Innovare Academic Sciences

International Journal of Pharmacy and Pharmaceutical Sciences

ISSN- 0975-1491 Vol 7, Issue 11, 2015

Original Article

EVALUATION OF REPEATED DOSE-90-DAY ORAL TOXICITY STUDY OF L-DOPA AND HYOSCINE HYDRBROMIDE COMBINATION IN RATS

SABIR HUSAIN ATTAR, DHARMENDRA KUMAR KHATRI, JAYANT SANCHETI, ARCHANA RAMESH JUVEKAR*

Pharmacology Research Laboratory-1, Department of Pharmaceutical Sciences and Technology (DPST), Institute of Chemical Technology, N. P. Marg, Matunga, Mumbai 400019, India
Email: juvekar.archana@gmail.com

Received: 18 Jun 2015 Revised and Accepted: 03 Oct 2015

ABSTRACT

Objective: The present study was done to evaluate repeated-dose 90-day oral toxicity studies of l-dopa and hyoscine hydrbromide combination in rats.

Methods: Repeated-dose 90-day oral toxicity study was performed according to the Organisation for Economic Co-operation and Development (OECD) guidelines 408. In the present study, combination of L-dopa and hyoscine hydrobromide was administered at 5 times the upper limit of therapeutic dose of each drug which is 1200 mg per day for L-dopa and 0.75 mg per day for hyoscine hydrobromide for adult human being and which was converted to required dose for Wistar rats (5 males and 5 females).

Results: The combination of L-dopa and hyoscine hydrobromide at 5 times the upper therapeutic dose produced no treatment-related signs of toxicity or mortality in any of the animals tested during 90 d of the study. The subchronic administration of the combination of L-dopa and hyoscine hydrobromide did not produce any significant difference in any of the assigned parameters between the control and all treatment groups.

Conclusion: It is established that the combination of L-dopa and hyoscine hydrobromide therapy is safe on repeated dose 90-day oral toxicity at 5 times the upper limit of therapeutics dose of each drug.

Keywords: Parkinson's, Toxicity, L-dopa, Hyoscine hydrobromide.

INTRODUCTION

Parkinson's disease (PD) is primarily a dopamine (DA) deficiency progressive neurodegenerative disease in which there is extensive loss of nigrostriatal dopamine-containing neurons in the substantia nigra (SN) and this is the main cause of pathological change [1, 2] and the deposition of α-synuclein intracellularly throughout the nervous system [3]. This is characterized by muscle rigidity, tremors, bradykinesia, and postural abnormalities due to loss of dopamine [5]. Since dopamine cannot cross the blood–brain barrier, L-3, 4-dihydroxyphenylalanine (L-dopa), It's natural and direct precursor is the most effective and widely used gold standard medication for PD. L-DOPA was first used in clinical trials in 1967 wherein it was reported to result in the therapeutic success in the treatment of PD and a number of DA receptor agonists have been introduced for the treatment of PD [4-6].

On the other hand, L-DOPA therapy develops tolerance in Parkinson's patients on long term use resulting in the need for increased doses over time [7]. Eventually the drug raises various side effects particularly motor complications including L-DOPA-induced dyskinesias (LID) [8], motor fluctuations and the wearing off phenomenon [9]. This motor control fluctuation can be controlled by administering antimuscarinic drugs such as benztropine, scopolamine which act by blocking the excitatory cholinergic neurons in the neo striatum thereby assisting in establishing correct dopamine/acetylcholine balance. Scopolamine has the additional advantage of reducing nausea and vomiting, a prominent side effect of Levodopa [10].

Hyoscine hydrobromide, an antimuscarinic alkaloid has the strongest pharmacological effect, can be used to block the parasympathetic nerve. Hyoscine hydrobromide has been used for stiff and tremor symptom of parkinsonian syndrome; however, antimuscarinics generally have been replaced with dopaminergic drugs [11].

Despite their widespread use, little toxicological data is available regarding the safety of repeated use of the combination of L-dopa and hyoscine hydrobromide. Available data are insufficient to support the safety of the combination of L-dopa and hyoscine hydrobromide by oral route. The use of the combination of L-dopa and hyoscine hydrobromide by oral route in humans needs a safety evaluation. As part of a safety evaluation of combination of L-dopa and hyoscine hydrobrmide, a toxicological study was thus carried out to investigate its potential toxicity after repeated dose 90-day oral toxicity in Wistar rats of both sexes.

MATERIALS AND METHODS

Animals

Adult Wistar rats (100±15 g) of both sexes, were maintained in the animal house of National Toxicology Centre, Pune. The animals were acclimatized to laboratory conditions for 7 d prior to the experiments. The rats were maintained at a room temperature of 28±4°C, with 70±10% relative humidity. During acclimatization, the animals were housed in polypropylene cages, with free access to normal diet and water ad *libitum*. The food pellets for the experimental animals were purchased from Nav Maharashtra Chakan Oil Mills (India). The animal studies were approved by the Institutional Animal Ethics Committee (IAEC) (IAEC/2010/PH35), Institute of Chemical Technology, Matunga, Mumbai.

Drugs and solutions

L-dopa obtained from Devi's laboratory, Hyderabad and hyoscine hydrobromide procured from Sigma-Aldrich Co, St. Louis, MO with certificates of analysis were used in the present study. All other chemicals and biologicals, obtained from Sigma Chemical Co. (St. Louis, MO) were analytical grade with the highest purity. Solutions of L-dopa and hyoscine hydrobromide were prepared using distilled water and plain distilled water was used for administration of the animals under Control Group.

Repeated-dose 90-day oral toxicity

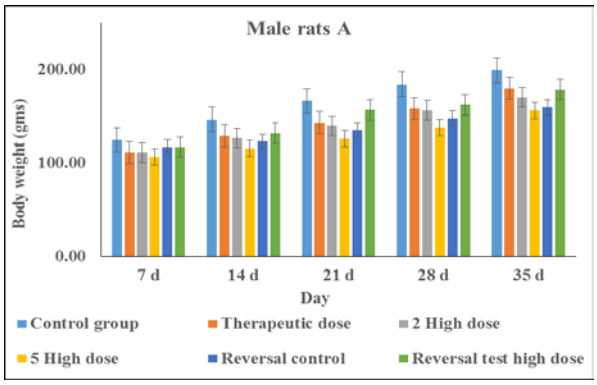
Adult Wistar rats were divided into 6 groups [Vehicle control, Therapeutic dose (TD), 2 times TD, 5 times TD, Reversal Control and Reversal Control of 5TD] of 10 male and 10 females each. OECD guideline 408 [12] was followed for the study with small modification. Combination of L-dopa and hyoscine hydrobromide were administered different strength was administered orally for 90

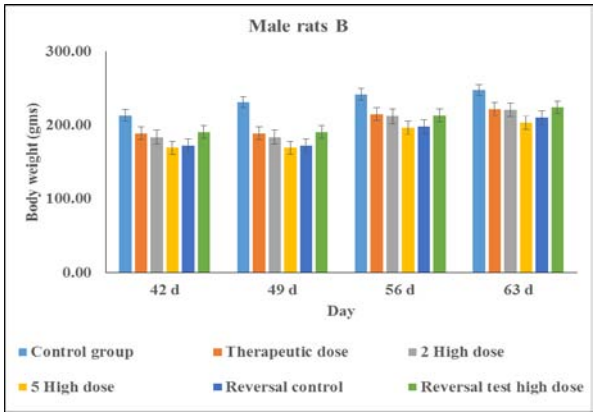
consecutive days. Out of the 3 treatment groups, i.e. II, III and IV the animals of Group II were administered dose of lower therapeutic limit of both drugs (1H) i.e. 18 mg/kg of L-Dopa and 0.027 mg/kg of hyoscine hydrobromide, animals of the group III were administered twice the upper limit of the therapeutic dose of each drug (2H) i.e. 108 mg/kg of L-dopa and 0.0675 mg/kg of hyoscine hydrobromide and animals of Group–IV were administered five times the upper limit of the therapeutic dose of each drug (5H) i.e. 540 mg/kg of L-Dopa and 0.3375 mg/kg of hyoscine hydrobromide. Animals of the Group V were administered only vehicle. Animals of Group VI were administered the same dose as that of Group IV i.e. 540 mg/kg of L-Dopa and 0.3375 mg/kg of hyoscine hydrobromide.

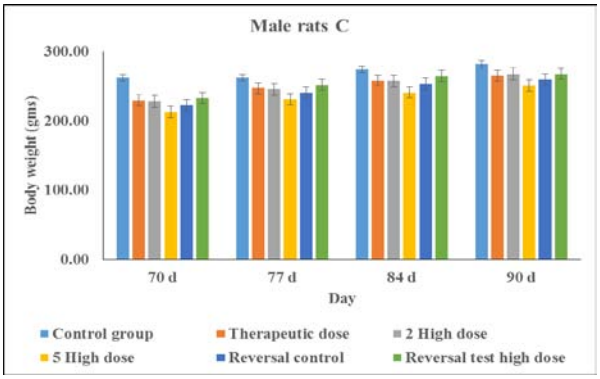
Animals of reversal control and Reversal Control of 5TD were kept for additional 30 d (total 120 d) without any treatment with free access to water and feed *ad libitum* to study recovery or persistence of toxicity. Cage side observation, body weight, food and water consumptions were monitored throughout the study period. Animals were fasted overnight prior to collect blood samples by retro-orbital technique on day 91 and on 121 d for (Reversal control and Reversal Control of 5TD group). The blood samples were analysed for haematological (haematocrit, haemoglobin concentration, erythrocyte count, total and differential leukocyte count, platelet count) and biochemical (glucose, cholesterol, alkaline phophatase, alanine aminotransferase (ALT), aspartate transaminase (AST), creatinine, blood urea nitrogen, total protein, albumin etc.) parameters using Pathozyme smart-7 (Semi-auto analyzer), BSA-3000 chemistry analyzer. After blood collection animals were sacrificed and subjected to gross necropsy. Once gross necropsy is done the organs like brain, lung, liver, heart, adrenal gland, kidney, uterus, ovaries, testis were surgically removed, weighed and stored for histopathological studies [13, 14].

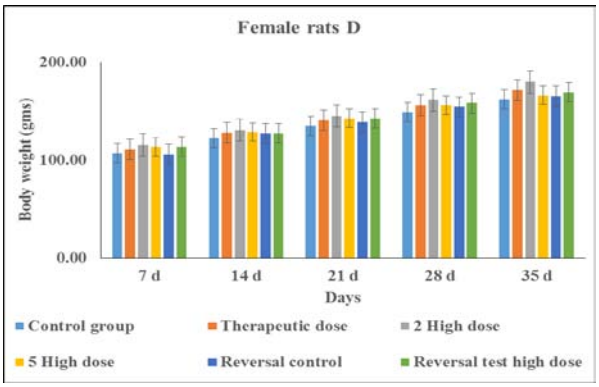
Statistical analysis

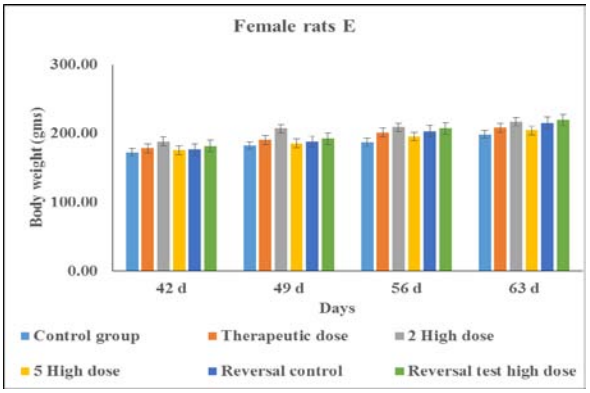
The results were expressed as mean±standard error of the mean (SEM). Statistical analysis was performed by one-way analysis of variance (ANOVA) followed by Bonferroni multiple comparison test as a post-ANOVA test to evaluate significant differences between groups. $p<0.05$ was considered statistically significant. All statistical analyses were carried out using the in statistical package (Graph Pad Software Inc., USA).











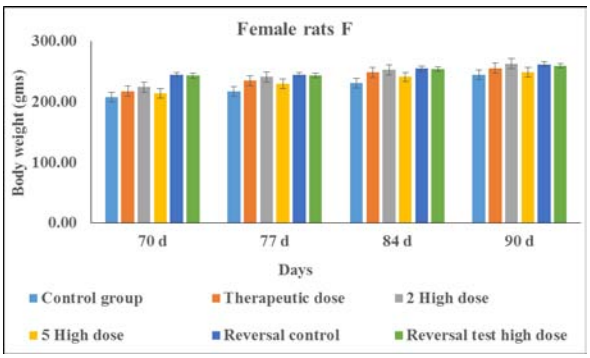


Fig. 1: Effects of oral administration of the L-dopa and hyoscine hydrobromide in combination for 90 d on mean body weights of male (A,B,C) and female (D,E,F). Body weight was calculated and data was expressed as mean of three replicates and±SD. The animals showed no significant differences in body weight between the control and treatment groups

RESULTS

Repeated dose 90-day oral toxicity

The sub-chronic effect of L-dopa and hyoscine hydrobromide given on repeated dose 90 d at dose 540 mg/kg and 0.3375 mg/kg respectively in combination did not produce any mortality nor alter the behavior patterns of the Wistar rats during the observation period. The animals showed no significant differences in body weight between the control and treatment groups. Body weight increased gradually throughout the study period in males and females of all groups (fig. 1ABC and 1DEF).

Behavior of the animal and food and water intake

None of the rats belonging to different treatment groups showed any signs and symptoms of toxicity during cage side observation (Results not shown). There were no change in their skin, fur, eyes, gait and posture when compared to the vehicle control group. No signs of toxicity and mortality were observed on any of the administered dose when compared with the vehicle control group. Their food and water consumptions were also normal (Results not shown).

Effect on the body weights

In the sub-chronic repeated dose 90-day oral toxicity study, the combination of L-dopa and hyoscine hydrobromide at the TD, 2TD and 5TD did not produce significant change in body weight compared to the vehicle control group. In addition, there was no significant difference in reversal control and reversal high dose (5TD) (fig. 1ABC and 1DEF).

Hematology

The hematological investigation of repeated dose-90 d oral administration of the combination of L-dopa and hyoscine hydrobromide on hematological parameters in rats was studied.

The analyzed hematological parameters for male (table 1) and female (table 2) rats included percent of total white blood cells (WBC), percent of lymphocytes (LY), percent of monocytes (MO), percent of granulocyte (GR) total red blood cells (RBC), platelet count, (HCT = hematocrit; HBG= hemoglobin), mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC), red blood cell distribution width (RDW), Platelet hematocrit (PCT),platelets (PLT),mean platelet volume (MPV), platelet distribution width (PDW) using a hematology analyzer MEK-6318K (Nihon Kohden Co., Ltd.).

The hematological investigation of sub-chronic toxicity study demonstrated that there were no significant change in hematological parameters between vehicle treated and the combination of L-dopa and hyoscine hydrobromide treated groups of either male and female rats. However, the group of male rats receiving the formulation (2TD) had significantly ($p<0.05$) increased MCV and MCH levels than that of the vehicle treated group. Whereas, the group of female rats receiving TD and 2TD dose decreased MCHC ($p<0.05$) and granulocyte levels respectively and group of female rats receiving 2TD dose increased ($p<0.05$) lymphocyte levels. Both male and female rats at higher dose (5TD) showed no significant change in any hematological parameters hence, changes in hematological parameters in male and female rats at lower dose may not be due to effect of combination of L-dopa and hyoscine hydrobromide (table 1and 2).

Table 1: Effects of sub-chronic 90 d oral administration of combination of L-dopa and hyoscine hydrobromide on hematological parameters in male rats

Parameters	Vehicle control	Combination of L-DOPA and Scopolamine			Reversal control group	5TD reversal group
		TD	2TD	5TD		
RBC (10[12]/l)	7.30±0.97	7.04±0.98	7.12±0.50	7.66±0.38	7.03±0.15	7.95±1.38
HGB (g/l)	11.48±1.51	11.06±1.47	11.99±0.72	12.27±0.64	11.08±0.34	11.80±0.75
HCT (%)	38.69±5.15	38.03±4.73	41.31±4.42	41.76±2.45	39.16±6.75	41.56±4.45
MCV (fL)	53.14±2.73	54.32±3.88	58.1±4.65*	54.66±3.46	54.92±2.79	53.04±3.75
MCH (pg)	15.69±0.38	15.66±0.44	16.8±1.00*	15.97±0.71	15.42±0.73	15.15±1.68
MCHC (g/l)	29.66±1.16	29.04±1.73	29.12±1.70	29.25±1.05	30.54±2.37	28.28±1.63
Platelet (10^9/l)	517.90±8.92	454.20±10.86	526.70±6.90	530.70±9.07	523.00±11.87	563.20±13.14
MPV (fL)	6.34±0.53	6.30±0.73	5.97±0.24	5.92±0.13	6.01±0.16	6.06±0.24
PCT (%)	0.33±0.09	0.29±0.07	0.315±0.05	0.31±0.06	0.37±0.05	0.35±0.04
WBC (10^9/l)	10.62±2.24	11.38±2.51	10.22±2.76	10.94±3.54	11.44±2.18	11.21±2.13
LYM (%)	61.78±9.96	60.46±15.53	68.63±9.51	65.32±12.01	72.12±2.49	65.26±11.67
MON (%)	4.14±1.11	4.23±1.14	4.21±1.43	3.93±1.19	5.08±0.37	3.21±0.66
GRAN (%)	34.08±9.26	35.31±14.47	27.16±8.41	30.75±11.21	29.71±0.86	21.53±10.90

Note: Each value represents the mean±standard deviation (n=6) * $P < 0.05$ comparison of group I with group II, III, IV using one-way ANOVA followed by Bonferroni multiple comparison test as a post-ANOVA test.

Table 2: Effects of sub-chronic 90 d oral administration of combination of L-dopa and hyoscine hydrobromide on hematological parameters in female rats

Parameters	Vehicle control	Combination of L-DOPA and Scopolamine			Reversal control group	5TD reversal group
		TD	2TD	5TD		
RBC (10[12]/l)	7.37±0.93	7.25±0.71	8.07±1.02	7.18±1.47	7.18±1.00	7.32±0.62
HGB (g/l)	11.73±1.24	11.82±1.02	12.17±0.63	11.72±2.46	11.37±1.04	11.85±1.02
HCT (%)	39.44±3.82	42.44±5.09	43.01±3.82	41.09±9.98	38.19±5.04	40.41±3.16
MCV (fL)	53.81±3.45	58.78±6.18	53.74±4.55	55.96±6.02	54.60±3.97	55.29±3.81
MCH (pg)	15.92±0.96	16.29±0.94	15.21±1.61	16.24±0.91	15.64±2.34	16.04±0.82
MCHC (g/l)	29.66±0.68	27.94±1.73*	28.38±1.82	29.25±1.63	28.63±1.59	29.47±1.09
Platelet (10^9/l)	556.70±114.70	637.80±145.07	527.30±108.48	443.60±108.04	531.40±52.87	497.8±99.07
MPV (fL)	5.98±0.33	6.31±0.61	5.96±0.29	6.21±0.63	6.31±0.23	6.28±0.42
PCT (%)	0.34±0.09	0.41±0.13	0.341±0.14	0.27±0.07	0.40±0.11	0.33±0.06
WBC (10^9/l)	11.72±2.87	13.25±2.05	11.07±1.65	12.17±3.30	11.08±0.71	9.87±1.78
LYM (%)	57.01±13.23	67.96±20.68	76.29±10.13*	60.33±21.94	60.81±14.95	68.97±9.38
MON (%)	4.93±2.52	4.17±2.44	3.38±1.04	4.59±2.03	4.18±1.80	3.81±1.08
GRAN (%)	38.06±11.94	27.87±19.48	20.33±9.19*	35.08±20.20	28.97±6.91	26.95±9.09

Note: Each value represents the mean±standard deviation (n=6) * $P < 0.05$ comparison of group I with group II, III, IV using one-way ANOVA followed by Bonferroni multiple comparison test as a post-ANOVA test.

Table 3: Effects of sub-chronic 90 d oral administration of combination of L-dopa and hyoscine hydrobromide on biochemical parameters in male rats

Parameters	Vehicle control	Combination of L-DOPA and Scopolamine			Reversal control group	5TD reversal group
		TD	2TD	5TD		
Glucose	128.60±15.50	132.70±20.64	157.90±32.37*	137.70±14.30	133.7±17.47	149.00±18.35
Creatinine	0.58±0.08	0.59±0.13	0.62±0.14	0.55±0.07	0.58±0.08	0.58±0.07
Urea	27.75±2.17	25.97±6.68	24.27±4.99	26.57±4.02	27.07±2.72	23.87±2.57
Total protein	7.46±0.54	7.22±0.67	7.72±0.61	7.69±0.62	7.66±0.44	8.07±0.19
SGPT	64.40±8.36	55.60±15.51	61.70±16.72	62.70±9.14	67.22±9.46	58.82±3.53
SGOT	107.04±17.42	107.50±19.29	111.70±16.92	121.53±11.73	104.3±20.03	116.2±19.33
ALP	410.70±136.81	366.70±132.43	431.70±216.40	492.50±141.21	398.98±91.45	313.18±105.74
T. Bilirubin	7.40±3.56	7.279±3.09	4.231±1.40*	4.36±2.68*	7.61±0.16	4.15±0.21*

Note: Each value represents the mean±standard deviation (n=6) * P< 0.05 comparison of group I with group II, III, IV using one-way ANOVA followed by Bonferroni multiple comparison test as a post-ANOVA test.

Table 4: Effects of sub-chronic 90 d oral administration of combination of L-dopa and hyoscine hydrobromide on biochemical parameters in female rats

Parameters	Vehicle control	Combination of L-DOPA and Scopolamine			Reversal control group	5TD reversal group
		TD	2TD	5TD		
Glucose	166.90±49.26	135.60±28.69	134.30±26.05	149.90±38.19	169.80±34.74	162.40±21.20
Creatinine	0.61±0.14	0.60±0.09	0.55±0.07	0.57±0.07	0.58±0.07	0.59±0.05
Urea	28.11±4.86	26.11±3.76	29.56±5.07	25.98±3.69	26.80±2.07	25.92±3.00
Total protein	8.30±0.61	6.98±0.54**	7.95±0.74	7.86±0.79	8.32±0.78	8.05±0.53
SGPT	63.80±7.41	56.00±8.89	63.8±12.76	64.60±10.39	60.79±6.33	63.88±12.19
SGOT	94.39±22.78	108.78±14.13	111.80±20.60	122.25±17.11**	95.10±16.88	122.90±16.99**
ALP	410.20±110.13	412.40±154.40	269.70±64.45*	351.30±122.26	351.19±62.98	322.65±87.65
T. Bilirubin	7.29±3.58	4.10±1.43**	4.419±1.56*	3.61±1.12**	7.43±0.23	4.409±0.14**

Note: Each value represents the mean±standard deviation (n=6) * P < 0.05, ** P < 0.01 comparison of group I with group II, III, IV, using one-way ANOVA followed by Bonferroni multiple comparison test as a post-ANOVA test.

Chemical chemistries

Blood biochemistry analysis was done on serum from blood samples collected in separator tubes using a BS-200 automatic biochemistry analyzer (Mindary Co., Ltd.) including glucose (Glu), creatinine (Cr), total protein (TP), serum glutamic-pyruvic transaminase (SGPT) and alkaline phosphatase (ALP) total bilirubin (TB).

Blood biochemistry analysis of male and female rats showed no significant change in serum biochemical parameters between vehicle treated and the combination of L-DOPA and hyoscine hydrobromide treated group except few. Male rats treated at 2TD dose showed an increase ($p<0.05$) in glucose level and at 2TD, 5TD, 5TD reversal group showed decrease ($p<0.05$) in T. bilirubin level. Female rats showed significant ($p<0.05$) decrease T. bilirubin level in TD ($p<0.01$), 2TD ($p<0.05$), 5TD ($p<0.01$), 5TD reversal group whereas decrease in total protein ($p<0.01$) and ALP ($p<0.05$) level at 2TD. Female rats also showed significant increase ($p<0.01$) in SGOT level at 5TD and 5TD reversal group. The changes in glucose level in male rats and ALP, total protein levels in female rats are not significant at higher dose (5TD) hence, these changes may not be due to the effect of drug (table 3 and 4).

Macroscopic and histological observations

Histopathological examination showed that, all the organs were normal and did not show any pathological changes. The histological pictures of some of the important organs were shown in figure. Taken together, body weight, hematological and biochemical parameters and histological data indicate that there is no toxicity in chronic study of the combination at any of the administered dose (fig. 2).

DISCUSSION

The concomitant and growing use of combination of traditional medicine with modern medicine in the present age has necessitated the thorough evaluation of safety and efficacy of the combination therapy [15]. Keeping in view the necessity of evaluation of safety and efficacy of the combination therapy, the present chronic toxicity study was carried out for the combination of L-dopa and hyoscine hydrobromide. The present 90 d chronic toxicity study was done to evaluate the possible health hazard likely from repeated exposure over a relatively long period of time.

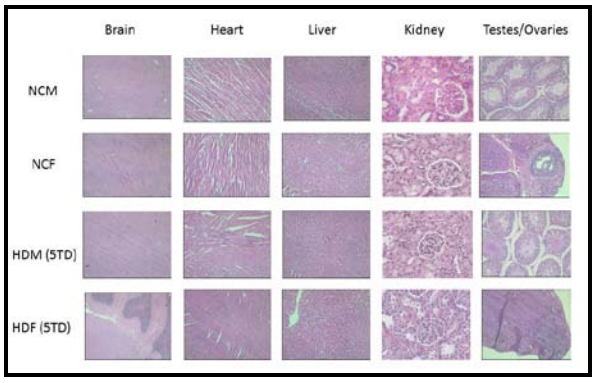


Fig. 2: Histopathological examination of Brain, Heart, Liver, Kidney and Testes/Ovaries from normal control male (NCM), normal control female (NCF) rats, High Dose treated male (HDM) and High Dose treated female (HDF) rats by hematoxylin and eosin staining 100 X zoom

None of the rats belonging to different treatment groups showed any signs and symptoms of toxicity during cage side observation (Results not shown). There were no change in their skin, fur, eyes, gait and posture when compared to the vehicle control group. No signs of toxicity and mortality are observed on any the administered dose when compared with the vehicle control group. Their food and water consumptions were also normal (Results not shown). All the treated groups gained weight during the study period and absence of significant changes in body weight suggested that the combination is fairly nontoxic. Gross necropsy finding also did not reveal any adverse effect in organ and hence combination is said to be safe [16, 17].

Blood is an important physiological and pathological status in animals and humans. Normally the parameters measured are RBC, platelets, WBC, differentiated leukocyte count, hematocrit, and hemoglobin. Toxicity induced by drugs can alter the normal range of these hematological parameters [18-20]. The haematological parameters of rats treated with TD, 2TD, 5TD and 5TD reversal group were comparable to that of the vehicle control group. Except, 2TD in male rats dose showed a significant increase in MCV and MCH levels whereas TD and 2TD decreased MCHC and granulocyte levels and 2TD dose increased the Lymphocyte levels. Female rats showed a significant decrease in MCH levels. Although these haematological parameters showed statistically significant difference but these differences are not dose related. Hence, these may be the result of some biological variation rather than the treatment effect.

Analysis of serum biochemical parameters is relevant for risk evaluation, as any changes in the biochemical system have higher predictive value for human toxicity when data are transferred from animal studies to human [21]. The Biochemical parameters of rats treated with different doses of combination of L-dopa and hyoscine hydrobrmide instead of were comparable to that of the vehicle control group. Except, male rats and female rats showed a significant decrease in T. bilirubin level and female rats showed a significant increase in SGOT levels.

SGOT and SGPT are trustworthy markers of liver parenchymal injury [22]. The increase in the level of SGOT and SGPT in serum may be due to leakage of these enzymes from liver cytosol into the blood stream [23]. The SGOT levels in female rats at 5TD and 5TD reversal group were significantly higher than vehicle treated animals indicating liver dysfunction [24]. The bilirubin concentration in the serum indicates the secretory and synthetic functioning of the liver and can be used to determine liver damage. Bilirubin is the major breakdown product of red blood cells. It is removed from the body by liver; hence it can be used as an indicator of liver toxicity. Increase in bilirubin concentration indicates increased production of bilirubin or decreased liver uptake (a result of liver toxicity).

The results of the study showed that the word combination of containing L-Dopa and Hysocine HBr significantly decreased the total bilirubin levels when compared to the vehicle control group. This observation along with normal microscopic data of animals from treated suggests that the formulation had no adverse effect on liver [25]. There were some more changes in biochemical parameters in male and female rat's viz. glucose and ALP. These changes were not reflected in the higher dose. Hence, may not be because of the drug but may be due to some other variation. The results of the study suggested that chronic administration of the formulation of L-DOPA and Hyoscine HBr produced no significant toxic effects in male and female Wistar rats, which could stand as an assurance for its medicinal use.

CONCLUSION

In Conclusion, the 90 d chronic oral toxicity of L-DOPA and Hyoscine HBr formulation is well tolerated in rats. The No-Observed-Adverse-Effect Level (NOAEL) for formulation is 5TD, the highest doses tested in male and female rats.

ACKNOWLEDGMENT

The authors are thankful to University Grant Commission, New Delhi for providing funds to carry out our research work.

CONFLICT OF INTERSTS

The authors hereby declare that there is no conflict of interests regarding the publication of this paper.

REFERENCES

1. Edwards TM, Myers JP. Environmental exposures and gene regulation in disease etiology. Environ Health Perspect 2007;115:1264-70.
2. Serra PA, Esposito G, Enrico P, Mura MA, Migheli R, Delogu MR, *et al.* Manganese increases l-DOPA auto-oxidation in the striatum of the freely moving rat: potential implications to l-DOPA long-term therapy of Parkinson's disease. Br J Pharmacol 2000;130:937-45.
3. Dickson DW, Braak H, Duda JE, Duyckaerts C, Gasser T, Halliday GM, *et al.* Neuropathological assessment of Parkinson's disease: refining the diagnostic criteria. Lancet Neurol 2009;8:1150-7.
4. Tolosa E, Marti MJ, Valldeoriola F, Molinuevo JL. History of levodopa and dopamine agonists in Parkinson's disease treatment. Neurology 1998;50:S2-S10.
5. Lee PH, Park HJ. Bone marrow-derived mesenchymal stem cell therapy as a candidate disease-modifying strategy in Parkinson's disease and multiple system atrophy. J Clin Neurol 2009;5:1-10.
6. Hauser RA. Levodopa: past, present, and future. Eur Neurol 2009;61:1-8.
7. Maharaj H, Maharaj DS, Scheepers M, Mokokong R, Daya S. l-DOPA administration enhances 6-hydroxydopamine generation. Brain Res 2005;1063:180-6.
8. Ostock CY, Dupre KB, Jaunarajs KL, Walters H, George J, Krolewski D, *et al.* Role of the primary motor cortex in L-DOPA-induced dyskinesia and its modulation by 5-HT1A receptor stimulation. Neuropharmacol 2011;61:753-6.
9. Buck K, Ferger B. Intrastriatal inhibition of aromatic amino acid decarboxylase prevents L-DOPA-induced dyskinesia: A bilateral reverse *in vivo* microdialysis study in 6-hydroxydopamine lesioned rats. Neurobiol Dis 2008;29:210-20.
10. Katzung BG. Pharmacological management of parkinsonism and other movement disorders. In: Basic and Clinical Pharmacology. 12th ed. Lange Medical Books/McGraw Hill Companies, Inc; 2001.
11. AHFS drug information 2005. McEvoy GK, ed. Antimuscarinics/ Antispasmodics General Statement. Bethesda, MD: American Society of Health-System Pharmacists; 2005. p. 1229-36.
12. OECD Guideline for the testing of Chemicals: Repeated Dose 90 d oral toxicity study in rodents; 1998.
13. Hor SY, Ahmad M, Farsi E, Yam MF, Hashim MA, Lim CP, *et al.* Safety assessment of methanol extract of red dragon fruit (*Hylocereus polyrhizus*): Acute and subchronic toxicity studies. Regul Toxicol Pharmacol 2012;63:106-14.
14. Singh G, Kumar V. Acute and sub-chronic toxicity study of standardized extract of *Fumaria indica* in rodents. J Ethnopharmacol 2011;134:992-5.
15. Firenzuoli F, Gori L. "Herbal medicine today: clinical and research issue". J Evidence-Based Complementary Altern Med 2007;4:37-40.
16. Veerappan A, Miyazaki S, Kadarkaraisamy M, Ranganathan D. Acute and subacute toxicity studies of agele Marmelos Corr., an Indian Medicinal plant. Phytomedicine 2004;14:209-15.
17. Olson H, Betton G, Robinson D, Thomas K, Monro A, Kolaja G, *et al.* Concordance of toxicity of pharmaceuticals in humans and animals. Regul Toxicol Pharmacol 2000;32:56-67.
18. Fernanda de A, Claudia A C de A, Marcelo M, Edson L da S. Safety assessment of yerba mate (IIes paraguariensis) dried extract; results of acute and 90 d subchrnoic toxicity studies in rats and rabbits. Food Chem Toxicol 2012;50:328-34.
19. Lillie L, Temple N, Florence L. Reference values for your normal Sprague-Dawley rats: weight gain, hematology and clinical chemistry. Hum Exp Toxicol 1996;15;612-6.
20. Weiss D, Wardrop K. Schlam's Veternary Haematology. 6th edition Wiley-Blackwell, Hoboken, NJ, USA; 2010.
21. Siva MG, Aragao TP, Vasconcelos CF, Ferreira PA, Andrade BA, Costa IM, *et al.* Acute and subacute toxicity of *cassia occidentalis* L. stem and leaf in Wistar rats. J Ethnopharmacol 2011;36:341-6.
22. Moss DW, Handerson AR, Kachmar JF. In: Fundamentals of Clinical Chemistry. 3rd Eds. (Ed. NW Tietz). Philadelphia: W. B. Saunders Company; 1987. p. 346-421.
23. Navarro CM, Montilla PM, Martin A, Jimenez J, Utrilla PM. Free radicals scavenger and antihepatotoxic activity of *rosmarinus tomentosus*. Plant Med 1993;59:312-4.
24. Fowler BA, Woods JS, Schiller CM. Ultrastructural and biochemical effects of prolonged oral arsenic exposure on liver mitochondria of rats. Environ Health Perspect 1997;19:197-204.
25. Ojo OA, Oloyede OI, Olarewaju OI, Ojo AB, Ajiboye BO, Onikanni SA. Toxicity studies of the crude Aqueous Leaves extract of *Ociumum gratissiumum* in Albino Rats. J Enviorn Sci Toxicol Food Technol 2013;6:34-9.