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Evaluation of 28 days subacute toxicity of spark EC 36 [combination pesticide] through biochemical markers in wister rats

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ABSTRACT

Presently mixed formulations [pyretheroids with organophosphates] are in agricultural practice to reduce the organophosphate toxicity and to have better pesticidal efficay due to the synergetic effect of its constituents. Hence a 28 days sub acute studies were designed by administering 1/10,1/20 and 1/40 th of LD50 dosage daily through oral gavage for 28 days.The Blood samples were collected from both experimental and control Rats to asses the biochemical markers like Creatinine ,glucose blood urea nitrigen,Alkaline phosphatase,ALT and AST.The results are quite interesting to note that both male and female experimental animals showed a significant increase in glucose, creatinine and ALP was observed in higher dosed groups [III and IV] when compared to untreated control whereas other biochemical markers did not showed any significane.From the results it is evident that the organs like pancreas[glucose],kidney[creatinine] and bone [ALP] showed marginal change in their biochemical markers only in the higher doses with out showing any significant change in the lower doses due to the reduced toxicity [which is due to the less composition of organophosphate and its best pesticidal property is due to synergetic effect of its constituents in the combination pesticide].such studies on combination pesticide will help us to identify a best combination pesticide showing remarkable pesticidal property and lower toxicity in human and domestic animals.

Key words: combination formulation,alkaline phosphatase,Creatinine,synergetic,blood urea nitrogen.

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INTRODUCTION

We are aware that there are a number of possible ways in which humans and domestic animals are exposed to pesticides in addition residues of pesticides contribute significantly to contamination of air, water, soil and food (Koeman, 1979) and thus the toxic effects of these substances may have consequences even for consumers of food indirectly. In the olden days more acutely toxic pesticides have been used for suicide and murder (Marrs, 1993) The Organo-phosphates after entering the body of an organism reaches the cholinergic sites of the nervous system and inhibit the activity of the AChE by binding at its active sites. AChE inhibition, thus leads to the accumulation of Ach at nerve endings which in turn cause the disruption of the nervous activity resulting in excitation, paralysis and finally the death of the organism (Lotti, 1995). Hence recently new combination of pesticides have been introduced to reduce environmental pollution and at the same time to have a maximum action in killing the pest animals.There are many many biochemical markers are screened to asses working of different organs.

Alkaline phosphotase estimations are used in the diagnosis of bone diseases associated with increased osteoblastic activity. Alkaline phosphotase is increased in osteomalacia, hepatic lesions and bone tumours. Creatinine is a waste product of creatine and is excreted by kidneys. Creatinine is synthesized from the amino acids glycine, arginine, and methionine by liver and pancreas. Glomerular filtration is responsible for removing creatinine from extracellular fluid.

Albumin has been reported to decrease with cirrhosis, nephritic diseases and malnutrition (Mc Pherson, 1984).An increase in blood glucose above normal may be due to insufficient production of insulin due to lesions in β cells of islets of Langerhans.ALT is present in high concentrations in liver and to a lesser extent in skeletal muscle, kidney and heart. Marked increase causes circulatory failure with shock and hypoxia, acute viral or toxic hepatitis. Moderate increase was observed in cirrhosis, infectious and liver congestion and cholestatic jaundice.AST is present in high concentrations in cells of cardiac and skeletal muscles, liver, kidney and erythrocytes. Damage to any of these tissues may increase plasma AST levels. Elevated levels are caused by circulatory failure with shock and hypoxia, myocardial infarction and skeletal muscle disease.AST is present in high concentrations in cells of cardiac and skeletal muscles, liver, kidney and erythrocytes. Damage to any of these tissues may increase plasma AST levels. Elevated levels are caused by circulatory failure with shock and hypoxia, myocardial infarction and skeletal muscle disease.

MATERIALS AND METHODS

The present study was carried out to evaluate the short-term sub acute (28 days) toxicity and neurotoxicity of combination pesticide Spark EC 36 [triazophos 35% + deltamethrin 1%] in Wistar rats.The acute toxicity of these combination pesticides were carried out to arrive at the LD50 dose [Finney, D.J. 1971] from the LD50 dosage the sub acute doses were fixed as 1/10, 1/20 and 1/40 th of LD 50 dosage (4, 8 and 16 mg/kg B.W.) These dosed pesticides were dissolved in water and administered orally using oral gavage for 28 days.On the 29 th day the animals were sacrificed to collect blood sample to screen the biochemical markers.AchEase

activity. AChE, a sensitive promarker of neurotoxicity which is widely distributed within the central nervous system (CNS) Sekar Babu Hari Ram 2004.

Principle of analysis

Alkaline phsphatase

Alkaline phosphotase are inhibited by phosphate, borate, oxalate and cyanide ions but activated by magnesium, cobalt and manganese substrates (Kachman and Moss, 1976).
Paranitrophenyl phosphate is colourless and the alkaline phosphotase splits off the phosphate group from it to form p-nitrophenol. Under alkaline conditions, p-nitrophenol is converted to p-nitrophenoxide ions, which exhibit yellow colour. The intensity of yellow colour is directly proportional to the enzyme and can be measured at 450nm using spectrophotometer.

Creatinine

Creatinine is a waste product of creatine and is excreted by kidneys. Creatinine is synthesized from the amino acids glycine, arginine, and methionine by liver and pancreas. Glomerular filtration is responsible for removing creatinine from extracellular fluid (serum)
Creatinine reacts with picric acid in alkaline medium to form reddish yellow complex, the intensity of which is directly proportional to the concentration of creatinine in the sample and can be measured at 520nm spectrophometrically (picric acid method).

Creatinine + NaOH + Picric acid --------→ Creatinine picric acid

Blood Urea Nitrogen: (BUN)

Urea is derived in the liver from aminoacids and therefore from protein whether originating from the diet or tissues. The source of ammonia (NH3) is the catabolism of amino acids or by absorption from large intestine (Duncan and Prasse, 1986). If the rate of production exceeds the rate of clearance, plasma concentration of ammonia rises. The procedure is based on the Berthelot reaction. Urease splits urea into ammonia and carbon dioxide. The ammonia reacts with phenol in the presence of hypochlorite to form indophenols, which in alkaline medium gives blue colour, the intensity of which can be measured at 620nm..

Protein:

According to Biuret method, proteins react with cupric ions in alkaline medium to form violet complex. The intensity of colour produced is directly proportional to proteins present in the sample and can be measured at 530 nm using a spectrophotometer.

Protein + Cu2 ----------→ purple blue complex

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Serum proteins have also been reported to decrease because of benzene, carbon tetrachloride or phosgene exposure (Duncan and Prasse, 1986

Albumin

Albumin present in the serum binds specifically with bromocresol green to form a coloured complex, the intensity of which can be measured spectrophotometrically at 640 nm.

Alanine aminotransferase (ALT)

Alanine aminotransferase (ALT) previously referred as glutamate pyruvate transaminases (GPT) catalyses the transfer of an amino group from the amino acid alanine to oxoglutarate to produce glutamate. The end product, pyruvate, undergoes oxidation in the presence of LDH to develop a coloured complex which can be measured at 520 nm.

L-alanine + 2-oxoglutarate ←---(LDH)-----→ Pyruvate + L-glutamate

Pyruvate + NADH + H ←----------→ L-lactate + NAD^+

Aspartate Aminotransferase (AST)

Aspartate Aminotransferase was once referred to as glutamate oxaloacetate transaminase (GOT). This enzyme catalyses the transfer of an amino group from the aminoacid aspartate to oxalobutarate to form L-glutamate. As with ALT, pyridoxal pyridoxamine-5' phosphate function as coenzyme which favours the production of aspartate invivo which can be measured at 520nm.

Principle:

L-aspartate + 2-oxoglutarate $\xrightarrow{MDH}$ Oxaloacetate + L- glutamate

Oxaloacetarte + NADH $\longrightarrow$ Malate + NADH

Pyruvate + NADH $\xrightarrow{LDH}$ L-Lactate + NAD

RESULTS

In the case of male rats a significant increase in glucose, creatinine and ALP was observed in groups III and IV when compared to untreated control. Protein, albumin, globulin, ALT and AST showed an increase only in group IV (16 mg/kg b.w.). BUN did not show any significant difference among the groups (Table 1).

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Table 1 Biochemical parameters of male rats treated with different concentrations of Spark EC 36

Group	Biochemical Parameters								
	Glucose mg/dl	Creatinine mg/dl	Protein g/dl	Albumin g/dl	Globulin g/dl	BUN mg/dl	ALP IU/l	ALT IU/l	AST IU/l
I	95.2^{a} ± 6.57	0.454^{a} ± 0.04	6.26^{a} ± 0.06	4.76^{a} ± 0.40	1.50^{ab} ± 0.28	20.01^{a} ± 2.56	69.19^{a} ± 6.56	32.26^{a} ± 7.64	62.36^{a} ± 3.74
II 4 mg/kg b.w.	97.6^{a} ± 6.07	0.534^{a} ± 0.07	6.22^{a} ± 0.45	4.81^{a} ± 0.43	1.41^{a} ± 0.19	20.60^{a} ± 2.19	68.63^{ab} ± 7.22	28.42^{a} ± 8.18	65.58^{ab} ± 9.13
III 8 mg/kg b.w.	119.8^{b} ± 6.53	0.716^{b} ± 0.12	6.90^{ab} ± 0.31	5.16^{ab} ± 0.33	1.74^{ab} ± 0.15	21.80^{a} ± 1.30	78.27^{b} ± 5.41	36.04^{a} ± 8.06	71.74^{ab} ± 8.71
IV 16 mg/kg b.w.	132.0^{c} ± 5.16	0.845^{c} ± 0.02	7.58^{b} ± 0.79	5.75^{b} ± 0.66	1.83^{b} ± 0.15	24.00^{a} ± 2.94	90.00^{c} ± 1.48	49.08^{b} ± 6.48	79.60^{b} ± 10.60

Values are presented as mean ± Standard Error

Values having similar superscripts are not statistically significant ($p>0.05$)

As in males, females also showed significant increase in glucose was observed in groups III and IV when compared with untreated control. But in the case of protein, albumin and globulin no significant difference was observed among the groups. An increase was noticed in the highest dose group (G IV) in the case of creatinine, BUN, ALP, ALT and AST (Table 2)

Table 2 Biochemical parameters of female rats treated with different concentrations of Spark EC 36

Group	Biochemical Parameters								
	Glucose mg/dl	Creatinine mg/dl	Protein g/dl	Albumin g/dl	Globulin g/dl	BUN mg/dl	ALP IU/l	ALT IU/l	AST IU/l
I	91.20^{a} ± 7.69	0.566^{a} ± 0.16	6.54^{a} ± 0.39	5.07^{a} ± 0.40	1.37^{a} ± 0.12	21.20^{a} ± 1.48	63.80^{a} ± 7.10	33.06^{a} ± 8.59	65.14^{a} ± 11.16
II 4 mg/kg b.w.	94.40^{a} ± 8.21	0.550^{a} ± 0.12	6.68^{a} ± 1.23	4.98^{a} ± 0.92	1.70^{a} ± 0.34	21.40^{a} ± 3.04	65.49^{a} ± 7.49	32.40^{a} ± 5.45	64.62^{a} ± 8.83
III 8 mg/kg b.w.	121.8^{b} ± 5.93	0.660^{a} ± 0.19	6.40^{a} ± 1.55	4.95^{a} ± 1.22	1.45^{a} ± 0.35	23.40^{ab} ± 2.70	72.45^{a} ± 13.55	31.58^{a} ± 6.09	73.56^{ab} ± 7.39
IV 16 mg/kg b.w.	136.0^{c} ± 6.88	0.945^{a} ± 0.25	7.33^{a} ± 0.74	5.60^{a} ± 0.57	1.73^{a} ± 0.21	27.75^{b} ± 1.50	91.50^{b} ± 15.96	51.45^{b} ± 5.17	86.18^{b} ± 9.78

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DISCUSSION

Acute OP poisoning causes various neurological signs in human and experimental animals (Wadia et al., 1947). This includes behavioral changes; sleep disturbances, tremors, convulsions, coma and respiratory/circulatory failures. Early signs and symptoms of OP poisoning like depression, emotional lability, insomnia and tremors [Sekar Babu Hari Ram 2010], exhibit as a result of the disturbances to the Central Nervous System and other important organs.It could be observed that the combination pesticide Spark EC 36, exhibit a different toxicological profile when compare to its constituents. There was a marked elevation in the plasma levels of total proteins and blood urea nitrogen, following triazophos intoxication (Sandhu and Bal, 1997). Long-term oral administration of triazophos at doses of 0.005 and 0.001 mg/kg/day for 150 days did not produce any toxic symptom in buffalo calves which is in accordance with the prsent study results .but significant changes were observed only higher dosed animals.whereas animals of G2 G3 did not showed any significant change .the results are quite contradictory with the result obtained from the dtudy on triazophos caused significant ($p < 0.05$) time and dose dependent reduction in the levels of total protein,acetylcholinesterase (AchE) and significant enhancement in the levels of total free amino acids,glutamine, adenosine monophosphate (AMP) eaminases, adenosine deaminases, glutamate dehydrogenase (GDH) [Abdul Naveed, P. et al, 2010] .So it could be understood that the toxicity of Spark EC 36 was not the same as that of the constitute pesticides namely triazophos and deltamethrin it may be due to the synergic effect of the constituents which attributes low mammalian toxicity with reference to biochemical markers.

SUMMARY

The combination pesticides exhibit toxicity in a different fashion compared to the toxicity of its constitute pesticides. From the earlier reports it is understood that toxicity of the combination pesticide in most of the cases vary compared to that of the individual pesticide in the combination .Hence long-term toxicity studies are very essential for combination pesticides. The present study is aimed to screen the biochemical markers to asses the extent of toxicity using 28 days short-term sub acute studies. From the present study it could be understood that the combination pesticides behave differently in its toxicological profile when compared to the toxicity of the individual pesticides in combination. Spark EC 36 which may be due to the synergetic effect of its constituents to reduce the toxicity. Similar studies can also help to identify combination pesticides that have eco-friendly with lesser mammalian toxicity.

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