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Neuro toxic effect of neurella-D on the psychopharmacological changes in wistar rats

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ABSTRACT

Present trend in agricultural practice to control pest animals by using a mixed formulation of synthetic or natural pyretheroids with different proportions of organophosphates to reduce the toxic effect of organophosphate compounds toxicity on the human, domestic animals and environment. The mixed formulation selected for the present study neurella-D (chlorpyrifos 50% + cypermethrin 5%) was used for sub acute and acute toxicity studies. Based on the LD 50 values the sub acute doses were fixed [1/40th, 1/20th, 1/10th of the LD 50 Dosage]. The present work has been designed to asses the neuro toxicity of a combination pesticide through behavioral parameters [Psychopharmacological observation] and instrumental analysis such as spontaneous motor activity for 28 days. It is evident from the results that 1/20 and 1/10 of LD 50 dosed animals of either sex showed marked differences in the behavioral patterns when compare to 1/40 of LD 50 dose animals. When compare the neurotoxicity symptoms of constituent pesticide results show a different toxicological profile when compare with the toxicity of the combination pesticide which may be due to synergetic effect. Our study evident that the short time sub acute studies coupled with psychopharmacological evaluations can provide major part of the information's obtained from the long-term studies.

KEYWORDS

Neuro toxicity;
Neurella-D;
Psychopharmacology;
Pesticide;
Synergetic effect.

INTRODUCTION

Man lives in the world of chemicals. Chemicals are produced in widespread commercial usage particularly as pesticides, medicines and industrial chemicals. Although these chemical are generally beneficial they have their negative aspects too. Many of these chemicals may pollute the environment and some eventually find their way to humans through contaminated food, water and air. It is now established that organophorous and carbamates are toxic because of their interference in the normal synaptic transmission of nerve impulse. Nowadays organophosphate esters are widely used as pesticide and have substantially replaced many of more persistent chlorinated hydrocarbon insecticide[1]. Recently natural pyrethroids have replaced organophosphates because of their low mammalian toxicity and lack of persistence in the environment[2]. Neuro toxicity is one

TABLE 1 : Behavioral parameters of rats treaded with different concentration of compound neurelle-D

Groups	Weeks	Behavioral parameters											
		Alertness		Passive/Active		Grooming		Aggressiveness		Restlessness		Tremors	
		M	F	M	F	M	F	M	F	M	F	M	F
I	01	+	+	A	A	+	+	N	N	N	N	-	-
	02	+	+	A	A	+	+	N	N	N	N	-	-
	03	+	+	A	A	+	+	N	N	N	N	-	-
	04	+	+	A	A	+	+	N	N	N	N	-	
II	01	+	+	A	A	+	+	N	N	N	N	-	-
	02	+	+	A	A	+	+	N	N	N	N	-	
	03	+	+	A	A	+	+	N	N	N	N	-	-
	04	+	+	A	A	+	+	N	N	N	N	-	-
III	01	+	+	A	A	+	+	N	N	N	N	-	-
	02	+	+	A	A	+	+	N	N	N	N	-	-
	03	+	+	A	A	+	+	N	N	N	N	-	-
	04	+	+	A	A	+	+	N	N	N	N	-	-
IV	01	+	+	A	A	+	+	N	N	N	N	-	+
	02	+	+	A	A	+	+	N	N	N	N	-	-
	03	+	-	A	A	+	-	N	N	N	N	-	+
	04	-	-	P	P	+	-	N	-	N	-	+	+

M: Male, A: Active, +: Presence of activity, N: Normal, F: Female, P: Passive, -: Absence of activity

of the major effects induced by the organo-phosphates[3] and pyrethroids[4,5], since lot of data available regarding neuro-toxicity of constituent pesticide [organophosphates or pyrethroids] but less information available regarding the toxicity of combination pesticide. It has been stated that organophorous pesticides like chlorpyrifos and profenos are compatible with pyrethroids like cypermethrin[6]. Data on particular combination of insecticides are scanty and general principles are necessary for predictive purposes. The possible types of toxicological interaction between two insecticides are (1) additive, (2) synergistic and (3) antagonistic or they may act independently of one another. Neuro toxicity is one of the major effects induced by the organa phosphates and pyrethroids, since lot of data available regarding neuro toxicity of constituent pesticide [organophosphates and pyrethroids] hence the present work has been designed to asses the neuro toxicity of a combination pesticide through psychopharmacological observation for 28 days sub acute studies.

MATERIALS AND METHODS

Wister rats weighing around 90-120 gms selected for the present study and they were acclimatized in the laboratory condition. The male and female animals were fed ad libitum with standard rat pellets obtained from Lipton India Ltd Bangalore. The new combination pesticide neurella-D obtained from Aventis Bombay. The selected pesticide is a combination of [chlorpyrifos 50% + cypermethrin 5%].

Acute toxicity studies

Acute Toxicity studies were carried out using forty rats was randomly divided into four groups, each group consisting of 10 animals [five females and five males]. The allocation of the groups and fixation of doses of the combination pesticides in the different groups were carried as given below.

Compound name

Neurela –D, [Combination of chlorpyrifos 50% [Organophosphate] + cypermethrin 5% [synthetic Pyrethroids].

Details of groups and dosage

Group I: Control [only distilled water]
Group II: 75-mgs/kg body-weight
Group III: 150 mgs/kg-body weight

TABLE 2 : Behavioral parameters of rats treaded with different concentration of compound neurelle-D

Groups	Weeks	Behavioral Parameters									
		Convulsions		Ataxia		Catalepsy		Gripping strength		Righting reflex	
		M	F	M	F	M	F	M	F	M	F
I	01	-	-	-	-	-	-	+	+	+	+
	02	-	-	-	-	-		+	+	+	+
	03	-	-	-	-		-	+	+	+	+
	04	-	-	-	-	-	-	+	+	+	+
II	01	-	-	-	-	-	-	+	+	+	+
	02	-	-	-	-	-	-	+	+	+	+
	03	-	-	-	-		-	+	+	+	+
	04	-	-	-	-	-	-	+	+	+	+
III	01	-	-	-	-	-		+	+	+	+
	02	-	-	-		-	-	+	+	+	+
	03	-	-	-	-	-	-	+	+	+	+
	04	-	-	-	-	-	-	+	+	+	+
IV	01	-	-	-			-	+	+	+	+
	02	-	-	-	-	-	-	+	+	+	+
	03	-	+	+	-	-	+	+	+	+	+
	04	-	+	-	-	-	+	+	+	+	-

M: Male F: Female +: Presence of activity -: Absence of activity

Group IV: 300-mgs/kg-body weight.

The control group [G1] received distilled water by oral intubations [single administration] at a dose of 10ml/kg body weight. Groups II, III and IV received different doses by oral gavages, [single administration] of the pesticide dissolved in distilled water. The animals were observed 14 days after dosing and basing on the mortality, the LD50 was calculated using Finney's probit analysis[7].

Sub acute psychopharmacological studies

Psychopharmacological studies reveal behavioral changes observed depending biochemical and physiological changes at various morphological sites within the body due to the pesticide toxicity. Similar to acute studies, sub acute psychopharmacological studies [28 days] were also carried out by randomly dividing the animals into four groups and each group consisting of 5 males and 5 females. Group I the untreated control receiving only distilled water at a dose of 10ml/kg body weight. The doses of the combination pesticide in the sub acute studies were based on the LD 50 values obtained from acute studies. Group II received 1/40th, Group III-1/20th, Group IV-1/10th of the LD 50 Dosage.

The allocation of group and doses were fixed as follows

Group I: Control [distilled water]
Group II: 6 mg/kg body weight
Group III: 13 mg/kg body weight
Group IV: 26mg/kg body weight

The above mentioned dosages of the compounds were dissolved in distilled water and administrated by oral gavages once daily for 28 days. During the study period, in-life parameters like body weight and feed consumption were recorded. The psychopharmacological parameters to assess the neuro toxicity of the combination pesticide were evaluated during the live phase of the sub acute studies. Hence psychopharmacological symptoms such as restlessness, convulsions and skeletal movements like ataxia, catalepsy, gripping strength and righting reflex were studied in the compound treated with reference to the respective untreated control. The above observations were made on the 7th, 14th, 21st & 18th days using the actophotometer [Inco] and digital Rotorod apparatus [Inco].

RESULTS

Animals administered Nurelle-D [chlorpyrifos 50% + cypermethrin 5%] showed a decrease in body weight gain at the doses of 13 and 26 mg/kg b.w., from the third week onwards. A general decrease in feed consumption was observed in all the pesticide treated groups. In the last two weeks of the study marked changes were observed in the psychopharmacological parameters and spontaneous motor activity in the pesticide treated animals. Nurelle-D, the incidence of Psychopharmalogical signs of toxicity like lack of grooming, aggressiveness, catalepsy, tremors and convulsions was observed only during the fourth week of treatment among the animals of Group 4(26mg/kg b.w.). [TABLE 1 & 2] Psychopharmalogical parameters reflect the magnitude of neutoxicity and in the present study, an incidence of the behavioral changes was observed from the third week in rats treated with Spark EC 36 at the doses of 8(G3) and 16(G4)mg/kg b.w [sekar babu 2004]. Severe signs of neurotoxicity like ataxia, catalepsy, convulsions and tremors were observed only in

TABLE 3 : Motor activity (counts/15min) in Wistar rats treated with different concentration of compound No. 2(Nurelle-D)

Group	Sex	Week			
		I	II	III	IV
I Control	Male	176.8^{a}±12.1	193.0^{a}±4.6	187.6^{a}±5.2	182.2^{a}±11.0
	Female	180.8^{a}±7.04	200.4^{a}±9.11	207.8^{a}±2.81	190.6^{a}±3.4
II 6.5 Mg/kg b.w.	Male	183.2^{a}±6.02	198.6^{a}±12.02	178.6^{a}±4.02	171.8^{a}±3.05
	Female	203.8^{b}±4.11	183.2^{b}±6.3	200.1^{a}±2.89	160.0^{b}±4.70
III 13.0 Mg/kg b.w.	Male	185.6^{a}±3.09	170.8^{b}±6.33	176.0^{a}±3.62	146.6^{b}±5.01
	Female	197.6^{a}±6.14	184.4^{b}±8.4	174.2^{b}±4.43	126.0^{b}±7.1
IV 26.0 Mg/kg b.w.	Male	196.6^{b}±2.01	173.2^{b}±9.2	165.0^{b}±4.39	135.8^{b}±3.04
	Female	179.8^{a}±11.2	167.4^{b}±9.13	140.8^{c}±4.18	114.0^{b}±5.92

Values are presented as mean ± Standard Error, Values (sex-wise) carrying similar superscripts are not statistically significant (p>0.05)

TABLE 4 : Gripping strength (counts/15min) in Wistar rats treated with different concentration of compound No. 2(Nurelle-D)

Group	Sex	Week			
		I	II	III	IV
I Control	Male	32.0^{a}±2.11	29.5^{a}±0.39	31.0^{a}±1.22	30.0^{a}±1.19
	Female	33.0^{a}±1.11	28.2^{a}±1.11	30.5^{a}±0.97	34.0^{a}±1.08
II 6.5 Mg/kg b.w.	Male	28.0^{a}±2.01	29.0^{a}±0.56	28.1^{a}±0.86	31.0^{a}±2.0
	Female	33.0^{a}±1.55	27.0^{a}±0.64	29.4^{a}±1.35	30.0^{a}±1.31
III 13.0 Mg/kg b.w.	Male	32.0^{a}±1.02	31.2^{a}±1.07	33.0^{a}±1.21	29.8^{a}±0.56
	Female	30.8^{a}±4.44	27.0^{a}±3.98	26.0^{b}±0.99	28.2^{b}±1.01
IV 26.0 Mg/kg b.w.	Male	28.0^{b}±0.98	26.0^{b}±1.09	21.0^{c}±1.38	19.4^{b}±1.02
	Female	28.5^{b}±0.83	24.0^{b}±1.13	18.0^{c}±0.49	12.0^{c}±0.79

Values are presented as mean ± Standard Error, Values (sex-wise) carrying similar superscripts are not statistically significant (p>0.05)

animals of group 3 and 4 from the fourth week of treatment, whereas the righting reflex and gripping strength remained normal through out the study.

A significant decrease in spontaneous motor activity [TABLE 3] and muscle relaxant property was observed in both male and female rats from the third week of treatment with Spark EC 36[8] whereas Neurelle-D showed a dose dependent decrease in motor activity and muscle relaxant property [TABLE 4] among the group 111 and 1V animals. Compound 2-Nurelle-D.

DISCUSSION

Organophosphates affect insects and mammals primarily by phosphorylation of the acetyl cholinesterase enzyme [Ach Ease] at nerve ending leading to paralysis. On the other side pyrethroids also neuro toxic in virtue of their action voltage dependent sodium channels of the nerve membrane. Because of very low mammalian toxicity and lack of persistence in the environment have gained the pyrethroids a niche in pest control especially in households. A more recent trend is to mix Organo phosphorous and pyrethroids to arrive at combination pesticide. These combination pesticides have broad spectrum of activity when compared to individual compounds. Regarding toxicity especially long term toxicity of these combination pesticides remains unexplored entity. In this present study animals administered with Neurella-D showed a decrease in body weight gain at the does of 13, 26mgs/kg body weight from the third week of the treatment. In connection with psychopharmacological studies marked changes were observed behavioral pattern and spontaneous motor activity.

Extensive studies have been carried out on chlorpyrifos the OP constitutes of Nurelle-D. When rats were treated with chlorpyrifos at dietary concentrations of 10, 30, 84, 240 or 694 ppm for 2 weeks, clinical signs of toxicity like irritability, ataxia and failure to groom were observed in the highest dose group (694 ppm equivalent to 66-95mg/kg b.w.). No clinical signs were observed in the other doses.

Cypermethrin the pyrethroid constituent of the combination pesticide Nurelle-D has also been studied extensively. When rats were fed with diet containing cypermethrin at the concentrations of 25, 100, 250, 750 or 1500 ppm in feed for 5 weeks, reduced body weight and food intake were observed at the highest dose. The animals of the highest dose also showed clinical symptoms like piloerection, nervousness and in coordinated movement.

Neurotoxicity studies in rats treated with cypermethrin at the doses of 1250, 2500 or 5000 ppm in diet for 14 days resulted in mortality at the 2 higher doses and growth inhibition in all the treated groups. Clinical signs of neurotoxicity were impaired ability to walk, splayed hind limbs, ataxia, paralysis, hypersensitivity, gross disorientation and convulsions.

From the 28-day study with Nurelle-D on Wistar rats, it can be seen that the toxicity induced by this combination pesticide is similar to the toxicity induced individually by the constituent pesticides namely chlopyrifos

and cypermethrin (as reported in the earlier literature). from the present result it is evident that the combination pesticide behave differently and exhibit different toxicological profile when compare with the toxicity of the individual pesticide in the combination which may be due to synergetic effect psychopharmacological evaluations can provide major part of the information's obtained from the long-term studies.

Summary

The present study was carried out to evaluate the short-term sub acute (28 days) toxicity and neurotoxicity of combination pesticide Nurelle-D in Wistar rats.

The acute toxicity of these combination pesticides was evaluated to arrive at the doses for the sub acute study. Sub acute psychopharmalogical studies (28 days) were carried out separately for each of the combination pesticides. The in-life parameters to AChE, gross pathology and histopathology were investigated. Behavioral parameters, spontaneous motor activity and muscle relaxant property were also observed to estimate the neurotoxicity of these pesticides.

A general decrease in the feed consumption was observed in all the pesticide treated groups. In the last two weeks of the study marked changes were observed in the psychopharmalogical parameters and spontaneous motor activity in the pesticide treated animals. Marked changes were observed in psychopharmalogical parameters, spontaneous motor activity and muscle relaxant property in the pesticide treated groups.

CONCLUSION

The combination pesticides are gaining popularity in pest control programmes as they exhibit a broad spectrum of activity coupled with better efficiency and economy. But for the registration of these pesticides, acute toxicity data is sufficient and therefore the long-term toxicity of these compounds remains unexplored. Alternatives are required for long-term studies as they are difficult to carryout, time consuming and expensive. From this study it can be understood that a well designed sub-acute study clubbed with neuro toxicological assessment can provide a major part of the information observed from the long term study. The study also revealed that the combination pesticide behave differently and exhibit a different toxicological profile when compared with the toxicity of the individual pesticide in the combination. Therefore it is very necessary that all the toxicological studies have to be carried out (as for a new pesticide) for a combination pesticide before it can be brought to the field. Similar studies can also help to identify combination pesticides that have lesser mammalian toxicity compared to the individual pesticides used in the combination while retaining or obtaining better efficiency.

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