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Original Article

SAFETY ASSESSMENT OF L-DOPA AND HYOSCINE HYDROBROMIDE IN COMBINATION: ACUTE AND SUB-ACUTE ORAL TOXICITY STUDIES

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ABSTRACT

Objective: To evaluate the safety of L-Dopa and hyoscine hydrobromide combination by determining its potential toxicity after acute and sub-acute oral administration in rats.

Methods: The acute and sub-acute toxicity study was performed according to the Organisation for Economic Co-operation and Development (OECD) Guideline 423 and 407 respectively. The 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/d for L-dopa and 0.75 mg/d for hyoscine hydrobromide for adult human being and which was converted to required dose for Wistar rats (3 males and 3 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 14 d of the study. In the repeated dose 28 d oral toxicity study, there was no significant difference in any of the assigned parameters between the control and all treatment groups.

Conclusion: It is established that the combination therapy of L-dopa and hyoscine hydrobromide is safe 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, brady kinesia, and postural abnormalities due to loss of dopamine [5]. Since dopamine cannot cross the blood–brain barrier, L-3, 4-dihydroxyphenylalanine (L-dopa), It is 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, the 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 anti muscarinic 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 an 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 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 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 single and 28-day repeated oral dosing in Wistar rats of both sexes. An additional aim was to identify no-observed-adverse-effect levels (NOAELs) for acute and sub-acute combination of L-dopa and hyoscine hydrobromide exposure.

MATERIALS AND METHODS

Drugs and reagents

L-DOPA obtained from Devi's laboratory, Hyderabad and hyoscine hydrobromide procured from Sigma-Aldrich Co, St. Louis, MO with certificates of analysis was 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.

Experimental animals

Male and female Wistar rats were used for the acute and sub-acute toxicology studies. The rats were obtained from National Toxicological Center, Pune, India. 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). All procedures in this study were performed according to the details

given in the Protocol No. ICT/IAEC/2012/P-29 approved by the Animal Ethics Committee.

Acute toxicity study

The acute oral toxicity of the combination of L-dopa and hyoscine hydrobromide was investigated according to the OECD 423 Guideline for Testing of Chemicals Acute Oral Toxicity–Acute Toxic Class Method [12]. After seven-day adaptation to laboratory conditions, six rats (three male and three female) were administered with the combination of five times of therapeutic dose of L-dopa (540 mg/kg) and hyoscine hydrobromide (0.3375 mg/kg) in a single dose by oral gavages. Animals were observed individually after dosing at least once after 30 minutes and at every 4 hours thereafter for next 24 hours. After 24 h animals were observed each day for 14 d. The observation was made with regard to clinical signs, gross behavioral changes and mortality. Signs and symptoms of toxicity included the following: (1) skin, fur, eyes and mucous membranes evaluations, (2) autonomic effects such as salivation, (3) central nervous system effects such as tremors and convulsions and (4) changes in the level of activity, posture, strength and bizarre behavior. The daily food and water consumption were also recorded.

Sub-acute toxicity 28 days study

The study was conducted according to the protocol described by OECD Guideline 407 [13] with minimal modification. Animals were divided in to 6 Groups, each of 6 Animals (3 Males and 3 Females). Out of the 6 Groups, first group was Vehicle Control, Group II. III and IV were treatment groups, Group V was Reversal Control and Group VI was Drug Treatment Reversal. Animals of the Group–I (vehicle Control) were administered plain vehicle. 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 the 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 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.

The dosing of the animals of Group–I to Group–IV was done for 28 d and at the end of 28th day all the animals were anaesthetized by ether and after collecting blood sample by cardiac puncture from each animal the animals were dissected and the organs were collected for taking their weight and histopathology study.

The dosing of Reversal Treatment group (Group VI) was stopped after 28 d and the animals of the said group and that of Reversal Control Group (Group V) were put on vehicle from 29th day to 42nd d. At the end of 42nd d animals of both these groups were anaesthetized by using ether and after collecting the blood sample by cardiac puncture from each animal the animals were dissected and the organs were collected for taking their weight and Histopathology study. The blood samples were collected for biochemical and hematological analyses.

The last 2 Groups (V & VI) were included in the study as per OECD guidelines to evaluate the reversal in the various parameters that take place consequent to withdrawal of drug dosing. The parameters of the Reversal Control Group (Group V) were compared with the Control Group (Group I) and those of Reversal Treatment Group (Group VI) with the Drug Treatment Group (Group IV).

The blood sample was collected from each anaesthetized animal via cardiac puncture into non heparinized and EDTA-containing tubes for biochemical and hematological analyses [14, 15]. The haematological parameters studied are WBC, LYM, MON, GRAN, LYM%, MOM%, GRA%, RBC, HGB, HCT, MCV, MCH, MCHC, RDW, PLT, MPV, PDW, and PCT. The said parameters were studied by using an Auto Hematology Analyzer (Mindray BC-2800). The biochemical parameters studied were glucose (GLU), urea, creatinine (CR), total protein (TP), alkaline phosphatase (ALP),serum glutamic-pyruvic transaminase (SGPT) which were performed using pathozyme smart-7 (Semi-auto analyzer), BSA-3000 Chemistry Analyzer. After weighing and macroscopic examination organs of each Animal were preserved in formalin (10%) for histopathological examination.

At the end of 6 weeks (42 days) all the Animals in the Reversal Control Group (Group V) Reversal Treatment Group (Group VI) were anaesthetized by using Ether and all the parameters as recorded for Group I to Group IV were studied.

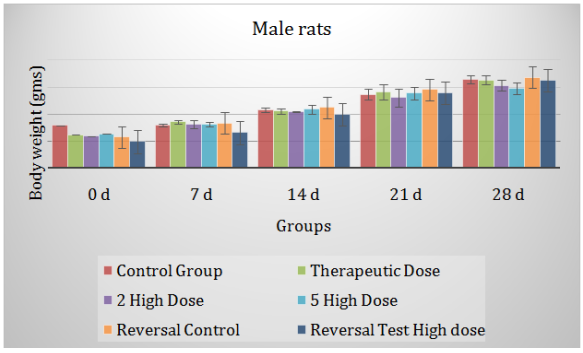
Statistical analysis

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 Tukey's 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).

RESULTS

Acute toxicity study in rats

The acute effect of L-dopa and hyoscine hydrobromidegiven as a single oral dose at 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. 1a and 1b). There was no significant difference in relative organ weights between the treated and control rats. Also no pathological changes were noticed in skin, fur, and eyes in comparison with the control groups. These observations suggest that combination of L-dopa and hyoscine hydrobromide is non-toxic. Therefore, the lethal dose (LD50) value for oral administration of the combination of L-dopa and hyoscine hydrobromide in male and female rats exceeds 5000 mg/kg.



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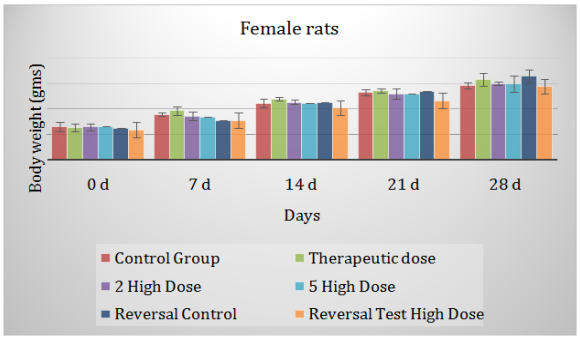

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Fig. 1: Effects of oral administration of the L-dopa and hyoscine hydrobromide in combination for 28 d on mean body weights of male (A) and Female (B). Body weight was calculated and data was expressed as mean of three replicates and±SE

Sub-acute toxicity study in rats

No mortality was observed in any of the 6 groups during 28 d of oral administration of the Vehicle and the L-Dopa and hyoscine hydrobromide in combination and no mortality was observed even after 28 d up to 42 d in the animals of the Reversal Vehicle Control (Group V) and Reversal Treatment Group (Group VI).

Behavioral observations

The general behavior of all the groups of animals was normal and there no symptoms of toxicity were observed during the 28 d treatment period in all the groups and up to 42 d in Group V and VI.

Food water consumption and body weight

The sub-acute oral administration of the combination of different doses of L-dopa and hyoscine hydrobromide resulted in insignificant changes in feed intake and water intake of the treated rats compared to control (Data not shown). The combination neither produced significant changes in the body weights nor relative organ weights of the brain, heart, liver, kidney, adrenal glands, lungs, sex organs and brain in the treated rats compared to control group. Both the control and rats treated with the combination were found to be normal till the end of the experiment as well as throughout the 28 d period and 42 d period with regard to Group V and VI.

Hematology and biochemistry

Effects of sub-acute oral administration of combination of L-dopa and hyoscine hydrobromide on hematological and biochemical parameters in rats were 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.).

Serum from blood samples collected in separator tubes was measured 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. The data are expressed as mean±S. E. M. and significant differences in each group versus the controls did not differ significantly in both males (table 3) and female (table 4).

Table 1: Effects of sub-acute oral administration of combination of L-dopa and hyoscine hydrobromide on biochemical parameters in male rats

Parameters	Groups					
	Control	Combination of L-dopa and Hyoscine hydrobromide			Reversal control	Reversal test high
		1H	2H	5H		
Glucose (mg/dl)	138.67±18.56	132.67±18.34	144.33±19.22	141.00±21.63	146.33±12.22	138.33±28.73
Creatinine (mg/dl)	0.53±0.06	0.53±0.06	0.50±0.00	0.50±0.00	0.53±0.06	0.50±0.00
Urea (mg/dl)	23.67±1.38	28.77±1.76	28.74±2.59	35.26±4.68**	21.69±6.18	18.00±2.29#
Total Protein (g/dl)	6.63±0.65	6.67±0.61	7.20±0.17	7.87±0.68	6.60±0.20	8.20±2.36
SGPT (U/l)	52.29±10.91	691.57±23.44	63.37±5.22	56.64±4.99	75.82±13.10	72.78±13.05
ALP (U/l)	631.93±64.43	508.97±22.06	571.05±19.12	602.75±12.13	597.83±14.51	534.83±18.19

Note: Each value represents the mean±standard deviation (n=6) * P < 0.05, ** P < 0.01, *** P < 0.001 comparison of group I with group II, III, IV, @ P<0.05, $P<0.01, #P<0.001 comparison of group IV with group VI and α<0.05, β<0.01, γ<0.001 comparison of group I with group V using one-way ANOVA followed by Bonferroni multiple comparison test as a post-ANOVA test.

Table 2: Effects of sub-acute oral administration of combination of L-dopa and hyoscine hydrobromide on biochemical parameters in female rats

Parameters	Groups					
	Control	Combination of L-dopa and Hyoscine hydrobromide			Reversal control	Reversal test high
		1H	2H	5H		
Glucose (mg/dl)	160.33±7.65	142.33±3.05	159.00±8.14	179.33±4.32	179.67±2.92	150.17±5.72
Creatinine (mg/dl)	0.53±0.06	0.53±0.06	0.53±0.06	0.57±0.21	0.57±0.06	0.57±0.06
Urea (mg/dl)	29.13±1.81	30.61±8.58	34.61±6.00	28.08±2.00	25.91±4.34	22.61±2.04
Total Protein (g/dl)	6.70±0.20	7.40±1.18 *	7.03±0.64	7.10±0.64	7.00±0.53	8.20±0.62
SGPT (U/l)	52.71±2.67	53.95±1.72	56.29±4.56	54.08±1.752	55.32±4.30	52.82±9.82 #
ALP (U/l)	487.00±7.49	431.17±9.05	472.57±9.12	615.37±12.13	472.33±14.51	523.67±8.19

Note: Each value represents the mean±standard deviation (n=6) * P < 0.05, ** P < 0.01, *** P < 0.001 comparison of group I with group II, III, IV, @ P<0.05, $P<0.01, #P<0.001 comparison of group IV with group VI and α<0.05, β<0.01, γ<0.001 comparison of group I with group V using one-way ANOVA followed by Bonferroni multiple comparison test as a post-ANOVA test.

Table 3: Effects of sub-acute oral administration of combination of L-dopa and hyoscine hydrobromide on hematological parameters in male rats

Parameters	Groups					
	Control	Combination of L-dopa and Hyoscine hydrobromide			Reversal Control	Reversal Test High
		1H	2H	5H		
WBC (10^3/uL)	14.53±2.07	14.60±1.56	9.87±2.73	10.40±3.70	10.44±3.53	10.72±3.62
LY (%)	75.20±10.41	73.80±7.19	64.93±3.48	77.03±7.15	79.47±8.87	85.30±4.50
MON (%)	3.37±0.78	2.93±0.31	3.53±0.64	3.17±0.31	3.38±1.14	4.10±1.35
GRAN (%)	21.43±9.67	23.27±7.13	31.53±3.84	19.80±6.82	16.70±10.00	10.63±5.01
RBC (10^6/µl)	5.65±0.58	6.82±0.48	6.98±.54	6.87±0.72	6.07±1.11	6.36±.66
HGB (g/dl)	10.60±0.82	10.67±0.06	12.20±0.87	12.40±1.28	12.63±1.37*	11.90±0.89
HCT (%)	34.30±2.82	34.40±1.04	39.50±2.94	40.60±4.37	44.87±3.71 β	50.90±4.97
MCV (fl)	60.87±3.29	59.40±3.75	56.70±1.20	59.17±0.23	65.00±9.46 #	80.33±8.39

MCH (pg)	18.77±1.01	18.33±1.46	17.47±.50	17.97±0.32	18.30±3.15	18.33±1.99
MCHC (g/dl)	30.83±0.15	30.97±0.85	30.83±0.32	30.47±0.58	28.37±43.43	29.43±.68
RDW (%)	14.20±2.49	15.37±2.58	13.60±0.79	15.90±0.75	15.10±1.18	17.23±1.55
PLT (10^3/µl)	502.00±8.83	501.33±6.79	423.33±3.17	606.67±6.61	436.67±6.87	393.00±2.04
MPV (fl)	6.30±0.40	5.97±0.15	6.07±0.35	6.07±0.15	7.03±1.74	8.27±1.17
PDW (%)	15.10±0.30	14.93±0.25	14.93±0.15	14.27±1.27	21.33±11.15	16.60±1.00
PCT (%)	0.31±0.11	0.30±0.07	0.36±0.04	0.37±0.02	0.30±0.05	0.40±0.10

Note: Each value represents the mean±standard deviation (n=6) * P < 0.05, ** P < 0.01, *** P < 0.001 comparison of group I with group II, III, IV, @ P<0.05, $P<0.01, #P<0.001 comparison of group IV with group VI and α<0.05, β<0.01, γ<0.001 comparison of group I with group V using one-way ANOVA followed by Bonferroni multiple comparison test as a post-ANOVA test.

Table 4: Effects of sub-acute oral administration of combination of L-dopa and hyoscine hydrobromide on hematological parameters in female rats

Parameters	Groups					
	Control	Combination of L-dopa and Hyoscine hydrobromide			Reversal Control	Reversal Test High
		1H	2H	5H		
WBC (10^3/uL)	10.53±5.00	9.93±0.21	15.63±0.67	13.70±1.640	13.70±1.49	9.29±4.33
LY (%)	78.23±1.36	79.53±6.49	74.90±10.76	75.33±9.18	84.27±6.09	77.53±2.37
MON (%)	2.70±0.20	2.53±0.50	4.00±1.55	3.67±1.10	2.93±0.50	3.20±1.31
GRAN(%)	19.07±1.42	17.93±6.02	21.10±9.21	21.00±8.09	12.80±6.26	19.27±2.54
RBC (10^6/µl)	6.23±0.31	7.25±1.12	6.59±1.93	5.52±0.20	6.10±0.56	7.72±1.59 @
HGB (g/dl)	11.17±0.64	13.50±2.25	12.83±4.12	10.87±0.15	11.83±1.55	12.57±1.36
HCT (%)	35.33±1.97	40.90±5.83	42.40±15.12	35.90±1.08	41.13±11.16	49.00±11.13
MCV (fl)	56.83±1.08	58.67±0.68	63.67±4.03	65.27±4.13	67.40±16.62	63.40±4.41
MCH (pg)	17.87±0.49	18.53±0.35	19.33±0.65	19.70±0.98	19.40±2.35	17.60±1.41 @
MCHC (g/dl)	31.53±0.21	33.63±3.39	30.43±0.982	30.23±0.60	29.40±3.64	26.33±3.78
RDW (%)	13.63±0.64	13.43±0.75	15.33±1.18	14.70±1.04 *	14.97±2.59	16.40±0.56
PLT (10^3/µl)	498.67±21.83	553.67±17.79	347.33±8.17	537.33±16.61	439.33±16.87	599.33±14.04
MPV (fl)	6.23±0.25	5.90±0.35	6.50±0.26	6.47±0.06	7.20±1.82	8.33±2.11
PDW (%)	14.93±0.15	14.73±0.15	15.30±0.35	15.23±0.21	21.70±11.78	18.07±11.66 @
PCT (%)	0.31±0.06	0.32±0.03	0.22±0.08	0.35±0.05	0.31±0.06	0.35±0.12 @

Note: Each value represents the mean±standard deviation (n=6) * P < 0.05, ** P < 0.01, *** P < 0.001 comparison of group I with group II, III, IV, @ P<0.05, $P<0.01, #P<0.001 comparison of group IV with group VI and α<0.05, β<0.01, γ<0.001 comparison of group I with group V using one-way ANOVA followed by Bonferroni multiple comparison test as a post-ANOVA test.

Histopathological examination

The histo architecture studies of vital organ like the liver, kidney and lungs showed no histological changes. None of the macroscopic observations was considered to be treatment related. Histopathological examinations of all tissues taken from all rats of both sexes revealed unremarkable appearance. Some examples of photomicrographs of liver, lung, adrenal, pancreas, spleen and kidney histopathology are shown in fig. 2.

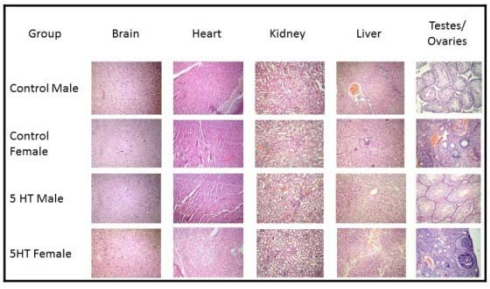

Fig. 2: Photomicrograph showing organ from representative animal. Brain, Heart, Kidney, Liver, Testes/Ovaries of Control group male, female and 5 times high dose group male and female with normal histology (hematoxylin and eosin stain) at 100 X zoom

DISCUSSION

Generally, reduction or changes in body weight and relative organ weights have been used as a simple and sensitive index of toxicity after exposure to potentially toxic drugs and chemicals [16, 17]. In the present acute toxicity study the combination of L-dopa and hyoscine hydrobromide did not produce any toxic effect when administered by oral route to experimental animals. There was no death or any significant variation of body weight and normal growth of rats of treated groups when compared to control rats, thus suggest acute and sub-acute administration of the combination of L-dopa and hyoscine hydrobromide had no effect on the normal growth of rats during the observation period of 1 week. The LD50 of the combination of L-dopa and hyoscine hydrobromide was found to be above the oral dose of 5 g/kg. Thus, based on the classification of Loomis and Hayes [18], viz. that substances with LD50 between 5000 and 15000 mg/kg body weight are regarded as being practically nontoxic, it can be concluded that the combination of L-dopa and hyoscine hydrobromide may be considered as practically non-toxic in acute ingestion.

Analysis of blood parameters is one of the most sensitive indexes for the evaluation of toxic compounds and also serves as an important parameter of physiological and pathological features in human and animals [19, 20].

After 28 d of treatment, with combination of L-dopa and hyoscine hydrobromide, there were no treatment-related changes in the hematological parameters between the control (group I) and treatment group (group II, III, IV), showing that the combination does not affect hematopoiesis and leucopoiesis in rats. The biochemical profile of treated rats showed no significant difference in both treated male and female rats, except urea which showed slight increase in male at the highest dose (5H Group IV) and a slight decrease in reversal treatment group (group VI).

Kidney functions are evaluated mainly by urea and creatinine and concurrently changes in these markers are associated with kidney dysfunction. The elevated urea levels could be a preliminary sign of the toxic properties of the combination of L-dopa and hyoscine

hydrobromide in kidney, although these were not accompanied by increases in creatinine levels [21]. This is further supported by histopathological examinations where no alterations found in the kidney tissue.

Since there no signs of abnormality were noted with respect to gross or histopathological examinations, hematology, clinical chemistry, and organ weights for all doses group, the NOAEL of the combination of L-dopa and hyoscine hydrobromide for both male and female rats was considered to be greater than 5000 mg/kg/d and hence the combination of L-dopa and Hyoscine hydrobromide was found to be safe.

CONFLICT OF INTERESTS

Declared None

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