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Pharmacological study of "treenoponchomul"-an Ayurvedic diuretic preparation

Sabera Haque[3], JMA Hannan[1], Masum Shahriar[2,*], M Naimul Islam[3], Mafruhi Sattar[3] and MSK Choudhuri[3]

[1]*Department of Pharmacology, Research Division, BIRDEM, Dhaka-1000;* [2]*Department of Pharmacy, Gono Bishwabidyalay, Nayarhat, Savar, Dhaka-1344;* [3]*Ethnopharmacology Laboratory, Department of Pharmacy, Jahangirnagar University, Savar, Dhaka-1342*

SUMMARY

The pharmacological effects of an Ayurvedic diuretic drug "Treenoponchomul" (TPM) was investigated in animal model. The pharmacological actions of the test drug along with that of the components thereof, on the Central Nervous System (CNS) were studied. The drug under study TPM showed little effect on the CNS, the same can not be said about the components. The most prominent CNS depressant effect was observed with *Saccharum officinarum* Linn. (EE) in that it lowered the spontaneous motor activity as well the exploratory -behavior of the animals. An exploration retarding effect of moderate degree, was evident with *Imperata cylindrica* Beauv. (UU), and *Phragmites maxima* Blatter & McCann (NN). Although the test drug did not alter the normal locomotor and / or exploratory behavior of the treated animals, it did significantly ($p<0.01$) lower the locomotion of the amphetamine induced hyperactive animals. TPM along with its components (especially *Desmostachya bipinnata Stapf*. Root, KU), significantly reduced the gastro-intestinal motility of the treated animals ($p<0.01$). The test drug and its components lowered the body weight of the treated animals, on being administered chronically (30 days), with EE being the only exception.

Key Words: Treenoponchomul; Diuretic; *Saccharum officinarum; Saccharum spontaneum; Desmostachya bipinnata*; *Phragmites maxima*; *Imperata cylindrica*

INTRODUCTION

The Ayurvedic diuretic preparation of Treenoponchomul (TPM) was first included in the Susruta Samhita and is also incorporated in the Bangladesh National Formulary of Ayurvedic Medicine (1992). Literature shows that this particular preparation is already being used in some countries (i.e., Nepal, India) and its clinical efficacy has been tested. The results of the preliminary pharmacological investigation of the drug Treenoponchomul (TPM) indicate that it has both preventive and curative action on urolithiasis and these effects of the drug has been attributed by the investigators to its diuretic action (Singh and Sachan 1979). The individual components of the drug has also been studied in different countries and encouraging results, in terms of diuretic activity, has also been reported (Agarwal *et al.*, 1979; Abed *et al.*, 1981; Hong *et al.*, 1986; Dat *et al.*, 1992). But there are no reports about other pharmacological effect of drug TPM and there are only few reports on pharmacological effect of individual component of the drug. The most interesting feature about this particular preparation was that it was composed of roots of five plants belonging to the family Gramineae. In order to get an as-precise-as-possible picture about the pharmacology of the drug, presence of other probable effects of it on the central nervous system was also investigated, on animal models.

MATERIALS AND METHODS

Composition of the drug treenoponchomul

The composition of the drug Treenoponchomul (TPM) was first given in the Susruta Samhita (Dev,

*Correspondence: Masum Shahriar, Lecturer, Department of Pharmacy Gono Bishwabidyalay, Gonoshayasthya Kendra, Savar, Dhaka1344 Bangladesh. Tel: 8802-7708004; Fax: 8802-9003228; E-mail: smasum@bdcom.com

1960; Bhisagratne, 1963). The same composition was adapted in the National Ayurvedic Formulary of Bangladesh (1992). It is essentially composed of the roots of five plants all belonging to the family Gramineae. The exact composition of the drug TPM is given below:

1. *Saccharum officinarum* Linn. Root (EE)
2. *Saccharum spontaneum* Linn. Root (KA)
3. *Desmostachya bipinnata* Stapf. Root (KU)
4. *Phragmites maxima* Blatter & McCann Root (NN)
5. *Imperata cylindrica* Beauv. Root (UU)

Animals employed

Swiss Webster mice [Crl : CFW (Swiss Webster)BR] of either sex, weighing between 20 to 25 gm, were employed in all the experiment weighing between 20-30 gm, were mainly employed. The animals were provided with food and tap water *ad libitum*. The animals were maintained at constant room temperature (22.0+1.0^{o}C), humidity 55-65%, 12 hours light and 12 hours dark cycle. The animals were carefully marked on different parts of their body in order to observe drug doses administered and also to note their corresponding responses.

Preparation and feeding of the drug

The individual roots were finely pulverized and 50 gm of the powder was weighed in an Sartorius optical balance. The weighed powder was then added to 400 ml of distilled water and boiled till the volume was reduced to one quarter of the original. The kvatha was fltered through thick cloth. The kvathas were administered as suspensions in 0.9% normal saline being the continuous phase. Each of these kvatha was given to the animals in a dose of 40 cc/kg. Unless otherwise stated, the route of administration of the kvatha was per oral.

Controls

A group of six animals were simultaneously employed in the experiment. They were administered with the vehicle (0.9% normal saline) and this group served as the control.

Experimental procedure

The following experiments were carried out:

Spontaneous motor activity test

To study the effect of the drug on spontaneous motor activity, a modified version of the method described by Siegmund and Wolf (1952) was employed. Immediately after administering the test drugs, p.o., the animals were placed on a wire netting covered with brick chips. The amount of brick chips displaced, due to the spontaneous motor activity of the animals, through the wire netting, was measured for a total period of 1 hour.

Hole cross test

This experiment was carried out by the method of (Takagi *et al.*, 1971). The observation was conducted in groups of 6 mice, after oral administration of test drugs (TPM, EE, KA, KU, NN, UU), Normal saline was given to the control group. Spontaneous movement of the animals through the hole, constructed at the middle of the dividing wall of a box having dimensions of 30×20×14 cm., from one chamber to the other was counted for a period of 2 minutes. Similar recordings were made for the control group.

Amphetamine induced hyperactivity

This experiment was also carried out by the modified version of the "Sand Displacement" method developed by Siegmund and Wolf (1952). The animals were pretreated with drugs (TPM, EE, KA, KU, NN, UU) one hour prior to the administration of d-amphetamine in a dose of 4 mg/kg (Vane, 1961). Recordings were made of the rate of displacement of brick chips at every 5 minutes interval for a total of one hour.

Climbing out test

The effect of test drugs on exploratory movement was observed in mice (of both sexes) in groups of 6. The climbing test described by (Sandberg, 1959) was employed. Groups of six mice were placed in the test cage for 10 min. The number of animals climbing out, as well as total time taken by all the animals belonging to the group was counted. Such recordings were made at different time intervals. Similar recordings were also made for the control animals.

Hot plate test

The apparatus employed for this experiment was

described by Woolf and MacDonald (1944). A laboratory hot plate was maintained at a constant temperature of $55^{o} \pm 1^{o}$C. The animals were introduced on that hot surface and the reaction time, indicated by the licking of hind and/or fore paws or by kicking of the legs or by trying to jump out, was determined at different time intervals after administration of graded doses of the test drugs. Similar recordings were made for the control groups.

Pentobarbital narcosis test

Groups of 5-10 mice were administered test drugs by the p.o. and 1 hour later a subhypnotic dose of pentobarbital (45 mg/kg) was administered by the i.p. route. This method was described by Tedeschi and Tedeschi (1968). The time interval between the loss and regain of righting reflex was deemed as the total sleeping time and was thus recorded. Similarly, the time between administration of pentobarbital and exhibition of its effects was deemed as the onset of sleep and was duly recorded. Similar recordings were made for the control groups. The average sleeping time was determined and significance of the difference between the drug treated and control group was determined.

Gastro-intestinal motility test

The method of Chatterjee (1993) was employed in this experiment. The "Barium milk" was given to mice (20-25 gms), in groups of 10, 15 minutes after the administration of test drug. The mice were sacrificed after 15 and 30 minutes of administration of Barium milk. The distance which the Barium milk meal traveled were measured and expressed as a percentage of the total length of small intestine (from pylorus to ileocecal junction).

Effect of tpm and its components on acute metabolism in mice

The effect of the test drug on the acute metabolic rate of the laboratory animals was studied by using a Nalgene metabolic cage. The animals were introduced into the metabolic cage and after an initial adjustment period of 72 hours, the rate of food and water intake as well as the rate of urination and defaecation was measured with an interval of one hour for a total period of 24 hours.

Effect of tpm and its components on body weight after chronic administration

Effect of the chronic administration of TPM and its five components on body weight was observed for one month. Drugs were administered p.o., once daily, in a dose of 40 gm/kg. Animals employed were mice of same sex, weighing 15-18 am. All the groups remained under similar environmental conditions and provided with enough food and water throughout the experimental time period. Recordings were made of the body weight of the animals, once every day for thirty days. Similar recordings were made for the control group.

Statistical method

The mean Standard error of the mean (SE) of the results were calculated and a student's ttest or paired ttest was applied: p=0.05 was taken to be the significant level. (Glasanapp and Poggio, 1985).

RESULTS AND DISCUSSION

Testing experimental drug using intact animal is considered to be the best method for investigating the action of drug on central nervous system. Classical research approach, found it convenient to get a primary indication of the spectrum of activity of the experimental samples on CNS, by observing its effect on the spontaneous motor activity of animals. In its broadest sense, motor activity refers to the whole repertoire of unconditioned behavior and in its narrowest sense, it refers to the whole body locomotor activity, such as running or walking. The experimental data, presented in Table 1 indicates that although the parent drug, TPM and three of its components did not have any lowering effect on the spontaneous locomotor activity of treated animals, the same can not be said about the other two components. It was obvious, from the experimental data, that EE and NN significantly ($p<0.001$) lowered the spontaneous movement of the treated animals. On the other hand KU treated animals showed a slight degree of elevation of locomotor activity but it was statistically in significant. This suggest that TPM was not supposed to cause a reduction in the locomotor activity and thereby somewhat enhanced its potential as a viable diuretic is at least devoid of this type of side effect.

Table 1. Tabular representation of locomotion test.

Groups	Displacement of Brick Chips (gm / gm body wt)	
	Spontaneous Mean±S.E.	Amphetamine induced Mean±S.E.
CONTROL (N=6)	0.071±.002	0.058± 0.006
TPM (N=6)	0.071±.0013	0.007±0.002[c]
ESKHU (N=6)	0.006±.001[b]	0.020±0.002[c]
KASH (N=6)	0.053±.012	0.060±0.005
KUSH (N=6)	0.083±.02	0.053±0.008
NOL (N=6)	0.047±.011[b]	0.055±0.004
ULU (N=6)	0.005±.001	0.052±0.008

[a]indicates $p<0.001$; [b]indicates $p<.01$; [c]indicates $p<0.05$

As spontaneous movements of the animals include, by definition, both the propulsive and non-propulsive movements of the animals, and as the fluctuating and multifarious nature of many overt movements patterns comprising spontaneous motor activity makes it improbable, if not impossible, to accurately measure the effects of a drug on the spontaneous motor activity of animals by using a single experimental procedure, the hole cross test was performed. Among the 5 components of TPM, NN and UU treated animals showed (Table 2) least interest in crossing the hole, as was manifested by the reduction in the number of holes crossed by the treated animals, and the data was found to be statistically significant ($p<0.001$). The NN treated animals, however, showed a period of marked hyperactivity, as was evident from the reading taken at half hour after drug administration, and this initial stimulation was followed by a depression (Table 2). The results of EE treated animals, only after 4 hours of drug administration, was also found to be significantly ($p<0.05$) lower than that of the control. TPM and its three other components did not show any significant deviation in terms of holes crossed, than the control animals. However, the treated animals of TPM showed a sudden rise in locomotor activity, across the hole, four hours after the administration of the drug.

The experimental data of the previous two experiments suggests that some of the components of TPM, namely NN, UU and EE had a sedating and / or quieting effect on the spontaneous locomotor activity of the treated animals. The question arises whether or not those components could also sedate the hyperactivity induced by amphetamine, one of the most potent sympathomimetic amines in respect to the degree of excitation that it can generate on the central neurons. This is exactly why, the next experiment performed, was the amphetamine induced hyperactivity test. The experimental data (Table 1) suggests that the treated animals of the drug TPM and EE did lower the hyperactive state of the animals pre-treated with a stimulant dose of d-Amphetamine. It is noteworthy here, that the drug TPM did not reduce the spontaneous locomotor activity of the test animals in the previous

Table 2. Tabular presentation of hole cross test

Groups	Name of the study	Number of hole crossed				
		0 min Mean±S.E.	+30 min Mean±S.E.	+60 min Mean±S.E.	+120 min Mean±S.E.	+240 min Mean±S.E.
Hole cross test	CONTROL (N=6)	3.7±0.23	2.8± 0.72	2.7±0.37	3.5±0.73	2.3±0.54
	TPM (N=6)	3.5±0.24	2.3±0.37	2.8±0.96	3.3±1.05	3.5±0.52
	ESKHU (N=6)	3.5±0.55	2.2±1.15	3.3±1.08	1.8±0.78	1.0±0.56[c]
	KASH (N=6)	3.8±0.52	1.7±0.83	2.6±0.54	3.7±1.29	2.8±0.98
	KUSH (N=6)	3.8±0.34	1.7±0.83	3.2±1.31	2.7±1.22	2.5±1.16
	NOL (N=6)	3.7±0.50	4.5±1.62	0.5±0.25[a]	1.7±0.97	0.7±0.54
	ULU (N=6)	3.8±0.87	1.2±0.52[c]	0.8±0.34[a]	0.8±0.52[a]	0.8±0.91

[a]indicates $p<0.001$; [b]indicates $p<.01$; [c]indicates $p<0.05$

Table 3. Tabular presentation of hot plate test

Name of the study	Group	Time of study				
		0 min Mean±S.E.	+30min Mean±S.E.	+60 min Mean±S.E.	+120 min Mean±S.E.	+240 min Mean±S.E.
Hot plate Test (Sec)	CONTROL (N=6)	12.0±1.9	15.7± .05	14.3±1.1	13.1±0.7	13.2±1.2
	TPM (N=6)	12.2±1.3	17.0±2.1	16.2±2.2	17.5±2.8	13.7±3.5
	ESKHU (N=6)	12.2±2.1	10.3±2.5[c]	9.7±1.8[c]	10.3±2.1	13.6± 2.7
	KASH (N=6)	11.7±1.6	8.7±2.5[b]	9.5±1.0[b]	9.8±1.0[b]	14.2±0.7
	KUSH (N=6)	11.5±1.3	13.0±2.6	11.8±0.8	9.7± 2.0	12.2±2.2
	NOL (N=6)	11.8±1.2	15.2±2.8	14.8±4.0	13.3±2.4	11.2±1.4
	ULU (N=6)	12.0±2.9	16.5±2.2	15.5±3.0	13.2±1.5	11.7±1.3

two experiments. This perhaps indicate that the drug, in spite of not lowering the spontaneous locomotor activity of the test animals, it does possess the ability of calming the excited animals.

The results of the experiments, performed so far, clearly indicates that certain components of TPM, namely NN, UU and EE, has got lowering effects on the spontaneous locomotor activity of animals. This pointed towards the possible direction of these components of TPM, if not all, of possessing depressant activity on the central neurons. Any assessment of the neurological effects of any compound must include information about its effects on the normal behavior of the animals. One of the primary objectives of the behavioral pharmacologists, that have still been elusive to them, is to obtain a precise picture of the effects of any compound on the behavior of animals, using a single experimental model. Although a set of inferences can be drawn from the experimental data obtained from the above test, still it would be difficult to come to an conclusion that can be regarded as a valid index of the effects of the components on the behavior of the animals, from the performance of the treated animals in a single experimental model (Robbins, 1977). This is exactly why the ethologists have advocated for performing a series of tests to explore the behavioral effects of a test compound and this was the precise reason behind performing the next experiment, i.e., the climbing out test, which was aimed at evaluating the effects of TPM and its components on exploratory behavior. The experimental data suggests that the TPM treated animals did not deviate significantly from the control animals in terms of climbing out time (Fig. 1). EE treated animals took longer time

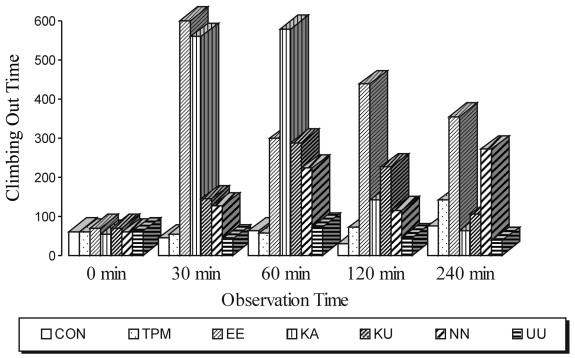

Fig. 1. Graphical presentation of climbing out test.

(significant $p<0.01$) to climb out from the box, and also the percentage of animals that did come out, was also lower than that of the control. An increase in mean time to climb out as well as a decrease in the percentage of animals to have come out was observed in case of the KA treated animals but only results of half hour and one hour after administration of drug was found to be statistically significant ($p<0.001$, $p<0.05$ respectively). A significant decrease in exploratory behavior of KU treated animal (two hours after drug administration), NN treated animal (four hours after drug treatment) was also observed. Interestingly, an increase in exploratory behavior was observed in UU treated animals, as they took less time to come out of the box (after 4 hours of drug administration) than that of the control. The data, obtained in this specific model, for UU was somewhat contrary to the data obtained in the previous models. Perhaps this indicates that UU lowers the normal locomotor activity of animals without lowering the exploratory behavior of the same animals.

The next test performed was the hot-plate test. This test is essentially designed to explore the

presence of narcotic analgesic activity of the test compounds (Peck and Wallace, 1980). Prostaglandins play a major role in modulating renal blood flow and maintaining normal renal function, in man (Liverson, 1982), and drugs which interfere with normal diuretic activity of human beings, may also have significant effect on arachidonic acid metabolism (Henrich, 1983; Clive, 1984). This very understanding, apart from the fact that few components of the test drug has exhibited sedative effect on the central neurons, influenced the researcher to perform the hot-plate test, to find out whether the test drug or its components have any analgesic activity, be it due to its effect on the mechanisms involved in diuresis or on the central neurons. KA, KU and EE treated animals were found to be more susceptible to pain stimulus as the pain perception time were less, in case of these drug treated animals, than that of the controls. However, the treated animals of TPM showed a consistent but marginal degree of increase in pain perception time. But the results, therefore, were not found to be statistically significant. NN and UU treated animals also showed an inconsistent as well as marginal degree of increase in pain perception time. The results are presented in Table 4.

As many central depressants act on the cerebral cortex and thus produce their action (Bowman and Rand, 1980) and bear the odds of fatal consequences, in case of poisoning and overdose, because of the fact that most of the controlling centers of the vital organs are located in the cerebral cortex (Guyton, 1986). As such, the next experiment performed was aimed at investigating whether TPM and its components had any effect on the cerebral cortex

Table 4. Tabular presentation of effect of tpm and its constituent on pentobarbital sleeping time test

Groups	Onset Mean±S.E.	Duration Mean±S.E.
Control	4.1±.60	41.8±3.81
TPM	4.4±.28	41.3±6.15
ESKHU	5.0±.50[c]	58.2±6.19
KASH	7.4±2.4	34.6±4.41
KUSH	3.2±1.95	54.2±3.23
ULU	14.0±2.21[b]	48.6±8.32
NOL	6.0±2.21	42.8±5.13

[a]indicates $p<0.001$; [b]indicates $p<.01$; [c]indicates $p<0.05$

Table 5. Tabular presentation of effect of rrr on body weight gain study

Group	Body Weight(gm)	
	Initial	Final
Control	19.0 ± 1.5	26.6 ± 1.6
TPM	18.0 ± 2.1	27.0 ± 1.13
ESKHU	18.7± 1.22	29.1 ± .68
KASH	18.0 ± 1.56	25.0 ± 2.13
KUSH	19.2 ± 1.11	23.4 ± 1.87
ULU	19.0 ± 1.34	27.0 ± .98
NOL	18.5 ± 2.5	23.3 ± 1.68

[a]indicates $p<0.001$; [b]indicates $p<.01$; [c]indicates $p<0.05$

or not. The results obtained in the pentobarbital sleeping time experiment indicate that while TPM had no effect on both the onset and the duration of action of pentobarbital narcosis (Table 5). KA exhibited a prolonging effect on the onset of pentobarbital action ($p<0.05$) and also had a lowering effect on the duration of action of the hypnotic ($p<0.05$). EE, UU and NN increases the onset of action, but the results of the former two were found to be significant ($p<0.05$ and 0.01 respectively). On the other hand, KU reduces the time taken for the onset of action of the hypnotic. Apart form these, no significant effect of the other components of TPM were found to exert significant effect on the duration of action of pentobarbital.

Many, if not all of the drugs, those which can increase the rate of formation of urine, are also capable of increasing the normal propulsive movement of the gut. The underlying mechanism is perhaps the fact that both the urinary bladder and the gut are primarily innervated and controlled by the cholinergic version of the autonomic nervous system. This prompted the author, once that the drug had proved its worth as a diuretic agent, to investigate the possible effects of the drug on the normal propulsive movements of the intestine of animals. In this experiment, the results (Fig. 2) indicate that TPM and its constituents reduced gastro-intestinal motility in the treated animals. However, statistical tests predicted that the deviation between the mean values for control and TPM, KU treated and KA treated groups after 15 minutes of treatment, were significant at $p<0.05$ and $p<0.01$ respectively. This effect was somewhat contrary to the normal postulation, in layman's term, that the diuretics

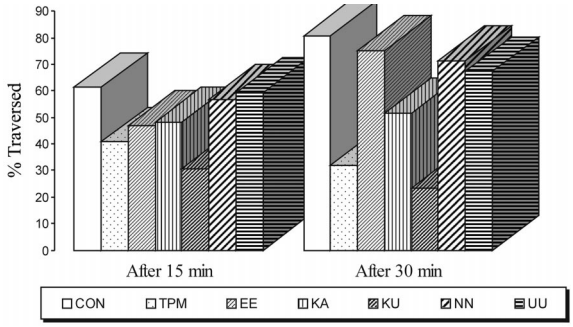

Fig. 2. Graphical presentation of gastrointestinal motility test.

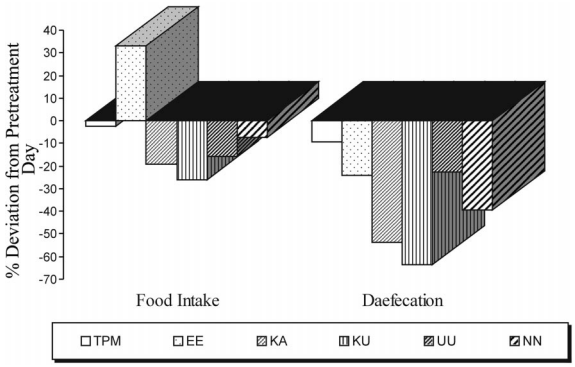

Fig. 3. Graphical presentation of acute metabolism test.

also increase the normal propulsive movements of the gut. The results obtained after 30 minutes of treatment were higher than that of 15 minutes of treatment, for groups including control, but excluding TPM and KU treated groups. In the case of TPM and specially KU treated groups GI motility was not only reduced with the passage of time but was also reduced significantly ($p<0.01$) from that of control. It should be mentioned here that, TPM and its five components significantly lowered the length passed by "Barium Milk" front after 30 minutes of study. We have seen that KU did not possess any diuretic activity of its own rather it worked as a "synergist". A connection may exist between reducing GI spasm and potentiating the effect of other roots, possibly, by aiding in the process of the absorption of fluids.

In an effort to find out about the implications of the acute administration of the test drug on the rate of food intake as well as the rate of formation of stool. The experimental data (Fig. 3) suggests that food intake was increased only in the group receiving EE, in comparison to that of the control readings. It was also evident that the rate of stool formation decreased in all the treatment groups, in comparison to that of the control group. However, the effects of KU and KA were found to be statistically significant ($p<0.01$).

It is clear, therefore, that the effects of these compounds upon the relative excretion of sodium and water may vary greatly and that quantitative assays based on either natriuretic or acute weight loss responses alone may yield quite different and even contradictory estimates of diuretic efficacy, specially if representatives of different groups are being compared (Lant *et al.*, 1966). This observation couple with the fact that these diuretic agents are generally used for a considerable period of time, made the researcher to think it to be prudent to perform the next test, aimed at evaluating the effects of the test drug on the body weight of laboratory animals after being administered chronically. After 7-10 days of initial period, all the groups gained body weight during the following three weeks. EE treated group gained body weight higher than that of the control group after 30 days of treatment in body weight, however, this difference proved to be statistically insignificant.

In conlusion it can be said that though TPM have little effect on CNS some of components have marked central nervous system depressant effect. The test drug and its components did not have any detrimental effect on the weight gain pattern of the treated animal, on being administered chronically.

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