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# Brain Targeted Delivery of Amino Acid Conjugates of Dopamine

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**In the present study dopamine was conjugated with amino acids, glycine, alanine, phenylalanine, and valine, with a view to enhance bioavailability by increasing permeability to brain, lower the rate of clearance by reducing metabolism and eliminate undesirable effects. These conjugates were synthesized and characterized by infrared spectroscopy. The partition coefficient of dopamine and dopamine conjugates was determined in n-octanol and phosphate buffer saline pH 7.4 and the conjugates showed 7 to 10 fold increase in their partition coefficients. Moreover, these conjugates exhibited less protein binding as compared to dopamine and *in vitro* hydrolysis study revealed that the conjugates have long duration of action because of slow hydrolysis. The reduction in the degree of chlorpromazine-induced catatonia was significantly increased as compared with plain dopamine and combination of levodopa and carbidopa.**

Chemical conjugated brain specific drug delivery systems represent a rational drug design approach that is exploited to delivery of the drug to the target site of action (brain)[1-3] as well as reduces the toxicity and increase the treatment efficacy. As it is known that drug delivery to brain is quite difficult due to the presence of the blood brain barrier (BBB), which is formed by tight junction within the capillary endothelium of the vertebrate brain[4]. Because of the presence of BBB physicochemical properties of drugs, i.e. lipophilicity and molecular weight, determine extent to which drugs can across the BBB[5].

Parkinson's disease is a progressive neuro-degenerative disorder, which is attributed primarily to depletion of striatal dopamine in the basal ganglia as a result of the loss of neurons in the substantia nigra. Striatal dopamine deficiency results in loss of the normal functional balance between dopaminergic and cholinergic activity and therapeutic treatment aims to increase the former or decrease latter[6]. Deficiency of the neurotransmitter dopamine, which is produced by the brain, cannot be corrected by orally administered dopamine primarily because it is not orally effective, it does not cross BBB and it has very short half-life of about two min. The absorption of dopamine can be increased by increasing its lipophilicity[7,8]. Dopamine has been successfully administered to brain by encapsulating it into the liposomes[9]. Amino acid conjugation of the dopamine enhances brain uptake because of its affinity for the neutral amino acid transport to the brain[10].

Levodopa, a precursor of dopamine is given to restore the deficiency of dopamine and the carboxyl and b-amino group of levodopa allows it to compete for transport across the BBB by the large neutral amino acid carrier[11-13]. Levodopa is metabolised peripherally and only 5% of levodopa may reach intact into the brain. Therefore, large amount of drug is given to maintain effective drug concentration in the brain or combined with aromatic-L-amino acid-decarboxylase (AADC) inhibitor, such as carbidopa[14,15]. Schematic representation of the metabolism of DOPA is shown in fig.1.

Therefore, complementary strategies have developed to compensate these problems. The aim of the present study is to synthesize prodrug of dopamine using carbodiimide

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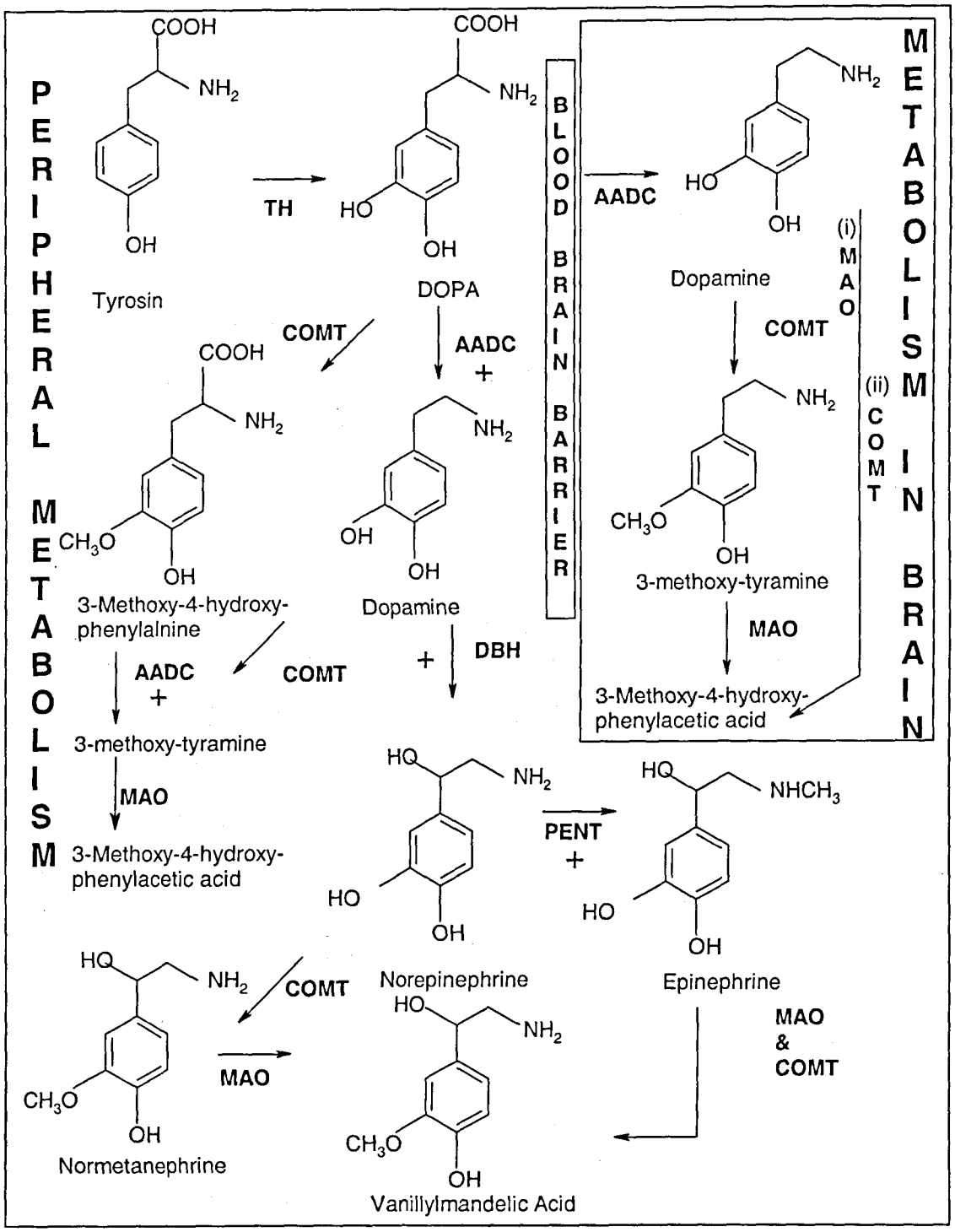


+ Metabolic steps not likely to act on prepared prodrug (Cojugates of dopamine and amino acids)

**Fig. 1: Biosynthesis and metabolism of levodopa and dopamine**

**AADC-Aromatic-L-amino acid decarboxylase, COMT-Catechol ortho methyl transferase, MAO- mono amine oxidase, TH-Tyrosine hydroxylase, DBH-dopamine beta hydroxylase and PENT- Phenylethanolamine-N-methyltrasferase**

chemistry with the objectives: nutrient like pharmacokinetic behaviour for oral absorption; enough lipophilic character for transport of drug into the brain; as well as should remains unionised form at physiological pH. Dopamine was conjugated with alanine, glycine, valine and phenylalanine, which transport the drug to the brain via amino acid transport system[16-18]. Furthermore, the delivery of such conjugates was hypothesized on the assumption of the release of drug in brain, as the hydrolytic metabolism is well known to occur, although, with a slight resistance in case of amides. As in previous studies by Bundgaard, plasma enzyme-catalyzed hydrolysis of N-acetyl-5-fluorouracil and N-1- ethoxy carbonyl-2-fluorouracil has been reported[19]. If the amide formation is done with amino acids, serum transpeptisases are known to hydrolyse the amino acid conjugates. This type of hydrolysis is particularly fast with L-glutamic acid, therefore, conjugation with L-glutamic acid was avoided. Amide bonds undergo hydrolysis in the serum, as reported in the literature[20] and prodrugs can undergo such type of hydrolysis in serum. Therefore, the dopamine conjugates will cross BBB by virtue of receptor-mediated endocytosis or by passive diffusion due to possible increased lipophilicity and manyfold decrease in metabolism by major enzymes COMT and AADC and deliver the drug to the brain.

## MATERIALS AND METHODS

Dopamine HCl, tert-butyl alcohol (t-BuOH), d-tertbutylcarbonate, acetonitrile, N,N-dicyclohexylcarbodiimide (DCC), glycine, alanine, phenylalanine, and valine were obtained from Spectrochem Pvt. Ltd. Mumbai, and all other reagents and solvents used were of analytical grade.

### Preparation of dopamine conjugates with amino acids:

Dopamine was conjugated with glycine, alanine, phenylalanine, and valine as per the method reported for the preparation of peptide via activation provided by carbodiimides, generally dicyclohexylcarbodiimide[21,22]. The process of synthesis is shown schematically in fig. 2.

Amino acid (0.01 M) was suspended in a 2:3 mixture of t- BuOH and water. The pH was then adjusted to 13 by drop-wise addition of aqueous solution of Sodium hydroxide (8 M). Then, di-tert-butyl carbonate (0.015 M) suspended in t-BuOH, was added to it at room temperature. The pH was adjusted to 11-12 and the reaction mixture was stirred for an hour following its dilution with water, then the pH was dropped down to 3 by adding citric acid. The organic phase was extracted with ethyl acetate after drying with anhydrous magnesium sulphate. The organic layer was separated and

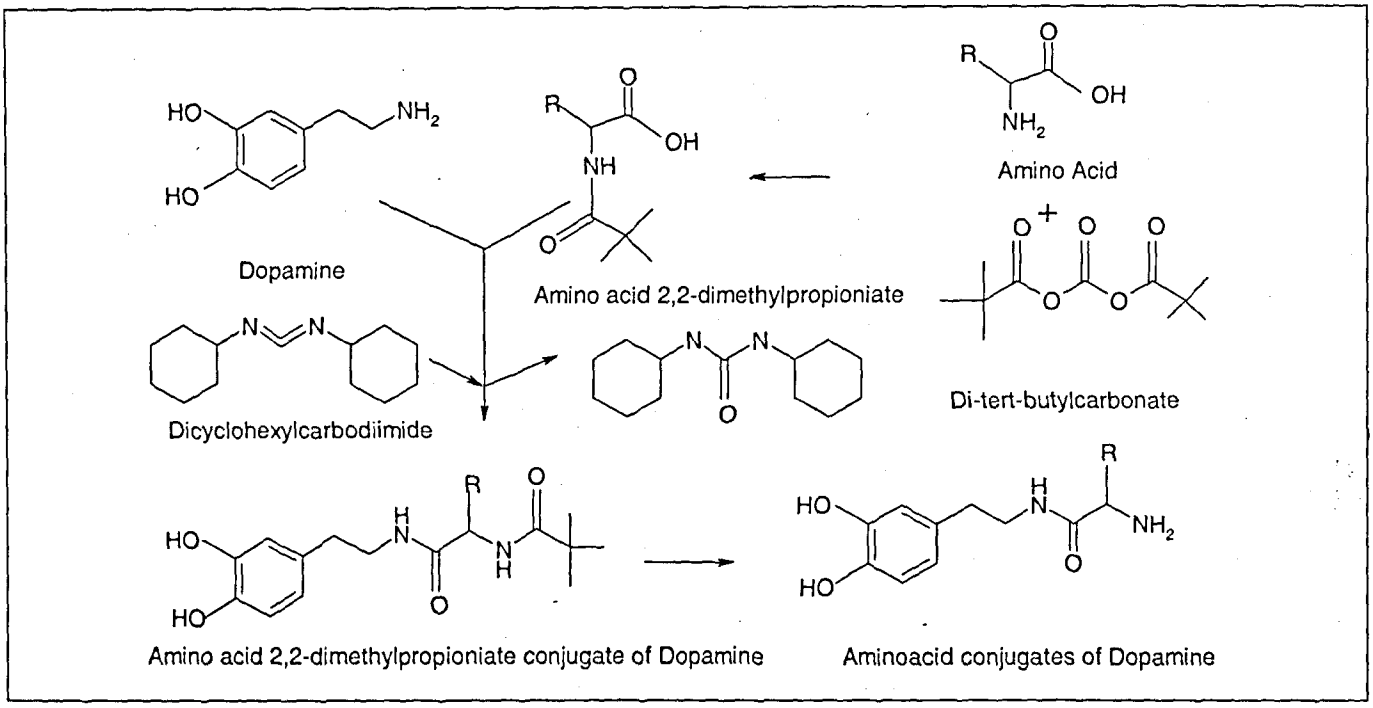


**Fig. 2: Schematic representation of preparation of amino acid conjugates of dopamine**

evaporated; the residue was taken in acetonitrile and filtered. The product was recrystallised from ethyl acetate-petroleum ether and dried. In next step, the syntheses of amide conjugates with amino acid and 2,2 dimethylpropionates of dopamine was taken up by adding DCC (0.01 M) to a pyridine solution containing dopamine HCl (0.01 M) and amino acid 2,2 dimethylpropionates at 0°. The reaction mixture was stirred at room temperature for 24 h and the formed dicyclohexylurea was removed by filtration and the residue was dried over phosphorus pentaoxide. The product was recrystallised from 2-propanol and dried at 100°. The protecting group (2,2-dimethylpropionate) was removed by suspending the end product of the previous step in 3% HCl in methanol and washed with cold water and dried at 100°.

Dopamine was conjugated with glycine, alanine, phenylalanine, and valine to synthesize dopaminylglycinate [compound DA-1: a-(3,4-dihydroxyphenyl)-b-(aminoacetamido) ethane], dopaminylalaninate [compound DA-2: a-(3,4-dihydroxyphenyl)-b-(2aminopropionamido) ethane], dopaminylphenylalanate [compound DA-3: a-(3,4-dihydroxyphenyl)-b-(2-amino-3-phenylpropionamido) ethane], dopaminylvalinate [compound DA-4: a-(3,4-dihydroxyphenyl)-b-(2-amino-3-methylbutanamido) ethane], respectively.

**Characterizations of the conjugates:**

The purity of the conjugates was ascertained by thin layer chromatography using silica gel-G as adsorbents and a mixture of n-butanol: acetic acid: water (4:1:1) as solvent. These compounds were scanned for their absorbance in phosphate buffer saline (PBS) pH 7.4 by using Shimadzu UV 1601 spectrophotometer.

**Physical properties:**

All the conjugates were found to occur as white crystalline solid. Their melting points and solubility studies were performed in different solvents with the help of reported method[23]. The melting point of synthesized conjugates was determined using melting point apparatus (Tempo, Mumbai). The solubility of the synthesized compounds was determined by shaking (2 mg) compound in different solvents (5 ml) at room temperature for 24 h and filtered. The filtrate was analysed spectrophotomertically by using Shimadzu UV 1601 spectrophotometer at corresponding maxima (Table 1, 2)

**Characterization of synthesised conjugates:**

All the synthesized conjugates were analysed for % nitrogen content by using nitrogen Analyzer (Heracus Carlo Erba 1108). The results are shown in Table 2. Infrared spectra of the synthesized compounds were recorded in potassium bromide pellets using an IR spectrophotometer (Shimadzu, Japan). The spectra were interpreted with the help of reported literature values of different type of molecular movement's characteristics to the present functional groups[24,25].

**Partition coefficient:**

For determination of partition coefficients the drug and

TABLE 1: MOLECULAR FORMULAE, YIELD, MELTING POINT AND UV ABSORPTION MAXIMA OF THE CONJUGATES.

| Compds | R<br>Yield (%) | Molecular formula | Molecular Weight<br>mp (°) | Elemental composition<br>Calculated (%) | <br>Found (%) | UV<br>$\lambda_{max}$ | $R_f$<br>Value* |
|---|---|---|---|---|---|---|---|
| DA-1 | -H<br>71 | $C_{10}H_{14}N_2O_3$ | 210.23<br>218-220 | N-13.32 | N-13.52 | 272.5 | 0.44 |
| DA-2 | $-CH_3$<br>76 | $C_{11}H_{16}N_2O_3$ | 224.26<br>212-214 | N-12.49 | N-12.32 | 269.0 | 0.42 |
| DA-3 | $-CH_2-C_6H_5$<br>72 | $C_{19}H_{20}N_2O_3$ | 300.36<br>204-205 | N-9.33 | N-9.25 | 289.5 | 0.39 |
| DA-4 | $-CH(CH_3)_2$<br>74 | $C_{13}H_{20}N_2O_3$ | 252.32<br>209-210 | N-11.10 | N-11.54 | 262.5 | 0.41 |

*Thin layer chromatography: adsorbent silica gel G, solvent system: n-butanol:acetic acid: water (4:1:1)

TABLE 2: SOLUBILITY AND IR SPECTRAL ABSORPTION BANDS OF THE CONJUGATES

| Compound | Solubility | | IR Spectral absorption bands ($cm^{-1}$) |
|---|---|---|---|
| | Solvent | Solubility | |
| DA-1 | Chloroform | +++ | 3515, 3325-3400, 3260, 3025, |
| | Acetone | +++ | 2925, 1620, 1580, 1540, |
| | Alcohol | ++ | 1100-1500, 1250, 1080, 650 |
| | Ether | ++ | |
| | Benzene | ++ | |
| | Water | + | |
| DA-2 | Chloroform | +++ | 3510, 3330-3400, 3280, 3030, |
| | Acetone | ++ | 2920, 1620, 1580, 1540, |
| | Alcohol | +++ | 1100-1500,1250,1100, 650 |
| | Ether | +++ | |
| | Benzene | ++ | |
| | Water | - | |
| DA-3 | Chloroform | ++ | 3520, 3310-3400, 3270, 3045, |
| | Acetone | +++ | 2925, 1710, 1620,1580, 1530, |
| | Alcohol | +++ | 1100-1500,1250,1080, 710, 650 |
| | Ether | ++ | |
| | Benzene | + | |
| | Water | + | |
| DA-4 | Chloroform | +++ | 3520, 3325-3400, 3270, 3240, 2925, |
| | Acetone | ++ | 1710, 1620, 1580, 1520, |
| | Alcohol | +++ | 1100-1500,1260, 1080, 630 |
| | Ether | ++ | |
| | Benzene | ++ | |
| | Water | - | |

+++ freely soluble, ++ soluble, + slightly soluble, - practically insoluble.

synthesized compounds (10 mg) were taken in glass-stoppered tubes containing equal volumes (10 ml) of n-octanol and PBS pH 7.4. The tubes were shaken for 6 h on wrist action shaker. After 24 h, aqueous phase was assayed for the concentration using a Shimadzu UV 1601 spectrophotometer at corresponding absorption maxima.

***In vitro* drug hydrolysis:**

The *in vitro* drug hydrolysis of the synthesized compounds was determined using diffusion cell containing donor and receiver compartment separated by treated cellophane membrane[25]. The compound equivalent to 40 mg of dopamine was placed in the donor compartment and PBS pH 7.4 (50 ml) was kept in the receiver compartment

maintained at 37±1° using circulating water bath. Samples (1 ml) were withdrawn periodically for 5 h after 1 h interval from the receiver compartment and replaced immediately with the same amount of fresh PBS to maintain the constant volume. These samples were assayed spectrophotomertically for drug concentration.

**Protein binding studies:**

The protein binding of synthesized conjugates was determined using the dialysis tube (MWCO 3500). Dialysis tube containing 6% egg albumin solution and dipped into the drug solution contained in a beaker. The system was thermostatically maintained at 37±0.5° and stirred at 100 rpm using magnetic stirrer. Sample (1 ml) was withdrawn periodically for 5 h after 1 h interval and analyzed using Shimadzu UV spectrophotometer 1601 for the quantitation of protein bound drug.

***In vivo* studies:**

The *in vivo* performance of each of the compounds was carried out by assessing the reduction in the degree of chlorpromazine-induced catatonia (rigidity, akinesia) in the Wistar rat[24] compared to plain levodopa and dopamine. In this method, antiparkinson's drug was administered intraperitonially followed 30 min later by chlorpromazine administration to induce extra pyramidal effects. The experimental protocols have been approved by the Institutional Animals Ethics Committee.

Rats (200-300 g) were divided equally into eight groups and kept fasted for 12 h before injecting the compounds. The animals of first group were kept as control (without antiparkinson's drug administrations) while plain dopamine solution (10 mg/kg), plain levodopa solution (10 mg/kg), and marketed levodopa preparation syndopa (levodopa+carbidopa, 2.5 mg/kg) were injected intraperitonially to animals of second, third and fourth groups, respectively. The animals of fifth, sixth, seventh and eighth groups were given compound DA-1, compound DA-2, compound DA-3, and compound DA-4 (equivalent to10 mg/kg dopamine) intraperitonially, respectively. Thirty min after administrating the drug conjugates, all the animals were injected chlorpromazine (5 mg/kg) intraperitonially to induce catatonia.

The reduction in the degree of catatonic (difficulty to move and change the posture) assessed by placing the front paw of the rat on 9 cm and 3 cm high block alternatively and scored according to observations (Table 3). For single rat, the maximum possible score (3.5) revealed total catatonia. The onset and severity of catatonia response in all groups were compared by observing the catatonia at 0, 15, 30, 45, 60, 90, 120, 150 and 180 min[27].

## RESULTS AND DISCUSSION

Dopamine was conjugated with various amino acids such as glycine, alanine, phenylalanine, and valine, and the yield, melting point and UV absorption maxima of the conjugates were determined (Table 1). Solubilities and IR spectral absorption bands of the conjugates are given in Table 2. The IR spectral peaks of these conjugates were in conformity with the expected values from the synthesis. The solubility studies showed that the synthetic conjugates were found either insoluble or slightly soluble in water but completely soluble in the organic solvents which is due to fact that synthesized compound is lipophilic in nature. Partition coefficient studies are important in prediction of absorption and distribution of drug throughout the body. The partition coefficients of synthesized conjugates are higher than the parent drug, which indicate that the prepared conjugates are 2-10 fold more lipophilic than parent (Table 4). Absorption and distribution of drug may increase at the

TABLE 3: SCORES AWARDED FOR DEGREE OF REDUCTION IN CHLORPROMAZINE-INDUCED CATATONIA IN WISTAR RATS

| Stage | Observations | Score |
|---|---|---|
| I | Rat moves normally when placed in the table | 0.0 |
| II | Rat does not move when touched or touched or pushed also, | 0.5 |
| III | Rat placed on the table with front paw's set alternatively on 3 cm high block if failed to correct the posture in 10 seconds | 1.0 |
| IV | Rat placed on the table with front paw's set alternatively on a 9 cm high block if failed to correct the posture in 10 seconds | 2.0 |

TABLE 4: PARTITION COEFFICIENT AND PLASMA PROTEIN BINDING OF SYNTHESIZED COMPOUNDS.

| Compounds | Partition coefficient | Fold increase in Partition coefficient | Plasma protein binding (%) | Decrease in Plasma protein Binding (%) | % Hydrolysis of compounds in 4.5 h in | |
|---|---|---|---|---|---|---|
| | | | | | PBS pH 7.4 | 10% plasma in PBS pH 7.4 |
| Dopamine HCl | 0.20±0.01 | - | 32.98±1.42 | - | - | - |
| DA-1 | 1.44±0.06 | 7.20 | 32.54±1.25 | 1.33±0.04 | 19.39±0.89 | 36.42±1.65 |
| DA-2 | 1.78±0.05 | 8.90 | 31.75±1.38 | 3.73±0.11 | 16.32±0.76 | 32.24±1.31 |
| DA-3 | 2.03±0.10 | 10.15 | 30.89±1.21 | 6.34±0.28 | 18.21±0.89 | 35.21±1.42 |
| DA-4 | 1.94±0.08 | 9.70 | 31.02±1.26 | 5.94±0.21 | 17.58±0.71 | 33.39±1.62 |

site of action by blood brain barrier due to lipophilicity of synthetic compounds. The partition coefficients of the conjugates were found to be in the order DA3>DA4>DA2>DA1> dopamine HCl. Protein binding affects the pharmacokinetic and pharmacodynamics properties of drugs. Plasma protein binding of each of the synthesized conjugates was found to be less (1.3-7.3%) as compared to parent drug (Table 4). The plasma protein binding of the conjugates was found to be in the order of dopamine HCl>DA1>DA2>DA4>DA3. The combined effect of the parameters must have certainly increased availability of the drug in brain, which is also supported by following *in vivo* studies. Our hypothesis of hydrolyses of the conjugates in brain is supported by the performed *in vivo* studies.

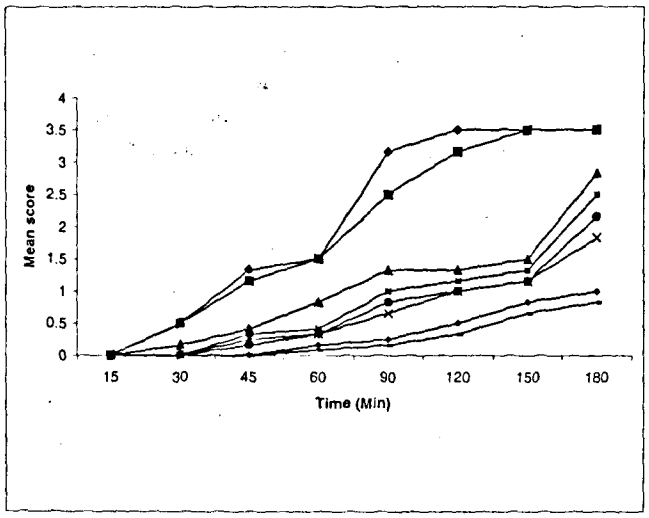


**Fig. 3: Mean score of reduction of catatonia in different time interval**

**Animals were intraperitonially administered with chlorpromazine HCL (-◆-), dopamine HCL (-■-), Levodopa (-▲-), Syndopa (-x-), DA-1 (-■-), DA-2 (-●-), DA-3 ( -♦-) and DA-4 (-_-).**

The *in vitro* hydrolysis of synthesized conjugates over varying time interval in PBS and PBS containing 10% plasma was determined (Table 4). *In vitro* hydrolysis study showed that the synthesized conjugates have slow rate of hydrolysis. Therefore, each of the synthesized conjugates should possess longer circulation half-life in biological fluid, which is also supported by *in vivo* studies. The order of the percent hydrolysis of the compounds in PBS pH 7.4 was found to be DA1 (19.39±0.89)>DA3 (18.21±0.89)>DA4 (17.58±0.71)>DA2 (16.32±0.76) and the percent hydrolysis of the compounds in PBS pH 7.4 containing 10% plasma was found to be DA1 (36.42±1.65)>DA3 (35.21±1.42)>DA4 (33.39±1.62)>DA2 (32.24±1.31) (Table 4).

*In vivo* assessment of the reduction in the degree of drug-induced catatonia was also carried out for the parent as well as synthesized conjugates. The results revealed that group of animals with plain dopamine showed negligible reduction in the degree of drug-induced catatonia upon intraperitoneal administration, which is due to decomposition of drug and non-permeability of drug to BBB whereas the synthesized conjugates showed almost complete abolition of drug induced catatonia (fig. 3).

In conclusion, it can be stated that the antiparkinson's activity of dopamine is effectively achieved by dopamine conjugated with amino acids which could be due to the: (i) high partition coefficient of the compounds indicating an increase in lipophilicity which in turn improve the ability of the drug to cross BBB, (ii) hydrolyses of the conjugates in brain to dopamine and corresponding amino acid and (iii) significant slow rate of hydrolysis and slightly less plasma protein binding of the compounds indicates longer durations

of action. Additionally dopamine conjugated with amino acid could be transferred across the BBB via receptor mediated transport systems for amino acids present in the BBB. However, this receptor-mediated endocytosis of the synthesized conjugates is to be established.


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