A Potential Antidotal Effect of *Datura stramonium* Seeds on the Toxicity of Malathion Insecticide in Rats

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ABSTRACT

The purpose of the present study was to determine the antidotal effect of *Datura stramonium* aqueous seed extract (DSE) in treatment of toxicity of malathion. Twenty-five albino rats were evenly divided into five groups including the control. The control group (5) and the other four groups (1, 2, 3 and 4) received initially 500 mg/kg body weight of malathion. After ½ an hour, groups 2, 3, and 4 received 5, 7.5 mg/kg of DSE and 17 mg/kg atropine sulphate, respectively; this treatment was repeated after 4 hours, and group 5 was left as unintoxicated, untreated (control). Blood samples were collected three times, after ½ an hour of the first treatment, after ½ an hour of the second treatment and after 24 hours. In the treated groups, the level of the enzyme cholinesterase significantly increased towards the normal level, while the levels of ALT, AST and ALP significantly decreased. The rats were sacrificed after 24 hours, and tissue specimens were processed for histopathology. The result of histopathology showed normal internal organs in the groups dosed with 7.5 mg/kg DSE and the group treated with the drug. The groups treated with 5 mg/kg DSE showed mild toxicity in the internal organs and slightly high levels of serum enzymes. The malathion group (untreated) showed typical signs of toxicity in the internal organs and high level of serum enzymes. The study concluded that the dose of 7.5 mg/kg DSE possesses antidotal effect against malathion toxicity and has the same effect of atropine sulphate in treatment of toxicity and indicates that the protective action of DSE against the insecticide tested was dose dependant. This effect is most probably due to the presence of the atropine-like alkaloids in *D. stramonium* that may possess central anticholinergic effects which minimize the action of acetylcholine at muscarinic receptor.

Key words: *Datura stramonium;* malathion; antidote; rats
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INTRODUCTION

Plants have always played a major role in the treatment of animals' traumas and diseases worldwide. They have been used as sources of modern drugs, either by providing pure compounds, starting materials for partial synthesis of useful compounds or models for

synthesis of new drugs. *Datura stramonium* which belongs to the family *Solanaceae* is distributed throughout the warmer portion of the whole world. In Sudan, *D. stramonium* is widely distributed in Kassala, Dongla, Barber, Nuba mountains and Khartoum Province (Ali, 1995). All parts of Jimson weed are poisonous. Leaves and seeds are the usual causes of poisoning, but are rarely eaten due to their strong odour and unpleasant taste (Cornell University, 2009). The toxic principles of the plant are tropane belladonna alkaloids which possess strong anticholinergic properties (Ruhwald, 2005). These alkaloids include, hyoscyamine (in stems, leaves, roots and seeds), hyoscine inroots and Atropine (d,l-hyoscyamine) and scopolamine (l-hyosci ne) (Miraldi *et al*., 2001;. Steenkamp *et al*., 2004). The plant has a long history of use as a herbal medicine and its medicinal properties are notable. The leaves, flowering tops and seeds are anodyne, antiasthmatic, antispasmodic, hallucinogenic, hypnotic, mydriatic and narcotic (Chiej, 1984; Launert, 1981; Bown, 1995). It is, also, used as analgesic, anthelmintic and anti-inflammatory in the treatment of stomach and intestinal pain due to worm infestation, toothache and fever from inflammations (Tsarong, 1994). Jimson weed extract was used as a protective agent in severe Organophosphate toxicity by Theodore *et al*. (2004). They have proved that pretreatment with Jimson weed extract significantly increased survival following severe dichlorvos exposure in white rats. Malathion is one of the most commonly used Organophosphate; it has a wide range of control uses in agriculture and has been used to control, stored grain and forest pests (Brenner, 1992). The objective of this study was to investigate the use of *D. stramonium* seed extract in treatment of malathion toxicity in correlation with doses compared to a reference drug Atropine sulphate.

MATERIALS AND METHODS

The aqueous extract of *D. stramonium* seeds was used as post treatment to toxicity induced by an organophosphorus (malathion)**.** Twenty five male and female white Albino rats of weighing 115-180 gm, were obtained from the Medicinal and Aromatic Plant Research Institute, National Centre for Research, Khartoum, Sudan. The animals were weight- distributed and allotted randomly to 5 groups of 5 rats each. Group 1 received only malathion at a dose of 500 mg/kg body weight (BW). Group 2 was exposed to 500 mg/kg BW and after half an hour treated with *D. stramonium* seeds extract 5 mg/kg BW, intraperitonially (I.P). Group 3 received 500 mg/kg BW malathion and after half an hour was treated with *D. stramonium* seeds extract at a dose of 7.5 mg/kg BW I/P. Rats in group 4 were exposed to toxicity by malathion 500 mg/kg BW orally and post treated with 17 mg/kg BW Atropine sulphate I/P. The treatment dose of Atropine sulphate and *D. stramonium* aqueous extract was repeated after four hours. Group 5 served as unintoxicated and untreated control group.

Clinical signs were observed and blood samples were collected from the ocular vein three times. The first blood samples were collected after 30 minutes after administration of *D. stramonium* seed extract and Atropine sulphate. The second collection was after 30 minutes after the second treatment with *D. stramonium* seed extract and atropine. The third collection was after twenty four hours from the second treatment (by *D. stramonium* seed extract and atropine). Sera were analyzed for the concentration of cholinesterase and for the activities of Alanine amino transferase (ALT), Aspartate amino transferase (AST) and Alkaline Phosphatase (ALP). The concentrations of metabolic indicators, total protein, albumin, globulin, urea and creatinine were also evaluated. The rats were scarified after 24 hours and tissue specimens of livers, hearts, kidneys, spleens and brains were fixed in 10% buffered formalin and processed for histopathology.

RESULTS

Signs were noted by observing access of rats to food and water since there were no obvious clinical signs except for dullness and slow movement during on the first 30 minutes. Two hrs and 30 minutes after the beginning of the experiment, the rats in groups 2, 3 and 4 treated with the plant extract and atropine sulphate showed normal access to water, but remained inappitant. The untreated group (malathion group) showed no response to the presence of water. Three hours later (one hour after the second dose), only the treated groups started to take food. Table (1) shows pathological changes and serum constituents of rats post-treated by *D. stramonium* seed extract (two doses 5 mg/kg BW/rat and 7.5 mg/kg BW/rat) with regard to Atropine sulphate

DISCUSSION

The present study attempted to determine the efficacy of *D. stramonium* seed extract as an antidote to the toxicity of an insecticide organophosphate evaluation of *D. stramonium* seed extract as a post treatment in malathion poisoning had showed significant increase in ALT during the first 30 minutes which indicated tissue damage. A single intraperitoneal or oral doses of malathion, trichlorofon or dioxathion in rats, an increase in the activities of liver tyrosine transaminase and alkaline phosphatase, as well as a decrease in the level of adrenal ascorbic acid (Murphy, 1966). The results of this experiment support the hypothesis that acute poisoning may produce metabolic alterations which are mediated through the pituitary-adrenal system. Thirty minutes after the second dose, the levels of AST, ALT and ALP in groups 2, 3 and 4 (*D. stramonium* 5 mg/kg, *D. stramonium* 7.5 mg/kg and Atropine sulphate) were reduced and were significantly different from the control group. After 24 hours, the levels of these enzymes in all the groups, were reduced almost to the normal level except for the malathion group. Group 2 (*D. stramonium* 5 mg) had slightly higher enzymes levels than groups 3 and 4 (*D. stramonium* 7.5 mg/kg and atropine). These results together

with histopathological changes were similar to pathological lesions of groups 3 and 4 (*D. stramonium* 7.5 mg/kg and atropine sulfate) indicating the effective action of the treatment.

The levels of total protein, globulin and albumin; were highly reduced in the malathion group and the levels of the metabolic indicators had lesser values compared to groups 3 and 4 (*D. stramonium* 7.5 mg/kg and atropine); another evidence of the extract protective action. There was an apparent reduction in cholinesterase activity in all the groups during the first 30 minutes after the first dose and this was recorded by Frawley *et al*. (1957), who studied simultaneous administration of malathion and ethyl pnitrophenyl thionobenzenephosphate (EPN) which potentiated cholinesterase inhibitory effect of malathion in mice, rats and dogs. The level of this enzyme began to increase after 24 hours especially in groups 3 and 4 (*D. stramonium* 7.5 mg/kg and atropine) which means that the body began to eliminate malathion from its tissues. The usual duration of anticholinergic effect following exposure to the *D. stramonium* extract is 12–48 hours (Levy 1977, Chang *et al*., 1999; Clancy *et al*., 2001). This long duration of clinical effect has a potential advantage in the treatment of OP toxicity since the effect of most OP is long. Patients treated with this extract may not require additional doses of antidote. *D. stramonium* crosses the blood–brain barrier, and has central anticholinergic effects and antagonizes the peripheral muscarinic receptor. (Clancy *et al*., 2001) The high concentrations of these anticholinergic alkaloids in the *D. stramonium* seeds render the extract of this plant a potentially useful agent for the treatment of organophosphorus toxicity. It is concluded that the protective action of *D. stramonium* seed extract against insecticide is dose dependant and that treatment with *D. stramonium* seed extract has a significant protective action in a rat model of organophosphorus poisoning.

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Table 1: Pathological changes in rats treated by *D. stramonium*

changes Groups	**Pathological changes**
Group 1	Pale liver with haemorrhages. The heart, spleen, brain, and kidneys were characterized by severe congestion and haemorrhages.
Group 2	Distended stomach, severe congestion in the heart, spleen with fatty changes in the liver and scattered spots of necrosis in it's upper surface.
Group 3	Distended stomach, fatty changes and congestion in the liver and kidney, no significant changes in the heart and brain
Group 4	Distended stomach with gases in three rats. The liver is slightly congested. The kidney showed fatty changes. No significant changes, in the heart and brain

Table 2. Histological changes in serum constituents of rats post-treated by *D. stramonium* seed extract (two doses 5mg/kg b.wt/ rat and 7.5 mg/kg b.wt/ rat) with regard to a reference drug –Atropine sulphate

changes Groups	**Histological changes:**
Group1	**Liver:** congestion, generalized necrosis land lymphocytic infiltration. **Heart-and kidney:** congestion with lymphocytic infiltration, and severe necrosis of the cortical and meddullary tubules plus necrosis of the glomeruli.. **spleen** :severe congestion **Brain** :severe leucocytic infiltration
Group 2	**Liver** :congestion in the central vein, fatty change and centrilobular necrosis.- **Heart, kidney and spleen**: was severely congested with lymphocytic infiltration.. **Brain** :mild infiltration
Group3	**Liver fatty change** -slight necrosis of the hepatocytes. **heart**-slight congestion **The kidneys** lymphocytic infiltration, shrinkage of glomeruli, with fatty of the central and meddullary tubules. Spleen-: slight haemosidrosis . **brain** no significant histopathological change
Group4	**Liver**: slight congestion, fatty change and slight necrosis. – **Heart-:**no histological changes. **Kidneys**: lymphocytic infiltration, shrinkage of glomeruli, with fatty changes of the meddullary tubules . **The spleens**: showed slight haemosidrosis. **The brain** : no significant histopathological change.

Changes in the serum constituents:

Table (3) and Fig. (1). Changes in serum constituents of rats post-treated by *D. stramonium* seed extract (two doses 5 mg/kg b.wt/ rat and 7.5 mg/kg b.wt/ rat) with regard to a reference drug –Atropine sulphate

Groups	**Changes**		
	After 30 minutes of administration of the first dose of *D. stramonium* seed extract and atropine	**After 30 minutes from the second treatment**	**After 24 hours**
Group1	cholinesterase, was significantly reduced AST ,ALP and urea, increased significantly -ALT increased significantly -No significant changes in total protien, creatinie and albumin	ALT, AST and ALP and urea increased significantly cholinesterase .Total protein and albumin was significantly reduced	ALT, AST, ALP urea and creatinine increased, cholinesterase , Total protein and albumin are insignificantly reduced ,
Group 2	cholinesterase, was significantly reduced AST ,ALP and urea, increased significantly -No significant changes in total protien, creatinie and albumin	reduction in the level of cholinesterase ,Total protein and albumin -ALT, AST and ALP and urea are reduced	normal levels of ALT and ALP, while the level of AST was significantly -increased . cholinesterase , Total protein and albumin are insignificantly reduced ,
Group 3	cholinesterase, was significantly reduced - AST ,ALP and urea, increased significantly -No significant changes in total protien, creatinie and albumin	cholinesterase was significantly increased - ALT, AST and ALP and urea are reduced ,total protein and albumin	normal levels of cholinesterase ,ALT, AST and ALP , level of total protein and albumin were not significantly reduced
Group 4	cholinesterase, was significantly reduced - AST and urea, increased significantly -No significant changes in total protien , creatinie and albumin	cholinesterase was significantly increased - ALT, AST and ALP and urea are reduced ,total protein and albumin	normal levels of cholinesterase ,ALT, AST and ALP , level of total protein and albumin were not significantly reduced

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Table 4. Analysis of variance and Average (mean ±SE) values of serum constituents of rats post-treated with *D. stramonium* seed aqueous extracts and intoxicated with malathion: (**TIME**) After 30 minutes from the first treatment dose

groups	**ALT(i.u/l)**	**AST(i.u/l)**	**ALP(i.u/l)**	**T,Protien(g/dl)**	**Globulin(g/dl)**	**Albumin(g/dl)**	**Urea (mg/dl)**	**Creatinine**(mg/dl)	**Cholinesterase(**
F value	3.43*	1.57*	2.99*	1.08	1.07	3.25	3.61*	3.27	
G1	51.0± 20.5 a	286.2±2.5.a	200.4±17.7 b	7.2 ±0.3a	3.2±0.4a	3.5 ±0.1ab	54.0±3.1a	0.620.1a	159.33 3
G 2	45.80±28.4ab	220.8±ab4.0b	197.2±27.3 b	7.3±0.3 a	3.7 ±a	3.3 ±0.1b	47.8 ±3.3bc	0.62a	12
G 3	51.0011.6±a	227.0±ab4.7b	182.0±26.6 b	7.9±0.2 a	3.5±0.1a	3.2±0.2 b	42.8±3.0 bc	0.72±0.02 a	361.04
G4	52.00±19.1a	242±ab2.8b	118.6±8.9a	6.80±0.5a	2.7 ±0.5a	3.6±0.2ab	50.6±3.7 bc	0.880.1 a	363.673
5G	33.33 bc	178.33ab	110.0a	7.6 a	4.1± a	4.13a	38.67c	0.83 a	4

(EMIT) After 30 minutes from the second treatment dose

groups	**ALT(i.u/l)**	**AST(i.u/l)**	**ALP(i.u/l)**	**T,Protien(g/dl)**	**Globulin(g/dl)**	**Albumin(g/dl)**	**Urea(mg/dl)**	**Creatinine**	**Cholinesterase(**
F value	**5.87**	**5.72**	**5.07**	**3.23**	**4.31**	**3.19**	**1.187**	**1.95**	
G1	82.4 ±15.2a	293.80 ±15.1a	196.2±5.3a	5.8±0.2 b	2.4±0.3b	3.16±0.3 b	48.80 ± 5.7a	0.8±0.1 ab	277.00 ±
G2	42.6±31.2 b	230.0 ±5.2 b	137.60 ±18.1b	6.9 ± 0.1ab	3.6±0.3a	3.2 ±0.1ab	47.80±3.3a	0.6±0.1 b	291.67±
G3	47.8±10.2b	219.00±3.4 b	115.80±20.5b	6.84±0.3 ab	3.2±0.2a	3.52±0.3ab	40. 0± 8.9a	0.9± 0.9a	351.67±40
G4	50.2±b9.5b	190.8 ±4.3b	117.00±10.5 b	7.00±0.6a	3.78 ±0.1a	3.1 ±b	50.25 ±a	0.72±0.3 ab	394.67 ±2
G5	29.75±4.7b	173.75 ±1.7 b	111.25±7.7 b	7.62±0.2 a	3.6±0.3a	4.05±0.1 a	38.25 ±0.6a	0.75±0 ab	486.00±1

(TIME) after 24 hours

groups	ALT(i.u/l)	AST(i.u/l)	ALP(i.u/l)	T,Protien(g/dl)	Globulin(g/dl)	Albumin(g/dl)	Urea (mg/dl)	Creatinine	Cholinesterase(u
F value	4.69	8.14	17.23	5.46	4.89	6.63	1.58	3.72	
G1	91.40 ±28a	317.60 ±68a	210.60 ±10.9a	4.8 ±0.7c	1.6 ±0.6b	2.72±0 b	62.60±5.8b	0.94±0.2 a	216.67±1
G2	45.60±23.7 b	257.4±5.5 b	110.20 ±15.2b	5.80 ±0.4bc	2.8±0 a	2384±0.2b	48.20±5.6 a	0.5±0.6 c	253.00±3
G3	38.00 ±2.07 b	218.4±5.6bc	105.60 ±13.2b	7.24 ±0.6ab	3.5±0.2 a	3.68±0.4a	40.20±6.3a	0.8 ±0.1ab	385.00 ±
G4	35.00± 8.3b	188.80± 1.2c	106.60±5.2 b	6.18 ±0.2abc	2.84±0.4 a	3.34 ±0.1ab	40.40± 3.8 a	0.58±0.1 bc	394.33
G5	29.75±3.8b	173.75±1.7 c	105.60 ±7.2b	7.650 ±0.2a	3.85±0.1a	3.97±0a	37.75±0.6 a	0.8±0.1ab	485.67

Means with the same letter are not significantly different (.P> 0.05) *At (4, 20) degrees of freedom. ** 5

G1=only Malathion 500mg/kg/body weight.
G2=Malathion 500mg/kg /body weight and *Datura* seed extract 5mg /kg/body weight
G3= Malathion 500mg/kg /body weight and *Datura* seed extract 7.5mg /kg/body weight.
G4 =.malthion 500mg /kg/body weight and Atropine Sulphate 17 mg/kg/body weight.
G5=Control

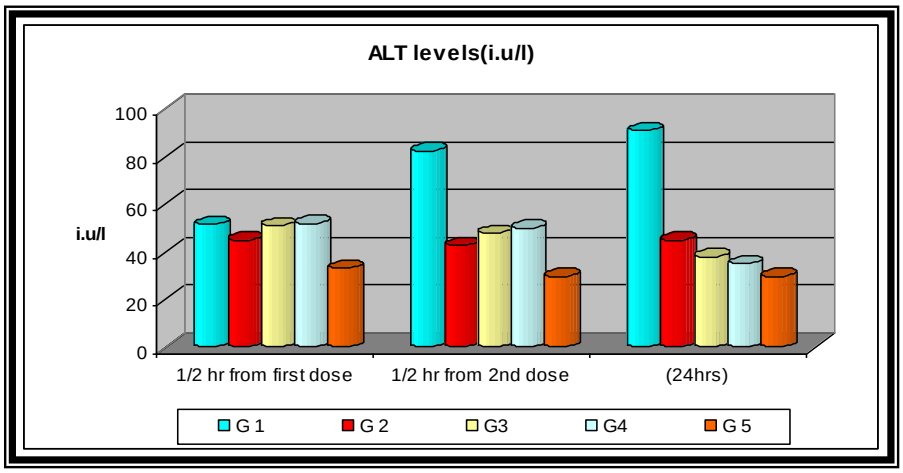

Fig. (1). Comparison between ALT levels in rats treated with malathion, *D. stramonium* seed extract and malathion and Atropine and malathion

G1= Only Malathion 500mg/kg/body weight
G3= Malathion 500mg/kg /body weight and Datura seeds extracts at a dose of 7.5mg/kg /body we
G2= Malathion 500mg/kg /body weight and Datura seed extract 5mg /kg/body weight
G4= Malathion 500mg /kg/body weight and Atropine Sulphate 17 mg/kg/body weight
G5= Control

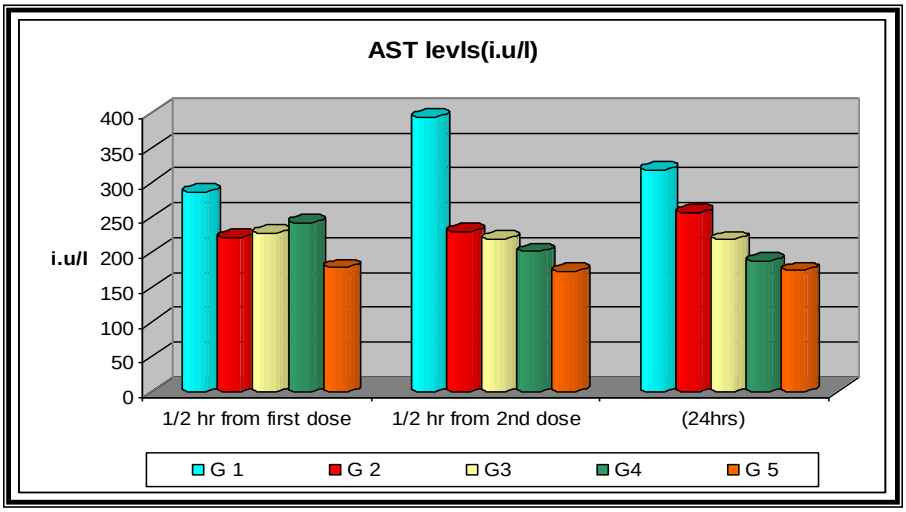

Fig. (2). Comparison between AST levels in rats treated with malathion, *D. stramonium* seed extract and malathion and Atropine sulphate and malathion

G1= Only Malathion 500mg/kg/body weight
G2= Malathion 500mg/kg /body weight and Datura seed extract 5mg /kg/body weight
G3= Malathion 500mg/kg /body weight and Datura seeds extracts at a dose of 7.5mg/kg/body weight
G4= Malathion 500mg /kg/body weight and Atropine Sulphate 17 mg/kg/body weight
G5= Control

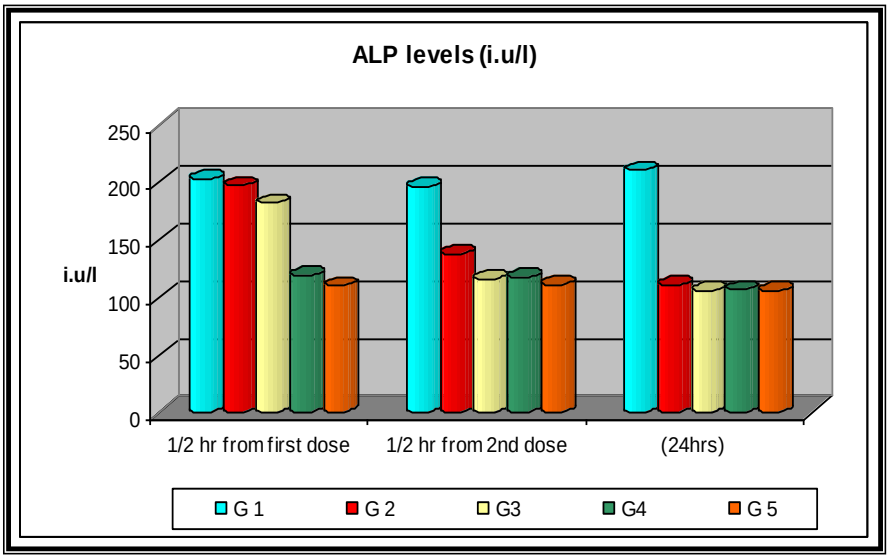

Fig. (3) Comparison between ALP levels in rats treated with malathion, *D. stramonium* seed extract and malathion and Atropine sulphate and malathion

G1=only Malathion 500mg/kg/body weight.
G3= Malathion 500mg/kg /body weight and Datura seeds extracts at a dose of 7.5mg/kg /body weight
G2= Malathion 500mg/kg /body weight and Datura seed extract 5mg /kg/body weight
G4= Malathion 500mg /kg/body weight and Atropine Sulphate 17 mg/kg/body weight
G5=Co

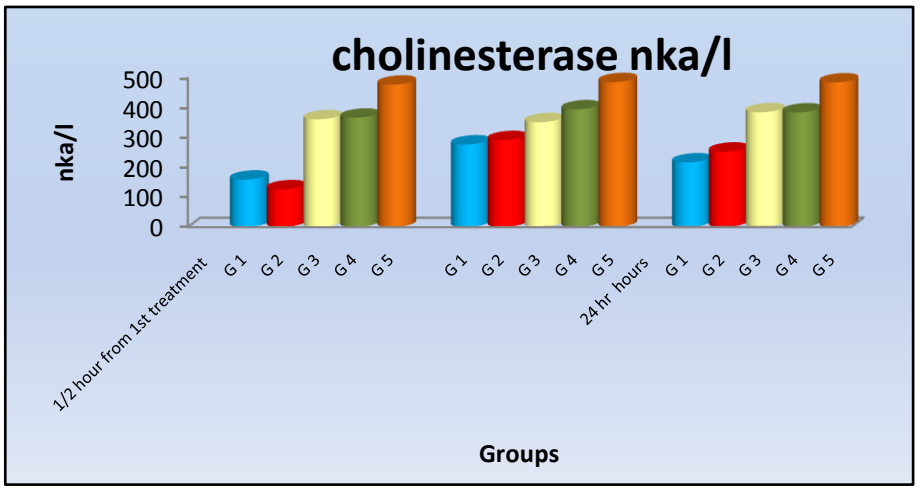

Fig (4) Comparison between cholinesterase levels in rats treated with malathion, *D. stramonium* seed extract and malathion and Atropine sulphate and malathion

G1= Only malathion 500mg/kg/body weight

G2= Malathion 500mg/kg /body weight and Datura seed extract 5mg /kg/body weight