Journal

BIOCHEMICAL EFFECTS OF GUAVA DIET ANTIOXIDANT AS A HYPOINTENSIVE AGENT FOR DIMETHOATE TOXICITY ON ENERGY AND CYTOCHROM-C RESPIRATORY SYSTEMS IN THE INTOXICATED ALBINO RATS

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ABSTRACT

Technical and formulated dimethoate sublethal doses (twentieth of LD_{50}) were administrated to male albino rats. The pesticide was used also, feeding on guava as antioxidant diet treated and reduced the pisticde toxicity by dermal or oral every 2 days. For guava, (which mixed with the normal diet) was fed *ad libitum* to rat for three months. Energy system (ATP, ADP, AMP and myokinase) and respiratory cytochrome-c- system (cytochrome-c, cytochrome-c-oxidase and succinate-cytochrome-c-reductase) in liver, brain and kidney tissues of adult male albino rats under these treatments were investigated.

The organs tissue contents of adenosine -5- triphosphate (ATP) was increased, whereas the contents of adinosine – 5- diphosphate (ADP) and adenosine-5- monophosphate (AMP) were decreased either under the effect of technical or formulated dimethoate of all tested organs, relative to control.

Furthermore, the effect of the both forms of dimethoate on mitochondrial cytochrome- c- level as well as the activity of cytochrom-c- oxidase and succinate cytochrome-c-reductase was determined. Considerable decrease in cytochrome-c level was noticed while significant stimulation in the activity of respiratory mitochondrial enzymes of liver, brain and kidneys were observed. The effect of adjuvant on the influence of formulated form was also discussed.

Generally, formulated dimethoate treatments were more effective than technical ones. Also, the effects of orally ingestion were more than dermally induction relative to control.

Feeding *ad libitum* on guava as antioxidant agent had antagonistic influences against the dimethoate toxicity. However, the present antioxidant treatment improved the studied organophosphours pesticide effect in which energy-system (ATP, ADP and AMP as well as myokinase activity) and respiratory system of cytochrome-c (cytochrome-c – content and cytochrome-c – oxidase and succinate – cytochrome – c- reductase activities) were normalized nearly around of that value in liver, brain and kidneys of normal control.

It is of interest to note that the guava as antioxidant reduced the toxic influences of dimethoate in the experimental animals and improved the disturbance of organophosphorous pesticide toxicity in respiratory system including energy and cytochrome-c- systems metabolism.

INTRODUCTION

Pesticides are chemicals commonly used to control and eradicate disease reactors which in turn improve agricultural production and protect stored agricultural products. Greater reliance on the use pesticides to maintain higher agricultural productivity appears inevitable as the demand for food increases with increasing population. In developing countries, the use of pesticides has become so important that their use is inextricably linked with improvement of human welfare (Osibanjo, 1989). Pesticides are usually applied in their formulated form where active ingredient is combined with organic solvents, emulsifying and wetting agents, which affect the pesticides penetration.

The W.H.O (1991) emphasized that the final toxic classification of any pesticide in intended to be by its formulation.

Dimethoate is main mode of toxic action is through the inhibition of acetylcholin esterase (A.Ch.E.) (Pesticide Manual, 2000).

Dimethoate belongs to the organophosphorous class of insecticides. Dimethoate is activated to the corresponding oxygen analog, which in turn is responsible for its mammalian toxicity through acetylcholinesterase (A.Ch.E.) inhibition. Dimethoate is known to produce oxidative stress resulting in the accumulation of lipid peroxidation products in deferent organs of rats

oreganophosphorous pesticides have been shown to damage DNA also (Shadnia et al, 2005).

Indeed, literature background revealed that a few reports dealt with the metabolic changes and with other side effects of formulated dimethoate as compared with technical one. Abou-Zeid et al (1993) observed that formulated malathion was more as toxic to rats as the pure technical ingredient, and acetylcholinesterase activity was inhibited and blood serum profile was changed by formulated dimethoate more than that by technical one with dermal treatment.

Links between oxidative stress and adverse health effects have been suggested for several groups of diseases such as cardiovascular, respiratory, neurological as well as for the general aging process. Several drugs, xenobiotics and environmental pollutants including pesticides are known to cause this imbalance between formation and known to cause this imbalance between formation and removal of free radicals. Biological antioxidants, including vitamins, can prevent the uncontrolled formation of free radicals and activated oxygen species or inhibits their reaction with biological structures. The destruction of most free radicals and activated oxygen species rely on the oxidation of endogenous antioxidants mainly scavenging and reducing molecules (Verma et al, 2007).

The present work aimed to comparative study between the effect of technical dimethoate and formulated one by oral and dermal on the contents of ATP, ADP and AMP and the activity of myokinase of liver, brain and kidneys tissue as will as the respiratory cytochrome-c-system such as cytochrome-c level, cytochrome-c- oxidase and succinate-cyochrome -c- reductase activity in mitochondria of the same three studied organs. Also studies were done to determine the effect of guava diet antioxidants as antitoxic agents on the dimethoate danger in respiratory systems.

MATERIALS AND METHODS

Dimethoate (O, O-dimethyl S-methylcarbamoylmethyl phosphorodithioate 2-dimethoxyphosphinothioylthio – N – methyl acetamide) technical (95% a,i), (Dimethoate LD_{50} oral = 387 mg/kg and Dermal = 2000 mg/kg body weight of rat) and formulated (40% E.C) was provided from the central Agricultural pesticides laboratory, ARC Ministry of Agriculture, Dokki, Egypt and used in this study.

Sixty healthy adult male albino rats, *Rattus norvegicus*. Sprague Dawley strain (each weighing 100±10g) were obtained from the animal house of Nutrition Institute, Cairo. The animals were kept under normal healthy laboratory conditions for two weeks in their cages prior to the starting of experiment for acclimatization. During this period, the animals were fed on normal diets *ad libitum*. The diet consisted of casein 18.8%, methionine 0.2%, cotton seed oil 10%, cellulose 5%, salt mixture 4% (Hegested et al, 1941) vitamins mixture 1% (Cambell, 1961) and rice starch 60%. Rats were allowed free excess of water and diet. They were then divided into ten groups (6 rats each). The first group served as control and the second group fed on normal diet with 20% dried guava. The third group was ingested with the sublethal dose of dimethoate which was twentieth (1/20) of oral LD_{50} of technical dimethoate. The fourth group was the same of 3^{ed} group but fed on normal diet with 20% dried guava. The fifth group was used for the dermal sublethal dose of dimethoate which was twentieth (1/20) of the dermal LD_{50} of technical dimethodate (Pesticide Manual 2000) which was applied on dorsal skin shaved area of 2x2 cm. One day before dosing an area of 2x2cm on the back of the dermally treated rats was shaved with care not to abrade the skin. The shaved area was washed with acetone. The doses were then applied evenly and carefully on the shaved area of rat skin (Abou-Zedi et al, 1993). The sixth group was the same of the 5th group but fed on normal diet with 20% dried guava. The seventh group was ingested orally with the sublethal dose of formulated dimethoate (equal 1/20 of oral LD_{50} for technical dimethoate). The eight group was the same of 7th group but fed on normal diet with 20% dried guava. The ninth group was used for the dermal sublethal dose of formulated dimethoate (equal 1/20 of dermal LD_{50} for technical dimethoate) which was applied like the same of group five. The last group was the same of 9th group but fed on normal diet with 20% dried guava.

Technical or formulated dimethoate was used without any additions for dermal induction but for oral the dose was emulsified with 0.5 ml distilled water. One was induced every 48 hours during the experimental period of 90 days, either for dermal or oral administration of the both forms of dimethoate. Diet and water were supplied *ad libitum* for all groups during the experimental period.

The animals were killed by decapitation at the end of the experimental period (90 days). Liver, brain and kidneys were

dissected. Mitochondria of liver, brain and kidneys were prepared and emulsified with 1% Triton X-100 (3ml) at 0°C for 30 min. Mitochondrial enzymes were liberated and assayed (Walliaux and C. de- Duve, 1956 and Astawrov, 1974).

Cytochrome -c- content was determined spectrophotometrically according to the method of Williams and Thorp (1969). Cytochrome -c- oxidase activity was determined spectrophotometrically according to the method of Smith (1955) and succinate-cytochrome -c- reductase activity was assayed spectrophotometrically as described by King (1963). Myokinase activity was determined according to the method described by Bergmeyer (1974) in the tissues homogenate. Adenosine-5- triphosphate (ATP) was determined as described by Lamprecht and Trautschold (1962). Adenosine-5-diphosphate (ADP) and adenosine-5- monophosphate (AMP) were determined according to the method described by Adams (1962) in the tissues homogenate. Total soluble protein content was determined according to the method of Bradford (1976).

Statistical analysis was achieved using the analysis of variance (t-test) as described by Snedecor and Cochran (1967).

RESULTS AND DISCUSSION

The effect of technical and formulated dimethoate as well as an antioxidant agent (guava) against the toxicity of the pesticide on the energy and cytochrome -c- respiratory systems was studied and shown in tables (1, 2, 3 and 4).

The influence of administrating formulated and technical dimethoate by oral and dermal on energy system (ATP, ADP and AMP contents as well as myokinase activity) in liver, brain and kidneys tissues of male albino rats was studied and the results are presented in tables (1, 2 and 3). The energy metabolites i.e, ATP, ADP and AMP in different organs tissue of normal and administrated rats by technical and formulated dimethoate were affected. Thus ATP contents were generally increased in all studied treatments. The highest increase was noticed in rats ingested by formulated dimethoate flowed by oral technical one and dermal formulated dimethoate then dermal technical dimethoate treatment, respectively. On the contrary, both DAP and AMP contents were reduced under the same condition treatments. It must be added that ADP contents were decreased in

administrated rats by dimethoate under all the studied treatments which was approximately similar to AMP content relatively to control.

These results might be related either to the high rate of ATP synthesis of the energy liberated during the metabolic processes through trapping inorganic phosphate with AMP and ADP to form ATP. These findings were in parallel with the our results of thyroid gland and acid and alkaline phosphatase (Abdel – Rahim 2007) and also were confirmed by those of Abd El-Rahim et al. (1994). They found that formulated organophosphorous pesticide treatments stimulated protein biosynthesis, and cytochrome-enzyme-system greater than the treatments of technical ones.

Table (1): Effect of guava as antioxidant agent on the dimethoate toxicity in ATP content of organs tissues in male albino rats.

Treatment	Liver		Brain		Kidneys	
	U mal/g tissue	%	U mal/g tissue	%	U mal/g tissue	%
Control	9.75±0.60	100	6.11±0.37	100	0.81±0.04	100
Control + guava	10.00±0.61	103	6.20±0.41	101	0.80±0.03	99
Oral						
technical p	12.00±0.60*	123	9.21±0.56*	151	1.40±0.044	173
technical p + guava	11.00±0.53*	113	9.00±0.42*	147	1.11±0.048*	137
formulated p	10.90±0.70*	112	8.69±0.70*	142	1.14±0.062*	141
formulated + guava	10.00±0.42	103	8.20±0.39*	134	1.00±0.059*	124
Dermal						
technical p	13.01±0.61*	133	10.10±0.50*	165	1.55±0.069*	191
technical p + guava	11.09±0.50*	114	9.89±0.50*	162	1.09±0.047*	135
formulated p	11.88±0.68*	122	9.01±0.51*	147	1.22±0.068*	151
formulated p + guava	10.01±0.47	103	8.61±0.38*	141	1.01±0.042*	125

% relative to control p = the pesticide

Different is significant at control ($P < 0.05$)

Table (2): Effect of guava as antioxidant agent on the dimethoate toxicity in ADP and AMP contents of organ tissues in male albino rats.

Treatment	ADP M mal/g tissue			AMP M mal/g tissue		
	Liver	Brain	Kidneys	Liver	Brain	Kidneys
Control	0.154±0.006	0.540±0.031	0.139±0.006	0.630±0.032	0.250±0.011	0.270±0.011
%	100	100	100	100	100	100
Control + guava	0.160±0.007	0.541±0.021	0.140±0.007	0.632±0.031	0.252±0.010	0.265±0.011
%	104	100	101	100	101	98
Oral technical p	0.095±0.005	0.370±0.024	0.095±0.004	0.450±0.031	0.150±0.011	0.197±0.008
%	62*	68*	68*	71*	60*	73*
Oral technical p – guava	0.101±0.004	0.4000±0.002	0.100±0.001	0.469±0.020	0.167±0.008	0.205±0.009
%	66*	74*	72*	74*	67*	76
Oral formulated p	0.102±0.006	0.490±0.023	0.123±0.006	0.561±0.031	0.213±0.011	0.232±0.010
%	66*	91*	88*	89*	85*	86*
Oral formulated p + guava	0.117±0.005	0.500±0.021	0.130±0.007	0.600±0.029	0.230±0.010	0.270±0.012
%	76*	93*	94	95	92*	89*
Dermal technical p	0.107±0.05	0.321±0.017	0.052±0.002	0.382±0.011	0.125±0.006	0.167±0.008
%	69*	59*	37*	61*	50*	65*
Dermal technical p + guava	0.110±0.006	0.410±0.021	0.062±0.002	0.100±0.021	0.130±0.006	0.181±0.009
%	71*	76*	45*	64*	52*	67*
Dermal formulated p	0.093±0.004	0.392±0.020	0.105±0.006	0.471±0.030	0.175±0.011	0.194±0.009
%	60*	73*	76*	74*	70*	72*
Dermal formulated p + guava	0.121±0.005	0.461±0.020	0.119±0.006	0.581±0.021	0.200±0.011	0.203±0.010
%	79*	85*	86*	92*	80*	75*

% relative to control p = the pesticide
Different is significant at control ($P < 0.05$)

Table (3): Effect of guava as antioxidant agent on the dimethoate toxicity in myokinase activity and cytochrome-c content organs tissues in male albino rats.

Treatment	ADP M mal/g tissue			AMP M mal/g tissue		
	Liver	Brain	Kidneys	Liver	Brain	Kidneys
Control	0.490±0.021	0.510±0.024	0.221±0.011	23.41±1.11	9.30±0.52	27.20±1.21
%	100	100	100	100	100	100
Control + guava	0.500±0.011	0.512±0.021	0.222±0.011	24.00±1.04	9.35±0.611	27.21±1.11
%	102	100	100	103	101	100
Oral technical p	0.318±0.017	0.300±0.012	0.144±0.006	21.00±1.01	7.90±0.410	23.89±1.12
%	65*	59*	65*	90*	85*	88*
Oral technical p + guava	0.360±0.012	0.351±0.018	0.157±0.008	21.60±1.111	8.11±0.421	24.11±1.32
%	74*	69*	71*	92*	87*	89*
Oral formulated p	0.343±0.016	0.372±0.019	0.166±0.007	19.01±0.51	7.01±0.350	221.81±1.00
%	70*	73*	75*	81*	75*	80*
Oral formulated p + guava	0.400±0.020	0.391±0.020	0.177±0.006	20.01±1.13	7.54±0.361	22.42±1.01
%	82*	77*	80*	86*	81*	82*
Dermal technical p	0.279±0.011	0.255±0.014	0.113±0.004	21.51±1.05	8.00±0.460	25.19±1.21
%	57*	50*	51*	93*	86*	93*
Dermal technical p + guava	0.320±0.011	0.301±0.017	0.139±0.007	22.00±1.02	8.21±0.421	25.41±1.09
%	65	59	63	92	88	93
Dermal formulated p	0.308±0.012	0.311±0.014	0.130±0.006	21.00±1.11	1.80±0.390	24.00±1.21
%	63*	61*	59*	90*	84*	88*
Dermal formulated p + guava	0.350±0.016	0.333±0.016	0.148±0.007	22.40±1.09	8.00±0.411	25.00±1.21
%	71*	65*	67*	96	86*	92*

% relative to control p = the pesticide Different is significant at control ($P < 0.05$)

Table (4: Effect of guava as antioxidant agent on the dimethoate toxicity in cytochrom-c-oxidase and succinate-cytochrome-c-reductase acivity in organs tissues in male albino rats

Treatment	ADP M mal/g tissue			AMP M mal/g tissue		
	Liver	Brain	Kidneys	Liver	Brain	Kidneys
Control	1.96±0.100	29.50±1.19	3.10±0.19	15.50±0.75	49.40±2.22	14.00±0.80
%	100	100	100	100	100	100
Control + guava	2.00±0.110	30.00±1.57	3.11±0.16	15.48±0.80	50.00±2.47	14.11±0.71
%	102	102	100	100	101	101
Oral technical p	3.71±0.16	58.80±2.98	4.28±0.27	17.77±1.00	59.00±3.00	15.61±0.67
%	189*	199*	138*	115*	119*	112*
Oral technical p + guava	3.33±0.13	50.12±2.46	4.00±0.18	17.51±0.91	56.11±2.86	15.00±0.68
%	170*	170*	129*	113*	114*	107*
Oral formulated p	3.96±0.20	60.00±3.00	4.69±0.23	18.12±0.90	60.00±2.98	16.11±0.77
%	202*	203*	151*	117*	121*	115*
Oral formulated p + guava	2.81±0.12	51.11±2.75	4.37±0.21	17.77±0.87	56.00±1.46	15.80±0.71
%	143*	173*	141*	115*	113*	113*
Dermal technical p	3.49±0.15	47.32±2.22	4.07±0.20	16.89±0.83	55.11±2.41	14.69±0.69
%	178	160	131	109	112	105
Dermal technical p + guava	3.01±0.17	41.79±2.11	3.72±0.17	16.09±0.81	53.27±2.61	13.79±0.54
%	154*	142*	120*	104*	108*	99
Dermal formulated p	3.80±0.16	57.00±3.00	4.19±0.21	17.59±0.84	58.15±2.88	15.52±.061
%	194*	193*	135*	113*	118*	111*
Dermal formulated p + guava	2.70±0.13	50.00±2.28	4.00±0.19	17.00±0.88	55.00±2.78	15.01±0.71
%	138	169	129	110	111	107

% relative to control p = the pesticide Different is significant at control ($P < 0.05$)

The results of ATP, ADP and AMP led to suggest that at any circumstance associated with diminished availability of the prime dietary source of energy, namely carbohydrate, will accentuate utitization of fatty acids for this purpose. In this respect, the stimulation of glycolyic metabolism (forms pyruvic acid and then acetyl CoA) led to accumulate ATP and creatinine stores (Murrey et al, 2006 and Goel et al, 2006). ATP is rapidly utilized in protein biosynthesis, which stimulated by the adenylate cyclase (Elliott and Elliott, 2001).

Table (3) showed a remarkable decrease in myokinase activity in all tissues of rats administrated either by formulated dimethoate or technical one. In general, the increase in ATP content due to dimethoate treatments was mainly attributed to the effect of pesticide on the respiratory system (Abd El-Rahim et al., 1994). The maintenance of tissues is likely accomplished through increase the glycolytic process.

The inhibition of myokinase activity in rats administrated by technical or formulated dimethoate may be due to the plenteous ATP. Myokinase catalyzed the following reaction

$$2\ ADP \longleftrightarrow ATP + AMP$$

This reaction was observed after the complete utilization of ATP. Also, the amount of AMP might be due to the cyclic AMP which was formed from ATP. This reaction was catalyzed by adenylate cyclase as follows:

$$ATP \longleftrightarrow cAMP + pp$$

The extent of coupling oxidation to phosphorlation, evident in mitochondria, provided a mean by which the role of oxidation of food stuffs by respiratory oxygen was regulated by the inquirement of the cell for useful energy. The utilization of ATP to drive the energy requiring process of the cell automatically increased the available supply of ADP and inorganic phosphate which, in turn, became available to react in the coupling mechanism and permit respiration to proceed (Murrey et al 2006 and Chatterjea and Shinde 2002).

In the dimethoate administration condition, the oxidative phosphorylation was stimulated due to the respiration oxygen and the increase of ATP formation (Abd El-Rahim et al, 1994).

The most effective of formulated dimethoate than technical one may be due to the formulations which may cause synergism to the toxicity of the active ingredient. Such formulations are expected to effect the dimethoate penetration distribution and retention through the body tissues and blood constituents are factors to determine the trend for their tissue deposition, partitioning and toxikinetics (El-Sebae, 1985).

Guava (*Psidium guajava*) edible portion contained large amounts of the antioxidant compounds such as vitamin A and C (2270 mg and 660 IU/100 dry weights respectively of adible portion) as well as polyphenol and polyflavonoide compounds. Guava also contains vitamin B_1 and B_2 (0.5 and 0.5 mg/100 dry weight respectively) and contains nutritional minerals such as K, Fe, Zn and P with amount of 2250 mg, 10mg, 2.6 mg and 440 mg/100 dry weight respectively of edible portion. Several of these compounds are used by animal body as antioxidant agents (Nutrition Institute, 1996 and Cheng and Yang, 1983)

Certain enzymes reactivors, such as oximes, constitute the most important means of preventive treatment following exposures to organophosphorus insecticides in human (Buckley et al, 2005). However, the possible protective roles of safer preventative compounds, offering least amount of side effects are warranted to be explored. A number of studies have suggested zinc as a beneficial agent during peroxidative damage (Cabre et al, 1999 and Sidhu et al, 2004). However no studies have been performed to data to study if antioxidant agents or zinc may have beneficial effects in organophosphate reduced toxicity in experimental models, which have implications in managing humans with accidental exposures to such compounds.

Feeding of guava antioxidant diet for dimethoate intoxicated rat was accompanied by normalizing the ATP content in brain, liver and kidneys (Table 1). The level of ATP in liver and brain was higher than that of kidneys. In connection, ADP and AMP contents were significantly increased relative to their intoxicated rats and normalized (Table 2). The high elevation of AMP might be due to the action of myokinase which was confirmed by myokinase results (Table 3). The inhibited activity of myokinase by dimethoate ingestion, was normalized and stimulated relative to that of the intoxicated rats. The increase of AMP might be also due to the great elevation of cAMP

which was produced by adenylate cyclase to require the stimulation of protein biosynthesis (Elliott and Elliott. 2001).

Different tissues show different levels of susceptibility to dimethoate and thus the overall response varies from tissue to tissue. Vitamin C is a well known antioxidant, when present in an equeous environment, efficiently inhibits *in vitro* lipid peroxidation due to a combination of direct radical interception (where aqueous radicals are involved) (Sharma and Buettner, 1993).

Dimethoate like other organophosphorus pesticides is an anticholiesterase compound which covalently modified acetyl cholinesterase thus inhibiting its activity. The protection of the enzyme activity in liver, kidneys, spleen and brain of dimethoate intoxicated rats is offered by pretreatment with antioxidant vitamins. Results of the present study are confirmed with this phenomen on that treatment with antioxidant agent (vitamin C and polyphenol compounds of guava diet) decreased dimethoate – ingestion peroxidation. Ascorbic acid is located in the extracelluar and hydrophilic regions of the cell. Thus, ascorbic acid in the extracellular matrix defends the cells first. Free radicals must pass across the membrane to interact with extracellular compounds. Thus, the membrane is damaged first and lipid peroxidation is initial and necessary consequence of oxidative stress. Treatment with antioxidant decreased generation of reactive oxygen species, thus prevents the dimethoate ingestion derangements in the activities of antioxidant enzymes. The rodox status of the tissues is improved in antioxidant diet fed rats (Verma et al, 2007).

Cytochrome-c was used a marker of mitochonolrial synthesis and turnover. Cytochrome-c content of liver, brain and kidneys tissue was determined in control and treated rats after the experimental period (90 days). The decrease in cytochrome-c content by dimethoate was 7-25% compared with the control (Table 3). These results suggested that cytochrome-c destruction was occurred predominantly during dimethoate induction. Cytochrome-c is extra mitochondrially to cytosal ribosome and is attached to the inner mitochondrial membrane (Elliott and Elloitt, 2001). It shoud be a good maker of inner mitochondrial membrane turnover. The results of cytochrome-c strongly support the hypothesis that dimethoate (as organophosphorous pesticide) treatment leads to damage and destruction of 7-25% of mitochondria (Goel et al, 2007).

The effect of dimethoate administration on the activity of mitochondra enzymes related to respiration was studied and the results are show in table (3 and 4). Formulated dimethoate caused a significant stimulation of the activity of both cytochrome-c enzymes (succinate – cytochrom – c- reductase and cytochrome -c- oxidase) more than that of technical form. The stimulation was not due to an overall enhancement of activity of the respiration system but it appeared to be due to the increase in the dehydrogenases activity and the rate limiting step of the oxidation of metabolites as succinate (Goet et al, 2007). This was further substantiated by the observed stimulation in the activity of succinate-cytochrome-c-reductase which includes the rate limiting step catalyzed by the primary dehydrogenases and the limited stimulation of the activity of cytochrome-c-oxidase which is known to be far in excess of overall rate of oxidation of metabolites (Goel et al, 2000 and 2005 and Murrey et al, 2006).

The present findings are in agreement with those obtained by Abdel-Rahim et al, (1994) they found that the content of cytochrome-c was reduced but the activity of respiratory enzymes was stimulated under the effect of organophosphorus insecticde (malathion) either in technical form of formulated one.

Furthermore, increases in celluler oxidation and in the activities of several oxidoreductases has been reported in animal tissue (Goel et al, 2006 and Abdel-Rahim et al, 1994). The present findings showed that the oxidative enzymes could result in an increase in the overall rate of oxidation of metabolites as succinate and represent a compensatory mechanism which overcome the initial lack of O_2 and provide the minimal energy requirement. Thus the specific stimulation in the activity of oxidative enzymes in rat organs tissue given pesticides could be due to animal physiological status.

All these alterations were observed much more greater formulated dimethoate than technical form. These changes may be due to the adjuvant which are added to technical pesticide and may increase the degree of absorption from the gastrointestinal tract or skin after oral or dermal dosing. On might add that the polarity of the formulation molecules has a great effect on pesticide absorption rate and alter the physical properties of the technical pesticides.

Respiratory system of mitochondria is connected with cytochrome-c and its enzymes which considered one of the important markers of mitochondrial biosynthesis and turnover (Murrery et al,

2006). As shown in table (3) the cytochrome-c levels of liver, brain and kidneys were significantly reduced at normal control by ingestion of dimethoate. These values were normalized for dimethoate intoxicated animals by feeding on guava antioxidant diet. In addition, the stimulated activity of both mitochondrial cytochrome-c-oxidase and succinate cytochrome-c-reductase of the same organs tissue was also normalized by feeding on the guava diet in dimethoate intoxicated rat, but the values were still far from those of normal control.

The present results are in agreement with our results (Abdel-Rahim, 2007), who found that the antioxidant guava diet reduced the toxic effects of dimethoate (organophosphorus pesticide) on growth rate and blood pricture of intoxicated albino rats. The oxidoreductase enzymes could enhance the metabolites oxidations such as pyruvate and succinate in which via cytochrome-c mechanism. These stimulation in the animal tissues could be due to the physiological status of animal (Chatterjea and Shinde, 2002).

Coo et al (2004) found that co-administration of zinc to chlorpyrifos (organophosphorus pesticide) intoxicated animals normalized the enzymatic activities of cytochrome P450, NADPH-cytochrome-c-reductase and NADH-cytochrome-c-reductase within normal range. Zinc supplementation is protective in the animlas subjected to organophosphorus pesticides intoxication, as it markedly helps regulating the activities of key drug metabolizing enzymes in conditions of dimethoate toxicity. Although zinc-induced metallothionein levels and its antioxidant effects may be control to its biological protective role.

Collectively, the present studies suggest that zinc and vitamin C with polyphenol antioxidant (in guava diet) play an important role in regulating the tissues activities of drug metablizing enzymes in dimethoate intoxicated animals.

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التأثيرات الكيميائية الحيوية للجوافة كغذاء مضاد للأكسدة كعامل مخفض لسميه مبيد الديمتوايت على الأنظمة التنفسية للطاقة وسيتوكروم جـ في الفئران المسمة بالمبيد

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استخدمت فى هذه الدراسة المعاملة بمبيد الدايمثويت النقى والمجهز بجرعات 20/1 من الجرعة نصف المميتة ($LD_{50}/20$ جرعة كل 48 ساعة) عن طريق الفم أو الجلد لمدة ثلاثة أشهر كذلك تم دراسة تأثير الجوافة كغذاء مضاد للأكسدة لخفض سمية الدايمتويت على نظام الطاقة (محتوى أنسجة الكبد والمخ والكليتين من ATP-ADP-AMP وكذلك نشاط إنزيم الميوكينيز ونظام سيتكروم جـ الكلى محتوى الميتوكوندريا من سيتوكروم جـ ونشاط إنزيمى اكسيديز سيتوكروم جـ وكذلك ريدكتيز سكسينات سيتوكروم جـ). وقد أظهرت الدراسة أن المعاملة بمبيد الدايمتويت سواء عن طريق الفم أو الجلد أدت لارتفاع فى محتوى أنسجة الجسم من ATP وانخفاض فى محتواها من ADP وكذلك AMP سواء باستخدام المبيد النقى أو المجهز وذلك مقارنة بالكنترول وكذلك انخفاض فى محتوى الميتوكوندريا من سيتكروم جـ كما لوحظ ارتفاع نشاط انزيمى السيتوكروم (اكسيديز سيتكروم جـ وكذلك وريد كتيز سكسينات سيتوكروم جـ) وعموما فإن تأثير المعاملة بالمبيد المجهز كان أقوى من المعاملة بالمبيد النقى وكان تأثير المبيد عن طريق الفم أكبر من تأثيره عن طريق الجلد.

وتغذية الفئران على عليقة تحتوى على 20% جوافة جافة (مستبدلة بدلا من نشا العليقة) كعامل مضاد للأكسدة أظهرت تأثير مضاد لسمية الدايمتويت ومن ناحية أخرى فإن مضاد الأكسدة المستخدمة فى هذه التجربة يقلل من تأثير المبيد الفوسفورى (الدايمتويت) على النظم التنفسية للطاقة (ATP-ADP-AMP – نشاط إنزيم الميوكينيز) وسيتكروم جـ (محتوى الميتوكوندريا من سيتكردم جـ ونشاط انزيمى اكسيديز سيتوكروم جـ وكذلك وريد كتيز سكسينات سيتوكروم جـ) حيث أدى هذا إلى تحسن لاقتراب مدلولاتها من الكنترول.

ومن المهم ملاحظة أن الجوافة كمعاملة مضادة للأكسدة تؤدى لخفض التأثيرات السامة الناجمة من فعل مبيد الدايمتويت فى حيوانات التجارب. كذلك تؤدى هذه المعاملة إلى تحسن أو تقليل الاضطرابات الناجمة عن سمية المبيدات الفوسفورية على النظم التنفسية والتى تشمل التمثيل الغذائى لنظام الطاقة ونظام سيتوكروم جـ.