et al 2005; Liu et al 2001). Taking care not to damage the vagus nerve, the right common carotid artery was isolated using silk thread. After stabilization of 5 minutes, the right common carotid artery was occluded with a sterile stainless steel bulldog clamp for 120 minutes to induce ischemia and was confirmed by visually observing blockage of blood flow. Subsequently, the clamp was removed and reperfusion was confirmed.
was sutured. The animal was allowed to recover and was sprinkled on the wound to prevent occurrence of infection. During the surgery and after the surgery, the animal was kept under halogen lamp to maintain body temperature until it started moving freely.

7.2.2.2 Evaluation of anti-ischemic activity

Thirty minutes before and after initiating transient global ischemia, the anesthetized rats were placed on their backs, and a total of 20μL solution per mice (10μL in each nostril), saline and PE-mAb-Tfr-NNp respectively, was given intranasally using micropipette (1μL to10μL) attached with low-density polyethylene tubing, having 0.1mm internal diameter at the delivery site (Vyas et al 2005). The anti-ischemic effect of NG was evaluated with respect to brain infarct volumes, reduction in brain oedema expressed as the ratio of hemisphere areas and brain glutathione levels.

**Group I:** Served as sham control. No transient global ischemia was induced.

**Group II:** Served as ischemic control group. Transient global ischemia was induced and received saline intranasally.

**Group III:** Drug treated group, received PE-mAb-Tfr-NNp intranasally 30mins after induction of ischemia.

**Group IV:** Drug treated group, received PE-mAb-Tfr-NNp intranasally 30mins before induction of ischemia.

7.2.2.2.1 Histological estimation of brain infarct volume

After 22 hr of reperfusion the animals were euthanised with ketamine (100mg/kg). The brains were quickly removed, washed with ice-cold 0.9% sodium chloride, blotted and freezed at -70⁰C for 4-5 hrs. Immediately the brain was coronally sliced (2-mm-thick) with fresh chromium blade and the slices incubated for 30 min at 37°C with TTC solution. All the slices with the caudal face upward were recorded with a digital camera, the images transferred to a computer (Figure 1) and analyzed using an image analyzer (Image J, NIH, USA). The measured infarct volume (MV), right hemisphere volume (RV) and left hemisphere volume (LV). Volumes were calculated by numerical integration of data from individual slices. Total infarct volume was the sum of the infarct volumes from all of the slices. To correct for the brain oedema in the lesioned, ischemic hemisphere, we determined the infarct volume, expressed as a percentage of the control hemisphere volume, in each rat by the following formula
and tabulated in table 7.4 and shown graphically in figure 7.2 (Yu et al 2005; Liu et al 2001).

Brain edema was evaluated by the ratio of ischemic/non-ischemic hemispheres and tabulated in table 7.4 and shown graphically in figure 7.3.

\[
\text{Infarct volume (\%)} = \frac{[LV - (RV - MV)]}{LV} \times 100
\]

2,3,5-triphenyltetrazolium chloride (TTC) solution (2%w/v) was prepared in phosphate buffer pH 7.4 immediately before use TTC being light sensitive and was masked with aluminum foil till used further.

### 7.2.2.2 Estimation of brain glutathione

Glutathione (GSH) is key factor involved in the detoxification of electrophilic metabolites and reactive oxygen intermediates (Ferrari et al. 1986) and as a co-substrate of glutathione peroxidase, it plays an essential role in protection from free radicals (Adibhatla et al. 2001). Hence, glutathione level in the brain was taken as the index for the antioxidant activity of nicergoline (Sortino et al 1999; Vairetti et al 2004).

GSH in the samples was determined by the method developed by Moran et al (1979). After 22 hr of reperfusion the animals were euthanised with ketamine (100mg/kg). The brains were quickly removed and homogenized. To 1mL brain homogenate 1mL trichloroacetic acid solution (chilled) was added and centrifuged at 2500rpm for 10min. To 0.5mL of resulting supernatant 4mL DTNB solution and 1.5mL phosphate buffer were added and the absorbance recorded at 412nm. To prepare blank instead of tissue homogenate distilled water was taken and was given the same treatment as that of tissue homogenate samples. The GSH level was then estimated using formula given below and then expressed in micrograms per gram of brain tissue and tabulated in table 7.4 and shown graphically in figure 7.4:-

\[
x = \frac{(y - 0.0046)}{0.0034}
\]

Where, \( x \) = GSH level in \( \mu g/mL \)

\( y \) = absorbance.

1. \( 2 \text{GSH} + \text{DTNB} \longrightarrow \text{GSSG} + 2 \text{TNB} \)

Glutathione
The combined reaction:

\[
\text{Glutathione reductase} \\
\text{DTNB} + \text{H}^+ + \text{NADPH} \rightarrow 2 \text{TNB} + \text{NADP}^+ \\
\text{GSSG/GSH}
\]

*Trichloroacetic acid solution (10%w/v):* Dissolve 10g trichloroacetic acid in 80 ml distilled water and make up the volume upto 100 ml with distilled water.

*DTNB (dithio-bis-nitrobenzene) solution (0.6 mM):* Dissolve 60 mg of DTNB in 100 ml of 1%w/v sodium citrate solution.

*Phosphate buffer (0.2 M, pH 8):* Mix 25ml of 2.72%w/v solution of potassium dihydrogen phosphate with 23ml of 0.8%w/v solution of sodium hydroxide and make up the final volume upto 100ml with distilled water.

*Method to prepare brain homogenate*

Brain (whole) was isolated carefully in ice-cold saline. The organ was then blotted and weighed (wet mass) and suspended in 0.25M sucrose solution (chilled) and quickly blotted on a filter paper. The tissue was then minced and homogenized in 10 volumes of chilled 10mM Tris-hydrochloride buffer with 20 strokes of Teflon pestle in glass homogenizer at a speed of 2500rpm. The homogenate was then centrifuged at 10,000rpm at 4C for 15 min using Sigma centrifuge. The clear supernatant was used for the estimations.

*Sucrose solution (0.25M):* 85.58gm of sucrose was dissolved in 200ml distilled water and diluted to 1000ml with distilled water.

*Tris-hydrochloride buffer (10mM, pH 7.4):* 1.21gm Tris was dissolved in 900ml distilled water and the pH adjusted to 7.4 with 1M hydrochloric acid. The resulting solution was diluted to 1000ml with distilled water.
The data are presented ± SEM, and differences between the groups were analyzed using one-way analysis of variance (ANOVA) followed by Bonferroni’s post-test for multiple means comparison or Student’s t test. Differences greater at \( P < 0.05 \) were considered insignificant.

### 7.3 RESULTS

#### Table 7.1 Composition of high fat diet given to animals to induce obesity

<table>
<thead>
<tr>
<th>Sr.no.</th>
<th>Ingredient</th>
<th>Content</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Cholesterol</td>
<td>0.5 %w/w</td>
</tr>
<tr>
<td>2</td>
<td>Butter</td>
<td>10 %w/w</td>
</tr>
<tr>
<td>3</td>
<td>Coconut oil</td>
<td>10 %w/w</td>
</tr>
<tr>
<td>4</td>
<td>Sodium cholate</td>
<td>0.1 %w/w</td>
</tr>
<tr>
<td>5</td>
<td>Grounded pellet rodent chow</td>
<td>79.4 %w/w</td>
</tr>
</tbody>
</table>

#### Table 7.2: Obesity induction: weight and serum lipid profile of animals at initiation and end (10 weeks) of high fat diet feeding period

<table>
<thead>
<tr>
<th>Sr.no.</th>
<th>Control (Initial)</th>
<th>Control (Final)</th>
<th>HFD treated (Initial)</th>
<th>HFD treated (Final)</th>
</tr>
</thead>
<tbody>
<tr>
<td>n</td>
<td>5</td>
<td>5</td>
<td>18</td>
<td>18</td>
</tr>
<tr>
<td>Body weight (g)</td>
<td>380.3 ± 18.2</td>
<td>394.2 ± 13.7</td>
<td>392.1 ± 15.6</td>
<td>430.5 ± 12.9*</td>
</tr>
<tr>
<td>Total cholesterol (mg/dL)</td>
<td>67.5 ± 3.1</td>
<td>69.3 ± 2.8</td>
<td>66.7 ± 2.3</td>
<td>92.3 ± 4.8*</td>
</tr>
<tr>
<td>Triglycerides (mg/dL)</td>
<td>101.4 ± 1.4</td>
<td>104.2 ± 1.9</td>
<td>100.9 ± 2.1</td>
<td>123.2 ± 12.6*</td>
</tr>
<tr>
<td>HDL (mg/dL)</td>
<td>28.3 ± 1.9</td>
<td>30.3 ± 1.3</td>
<td>27.9 ± 1.8</td>
<td>24.1 ± 3.3*</td>
</tr>
<tr>
<td>VLDL (mg/dL)</td>
<td>19.6 ± 1.2</td>
<td>21.4 ± 1.8</td>
<td>19.2 ± 1.2</td>
<td>25.2 ± 2.5*</td>
</tr>
<tr>
<td>LDL (mg/dL)</td>
<td>20.7 ± 3.5</td>
<td>22.2 ± 2.92</td>
<td>21.3 ± 1.4</td>
<td>42.3 ± 5.1*</td>
</tr>
</tbody>
</table>

Values are expressed as mean ± SEM

** Indicates that the variation between the control and HFD fed groups are significant (\( P < 0.05 \)).

#### Table 7.3: Anti-obesity activity: body weight, serum lipids, serum leptin and white adipose tissue mass of animals at the end of drug treatment period (45 days)

<table>
<thead>
<tr>
<th>Sr.no.</th>
<th>Control (Group I)</th>
<th>Treated (Groups II and III)</th>
</tr>
</thead>
<tbody>
<tr>
<td>n</td>
<td>5</td>
<td>5</td>
</tr>
<tr>
<td>Body weight (gm)</td>
<td>450 ± 15.3</td>
<td>413.5 ± 13.5*</td>
</tr>
<tr>
<td>Total cholesterol (mg/dL)</td>
<td>91.5 ± 4.7</td>
<td>72.7 ± 5.2*</td>
</tr>
<tr>
<td>Triglycerides (mg/dL)</td>
<td>110.1 ± 6.7</td>
<td>90.4 ± 5.1*</td>
</tr>
</tbody>
</table>
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<table>
<thead>
<tr>
<th>Serum leptin (µg/mL)</th>
<th>4.1 ± 0.6</th>
<th>2.65 ± 0.3*</th>
<th>2.65 ± 0.3*</th>
<th>3.74 ± 0.4*</th>
</tr>
</thead>
<tbody>
<tr>
<td>WAT (%w/w)</td>
<td>13.9 ± 3.8</td>
<td>7.6 ± 2.5*</td>
<td>45.32 ± 2.7</td>
<td>9.5 ± 3.3*</td>
</tr>
</tbody>
</table>

Values are expressed as mean ± SEM

- *' Indicates that the value of the treated group is x% less compared to the control group i.e. ‘%decrease’.
- ‘+’ Indicates that the value of the treated group is x% more compared to the control group i.e. ‘%increase’.
- ‘**’ Indicates that the variation between the treated and control groups are significant (P<0.05).
- ‘***’ Indicates that the variation between the intranasally treated (Group II) and orally treated (Group III) groups are significant (P<0.05).

Table 7.4: Brain infarct volume, ratio of hemisphere areas, glutathione estimated for anti-ischemic activity

<table>
<thead>
<tr>
<th>Sr. no.</th>
<th>Sham control (Group I)</th>
<th>Control (ischemic, Group II)</th>
<th>Treated PE-mAb-Tfr-NNp (i.n.; Post-treated, Group III)</th>
<th>Treated PE-mAb-Tfr-NNp (i.n.; Pre-treated, Group IV)</th>
</tr>
</thead>
<tbody>
<tr>
<td>n</td>
<td>3</td>
<td>3</td>
<td>3</td>
<td>3</td>
</tr>
<tr>
<td>Infarct volume (mm³)</td>
<td>---</td>
<td>25.2 ± 1.04*</td>
<td>8.7 ± 1.12**</td>
<td>22.3 ± 1.26</td>
</tr>
<tr>
<td>Ratio of hemisphere areas</td>
<td>0.99 ± 0.09</td>
<td>1.14 ± 0.07*</td>
<td>1.03 ± 0.08**</td>
<td>1.09 ± 0.06</td>
</tr>
<tr>
<td>Brain GSH (µg/g of tissue)</td>
<td>111.4 ± 4.7</td>
<td>86.9 ± 3.9*</td>
<td>99.5 ± 4.2**</td>
<td>91.1 ± 5.3</td>
</tr>
</tbody>
</table>

Values are expressed as mean ± SEM

- ‘**’ Indicates that the variation between group II and group I are significant (P< 0.05).
- ‘***’ Indicates that the variation between group II and groups III and IV are significant (P<0.05).

Figure 7.1: TTC stained brain slices

A Sham control, B Ischemic control, C NG post-treated group, D NG pre-treated group
7.4 DISCUSSION

Obesity was induced in the animals by feeding them high fat diet for 10 weeks and was confirmed by estimating serum lipids at the initiation and end of 10 weeks and
The observations are tabulated in table 7.2. The results tabulated in table 7.3 demonstrate the comparative anti-obesity activity of SB with respect to body weight, serum lipids, leptin and WAT mass following intranasal and oral drug administrations. The differences between the control and drug treated groups were found to be significant (P<0.05). However, more marked results were observed with intranasal drug treatment than oral and can be attributed to the significantly improved brain uptake of drug SB following intranasal administration of brain selective antibody conjugated SB nanoparticles. Thus, intranasal treatment with SB was found to be more effective for the management of obesity than oral administration when the drug is a centrally acting molecule.

Intravenous delivery has limited utility in managing ischemic stroke due to the presence of BBB and restriction of blood supply to the brain due to thrombus or embolism limiting drug ingress effectively into the brain illustrating intranasal route to be advantageous in such circumstances. Significantly high brain GSH levels were observed following treatment with NG 30min after induction of transient global ischemia compared to ischemic control while the difference between the pre-treated group and ischemic control was insignificant. Also, the infarct volume and brain oedema were found to be significantly less for post-treated group than pre-treated or ischemic control group. Also, the pretreated group demonstrated less infarct volume and brain oedema and higher GSH levels than the ischemic control illustrating a possibility of NG to be advantageous if taken prophylactically. These demonstrate the neuroprotective effect of nicergoline in cerebral ischemia with intranasal delivery enhancing their magnitude to many times by virtue of selective and effective delivery of NG to the brain following intranasal administration of antibody conjugated nicergoline nanoparticles. The neuroprotectives effect of NG is attributed to its multiple mechanisms of action viz. antioxidant activity, antiplatelet activity and increasing cellular ATP in brain.

7.5 CONCLUSION

The above results conclusively demonstrate intranasal drug delivery a rational strategy to manage debilitating brain disorder like cerebral ischemia and related obesity delivering drugs quickly and in higher concentrations to the target tissue.
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Moran MS, Depierre JW, Mannervik B. Levels of glutathione, glutathione reductase and glutathione S-transferase activities in rat lung and liver. *Biochim Biophys Acta.*, 1979, 582(1), 67-78.


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