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Original Research

Serum biochemical analysis of the subacute toxicity of Dimethoate and the ameliorative potential of *Datura metel* linn in Wistar rats

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ABSTRACT

There is an increasing concern regarding the widespread use of pesticides such as Dimethoate and their potential impacts on animal and public health. Dimethoate causes developmental toxicity such as decreased number of implantations and live fetuses, incidences of resorptions and decreased foetal body weights. The present study aimed at generating information on the subacute toxicity of dimethoate which has flooded the Nigerian markets, and ameliorative potential of *Datura metel* ethanol leaf extract (DMELE). Twenty-five (25) Wistar rats weighing 185 - 223 g were divided into 5 groups at random, with 5 rats in each group. Group I (control) was administred soya bean oil (SBO) at 2 mg/kg whereas, groups II, III, IV and V were administred 500 mg/kg of DMELE, 30 mg/kg of dimethoate (DM), DM+DMELE, and DM + atropine (AT), respectively, for four weeks. The median lethal dose (LD_{50}) of the DMELE and DM were found to be 3950 mg/kg and 250 mg/kg, respectively. Generally, DM induced significant (*$P \leq 0.05$*) changes in the alanine aminotransferase (ALT), aspartate aminotransferase (AST), lactate dehydrogenase (LDH) and albumin (ALB), mostly at the 3rd and 4th week. Non-significant changes observed in the mixture (DM + DMELE) suggest an ameliorative effect of DMELE on subacute toxicity of DM. Therefore, DMELE appeared to be a potential remedy against biochemical changes induced by DM, especially in animals that are exposed daily to low-doses of the chemical agent.

Keywords: Cattle, Ixodid ticks, Nigeria, Prevalence, Zaria

INTRODUCTION

Pesticide poisonings remain a serious public health problem worldwide. According to an estimate provided by World Health Organization, 3 million cases of pesticide poisoning (Class I and II organophosphorus, OP) occur every year, resulting in more than 250,000 deaths (Buckley *et al.* 2004; WHO, 2012). Extensive use of organophosporous (OP) such as Dimethoate (DM) in agriculture, industry, public health and residential houses cause many disturbances in both human and animal in many countries because of the need to feed the ever-increasing human population and protect them from vector-borne diseases (Crumpton, 2001; Bhaskar *et al.*, 2014; Gabr *et al.*, 2015). Dimethoate has been reported to cause developmental toxicity such as decreased number of implantations and live fetuses, incidences of resorptions and decreased foetal body weights (Farag *et al.*, 2006). Walsh *et al.* (2000) has shown that DM disrupted reproductive function in animals by inhibiting steroidogenesis primarily, by blocking transcription of the steroidogenic acute regulatory gene. Dimethoate was reported to inhibited cholinesterase activity, affected cognitive and neurological function among other health effects in humans and nontarget mammalian species (Gomes *et al.*, 1999; Sinha & Shukla, 2003; Young *et al.*, 2006). Dimethoates are widely used in agriculture in Nigeria. They are increasingly being used in veterinary applications on farm and pet animals, for the protection of stored foodstuffs, for control of endemics and parasites in public health programmes as well as for household applications in kitchens and bedrooms. Poisoning is treated with atropine (AP) and simultaneously with oximes such as pralidoxime (Buckley *et al.*, 2011). Atropine blocks acetylcholine from binding with muscarinic receptors, which reduces the pesticide's impact (Buckley *et al.*, 2011). In Nigeria, AP and oximes are not readily accessible especially in the rural areas where the predominant horticulture and livestock farming take place. However, the leaves of *D. metel* were reported to have high content of tropane alkaloids including atropine, hyoscyamine and scopolamine (Dabur *et al.*, 2005), which are competitive

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antagonists of muscarinic receptors that may alter the cholinergic effect of dimethoate toxicity.

Datura metel is a shrub-like annual or perennial herbaceous plant that belongs to the family Solanaceae. It grows as weeds in abandoned farmlands, road sides, and dumpsites in urban areas in northern part of Nigeria (Mann *et al.,* 2003). The common names of *D. metel* lnclude: English; Thorn's apple, Hausa; Zaƙami or Haukata yaro (Blench and Dendo, 2007), Igbo; Myaramuo, Yoruba; Apikan (Abdullahi *et al.*, 2003), Fulani; manga jidde. *Datura metel* enjoys patronage for its psychoactive property in Nigeria especially among the teenagers, and also criminals who seek for mood alteration effects (Halpern, 2004; Strahil *et al.,* 2006; Ertekin *et al.,* 2005; Babalola, 2014). Report of Drug Abuse in Nigeria by the United Nations Office on Drugs and Crime in 2007, showed 0.4% use of *D. metel* out of the various narcotic and psychotropic substances consumed in Nigeria. However, the whole plant, especially the leaves and the seed have been used in ethno-therapeutics all over the world from historical times. It is used as an anaesthetic, hallucinogenic, anti-asthmatic, anti-spasmodic, anti-rheumatic, anti-tussive, anti-tumors, narcotic, bronchodilator, anodyne, hypnotic and mydriatic effects (Gary *et al.,* 2005; Yusuf *et al.,* 2009; Das *et al.,* 2012; Shagal *et al.,* 2012). In traditional Chinese medicine, the *D. metel* is known as 'baimantuoluo' and used for skin inflammation and Psoriasis (Wang *et al.,* 2008). In Ayurvedic medicine, seeds of *D. metel* are used to treat skin rashes, ulcers, bronchitis, jaundice and diabetes (Agharkar, 1991).

MATERIALS AND METHODS

CHEMICALS AND REAGENTS

All reagents used were of analytical grade. Diagnostic kits used in the present study were obtained from Randox Laboratories Ltd, BT29 4QY, United Kingdom. These were liver biomarkers (alanine aminotransferase [ALT], aspartate aminotransferase [AST], alkaline phosphatase [ALP]), lactate dehydrogenase (LDH), albumin (ALB), total protein (TP) and kidney biomarkers (urea [Ur], creatinine [Cr]).

PLANT MATERIAL

Datura metel fresh leaves were sourced from Kudingi forest of Sabon Gari L.G., Zaria. The leaves were duly authenticated by a taxonomist (Mr Sanusi Namadi), from the Department of Botany, Ahmadu Bello University (A.B.U.), Zaria. Herbarium specimen (voucher number 049) was prepared and deposited for future reference.

PREPARATION OF *DATURA METEL* CRUDE ETHANOLIC LEAF EXTRACT

Approximately 1000 g of powdered leaf sample of *D. metel* was placed in a 5 L conical flask, completely soaked in 3 L of 70% (v/v) ethanol and then covered with aluminum foil. The mixture was allowed to stand at ambient temperature (27 ^{o}C ± 3) for a period of 72 h with frequent agitation in order to facilitate dissolution of the soluble matter. The mixture was strained using muslin cloth to remove solid material, and then clarified by filtration by gravitation using Whatman filter paper (1). The filtrates were then concentrated to solid extract on a water bath at 45 ^{o}C. The solid extract was packaged in extract sample bottles and stored in a desiccator. Aliquot of the concentration was prepared immediately before use, using ultra-filtrate water to obtain the required dosage.

ETHICAL CLEARANCE AND ANIMAL MANAGEMENT

The experimental protocol and procedures used in this study were approved by the Ahmadu Bello University Committee on Animal Use and Care (ABUCAUC), A.B.U., Zaria with Permit Number: ABUCAUC/2020/77 in conformity with the Guide and Care of the Use of Animals in Research and Teaching of the University. Forty-eight (48) clinically healthy Wistar rats with Mean weight 185 - 223 g were purchased from the Animal Unit of the Department of Veterinary Pharmacology and Toxicology, A.B.U., Zaria. The animals were housed and acclimatized for 2 weeks under good lighting and room temperature, adequate ventilation and hygiene, at the Laboratory Animal House of the Department of Veterinary Pathology, A.B.U., Zaria, and fed with Vital feed® (grower mash) and water *ad libitum*.

DETERMINATION OF MEDIAN LETHAL DOSE

The method described by Lorke (1983) was used to determine the LD_{50} of the DMELE

SUBACUTE TOXICITY STUDY

Twenty-five (25) Wistar rats weighing 185 - 223 g were divided into 5 groups at random, with 5 rats in each group (Table I).

Table I: Animal grouping and treatmet

Group	Treatment
I (SBO)	Soya bean oil at 2 mL/kg
II (DMELE)	*Datura metel* ethanol leaf-extract (500 mg/kg)
III (DM)	Dimethoate at 30 mg/kg
IV (DM+DMELE)	Dimethoate + *Datura metel* ethanol leaf-extract, 30 min later
V (DM+AT)	Dimethoate + Atropine (0.02 mg/kg), 30 min later

The different regimens were administered once daily by oral gavage using feeding syringe as described by Chang *et al.,* (1992) for a period of 4 weeks.

BLOOD SAMPLES COLLECTION AND LABORATORY ANALYSIS

Blood samples were collected via the retro-orbital sinus into 5 ml sterile plain vacutainer tubes. The blood samples were allowed to coagulate at room temperature for 1 hour and then centrifuged at 700 *g* for 15 min (Ogbu & Okechukwu, 2001). The supernatant sera were harvested and kept at −20°C for subsequent biochemical analyses ALT, AST, ALP, LDH, urea (Ur), creatinine (Cr), TP and ALB, using Automated Biochemical Analyzer (PKL® PPC

DATA ANALYSIS

Quantitative results of biochemical indices were expressed as the mean of 3 replicates ± S.E.M. Statistical evaluation of data was performed by Graph pad prism version 5.0 (for windows from Graph Pad Software, San Diego, California, USA) using Repeated Measure ANOVA. Post-Hoc test analysis was done using the Tukey's Multiple Comparison Test. Values were considered statistically significant at $P \leq 0.05$, confidence level of 95%.

RESULTS

MEDIAN LETHAL DOSE

Datura metel ethanol extract caused death of a rat only at 5,000 mg/kg after approximately 22 hours of DMELE administration and the LD_{50} was calculated as follows;

LD_{50} = $\frac{1}{2}$(Lethal dose + highest dose that does not cause mortality)

= $\frac{1}{2}(5000 + 2900)$ mg/kg = 3950 mg/kg

Therefore; 12.5% of the LD_{50} of DMELE = $\frac{12.5}{100}(3950$ mg/kg) ≈ 500 mg/kg (working dose).

For DM, 100% mortality was recorded at 1,000 mg/kg and the LD_{50} was calculated as follows;

LD_{50} = $\frac{1}{2}$(Lethal dose + highest dose tha doesn't cause mortality)

= $\frac{1}{2}(200 + 300)$ mg/kg = 250 mg/kg

Therefore; 12.5% of the LD_{50} of DM = $\frac{1}{8}(250$ mg/kg) ≈ 30 mg/kg.

SERUM ALANINE AMINOTRANSFERASE

The effects of oral administration of DMELE and DM on serum ALT of Wistar rats were summarized in Table II. There was a statistically significant (*$P \leq 0.05$*) increase in the ALT of group III (DM) in weeks 3 and 4 with values of 67.50 ± 7.50 and 72.50 ± 7.50 (U/L) compared to their respective control with values of 44.15 ± 2.05 and 46.25±5.95 (U/L) respectively. However, ALT were comparable *($P > 0.05$)* in groups II (DMELE), IV (DM + DMELE) and V (DM + AT) control group.

SERUM ASPARTATE AMINOTRANSFERASE

The effects of oral administration of DMELE and DM on serum AST of Wistar rats were summarized in Table III. There was a statistically significant (*$P \leq 0.05$*) increase in the AST of group III (DM) in week 4 with value of 25.50 ± 1.20 (U/L) compared to control with value of 16.85 ± 0.45 (U/L). However, AST were comparable *($P > 0.05$)* in groups II, IV and V to control group.

SERUM ALKALINE PHOSPHATASE

The effects of oral administration of DMELE and DM on serum ALP of Wistar rats were summarized in Table IV. All the ALP values were comparable *($P > 0.05$)* across the groups.

SERUM LACTATE DEHYDROGENASE

The effects of oral administration of DMELE and DM on serum LDH of Wistar rats were summarized in Table V. Group III (DM) showed significant (*($P > 0.05$)* increased in LDH in weeks 3 and 4 with values of 686.71±24.68 and 730.86±35.41 (U/L) compared to their respective control.

SERUM UREA LEVEL

The effects of oral administration of DMELE and DM on serum urea of Wistar rats were summarized in Table VI. All the serum urea values were comparable *($P > 0.05$)* across the groups.

SERUM CREATININE LEVEL

The effects of oral administration of DMELE and DM on serum creatinine of Wistar rats were summarized in Table VII. All the serum creatinine values were comparable *($P > 0.05$)* across the groups.

SERUM ALBUMIN LEVEL

The effects of oral administration of DMELE and DM on serum ALB of Wistar rats were summarized in Table VIII. There was significant (*($P \leq 0.05$)* decrease in the ALB of group III (DM) in week 4 with value of 4.15±0.35 compared to control with value of 4.90 ± 0.10 (g/dL). However, ALB were comparable *($P > 0.05$)* in groups II, IV and V with control.

SERUM TOTAL PROTEIN LEVEL

The effects of oral administration of DMELE and DM on serum protein of Wistar rats were summarized in Table IX. All the serum TP values were comparable *($P > 0.05$)* across the groups

Table II: Serum alanine aminotransferase (U/L) in Wistar rats administered dimethoate and treated with Datura metel ethanol leaf extract

Group	Mean ± S.E.M (n = 3)				
	SBO	**DMELE**	**DM**	**DM+DMELE**	**DM+AT**
Week 0	42.80 ± 1.90	46.90 ± 0.60	46.70 ± 0.60	44.10 ± 2.90	47.25 ± 4.15
Week 1	43.55 ± 2.55	44.45 ± 2.05	51.15 ± 6.94	45.45 ± 0.14	47.20 ± 1.60
Week 2	43.30 ± 4.20	47.65 ± 5.35	55.10 ± 4.80	47.20 ± 2.60	49.40 ± 4.30
Week 3	44.15±2.05^{a}	56.85 ± 3.14^{ab}	67.50 ± 7.50^{b}	60.70 ± 9.10^{ab}	53.55 ± 1.45^{ab}
Week 4	46.25±5.95^{a}	59.60 ± 6.00^{ab}	72.50 ± 7.50^{b}	56.55 ± 3.54^{ab}	55.00 ± 2.00^{ab}

S.E.M = Standard error of mean, n = Number of replicate, SBO = Soya bean oil, DMELE = *Datura metel* ethanol leaf extract (500 mg/kg), DM = Dimethoate (30 mg/kg), AT = Atropine (0.02mg/kg). Values with different superscript alphabets (a,b) across the group are significantly different ((P≤0.05).

Table III: Serum aspartate aminotransferase (U/L) in Wistar rats administered dimethoate and treated with *Datura metel* ethanol leaf extract. Mean ± S.E.M (n = 3)

Groups	SBO	DMELE	DM	DM+DMELE	DM+AT
Week 0	15.85 ± 1.45	16.80 ± 2.60	13.40 ± 1.00	16.35 ± 1.34	13.50 ± 0.40
Week 1	16.05 ± 1.75	16.65 ± 1.45	16.05 ± 3.55	16.05 ± 2.45	17.25 ± 3.45
Week 2	16.25 ± 0.45	17.90 ± 0.59	17.65 ± 2.45	15.65 ± 0.65	17.45 ± 0.65
Week 3	17.20 ± 0.8	20.40±1.10	23.00 ± 1.20	19.50 ± 1.50	18.95 ± 1.05
Week 4	16.85 ± 0.45^{a}	22.00 ± 1.29^{ab}	25.50 ± 1.20^{b}	19.90 ± 0.45^{ab}	19.80 ± 0.45^{ab}

Table IV: Serum alkaline phosphatase (U/L) in Wistar rats administered dimethoate and treated with *Datura metel* ethanol leaf extract

	SBO	DMELE	DM	DM+DMELE	DM+AT
Week 0	283.60 ± 26.70	283.00 ± 18.10	293.00 ± 04.20	296.80 ± 10.20	289.80 ± 19.59
Week 1	279.50 ± 35.50	322.40 ± 14.00	335.95 ± 14.45	315.70 ± 14.30	320.30 ± 18.50
Week 2	295.30 ± 08.00	321.95 ± 17.95	359.55 ± 11.15	330.90 ± 10.00	314.35 ± 14.05
Week 3	330.65 ± 37.35	330.00 ± 16.00	350.60 ± 19.70	329.20 ± 14.40	330.65 ± 09.35
Week 4	302.60 ± 08.00	342.25 ± 10.95	363.85 ± 09.64	326.10 ± 06.60	323.90 ± 15.00

S.E.M = Standard error of mean, n = Number of replicate, SBO = Soya bean oil, DMELE = Datura metel ethanol leaf extract (500 mg/kg), DM = Dimethoate (30 mg/kg), AT = Atropine (0.02mg/kg). Values with different superscript alphabets (a,b) across the group are significantly different ((P≤0.05).

Table V: Serum lactate dehydrogenase (U/L) in Wistar rats administered dimethoate and treated with *Datura metel* ethanol leaf extract (Mean ± S.E.M (n = 3))

	SBO	DMELE	DM	DM+DMELE	DM+AT
Week 0	439.40 ± 32.00	448.95 ± 19.25	444.90 ± 44.00	439.85 ± 26.95	428.18 ± 19.19
Week 1	470.60 ± 39.10	461.45 ± 34.65	477.35 ± 27.14	462.75 ± 10.54	469.09 ± 46.54
Week 2	426.20 ± 17.09	521.35 ± 76.55	581.65 ± 30.94	511.95 ± 53.75	498.85 ± 30.95
Week 3	453.90 ± 28.92^{a}	609.25 ± 31.05^{ab}	686.71 ± 24.68^{b}	559.85 ± 47.92^{ab}	566.63 ± 66.64^{ab}
Week 4	462.06 ± 22.57^{a}	621.60 ± 91.39^{ab}	730.86 ± 35.41^{b}	589.85 ± 62.87^{ab}	561.93 ± 53.82^{ab}

S.E.M = Standard error of mean, n = Number of replicate, SBO = Soya bean oil, DMELE = Datura metel ethanol leaf extract (500 mg/kg), DM = Dimethoate (30 mg/kg), AT = Atropine (0.02mg/kg). Values with different superscript alphabets across the group are significantly different ((P≤0.05).

Table VI: Serum urea value (mmol/L) in Wistar rats administered dimethoate and treated with Datura metel ethanol leaf extract (Mean ± S.E.M (n = 3)

Gropups	SBO	DMELE	DM	DM+DMELE	DM+AT
Week 0	4.15 ± 0.05	4.40 ± 0.09	3.85 ± 3.85	4.00 ± 0.19	3.90 ± 0.30
Week 1	3.95 ± 0.24	3.80 ± 3.80	3.80 ± 0.39	3.85 ± 0.05	3.85 ± 0.15
Week 2	3.70 ± 0.10	3.80 ± 3.80	4.15 ± 0.15	3.70 ± 0.20	3.60 ± 0.10
Week 3	4.00 ± 0.19	4.20 ± 0.20	4.05 ± 0.14	3.80 ± 0.10	3.85 ± 0.15
Week 4	3.50 ± 0.09	3.70 ± 0.10	4.20 ± 0.10	3.65 ± 0.15	3.85 ± 0.05

S.E.M = Standard error of mean, n = Number of replicate, SBO = Soya bean oil, DMELE = Datura metel ethanol leaf extract (500 mg/kg), DM = Dimethoate (30 mg/kg), AT = Atropine (0.02mg/kg). Values with different superscript alphabets across the group are significantly different ((P≤0.05).

Table VII: Serum creatinine (µmol/L) following in Wistar rats administered dimethoate and treated with *Datura metel* ethanol leaf extract. (Mean ± S.E.M (n = 3))

Groups	SBO	DMELE	DM	DM+DMELE	DM+AT
Week 0	51.60 ± 2.60	51.30 ± 2.29	49.90 ± 1.50	51.35 ± 0.75	51.61 ± 2.89
Week 1	50.20 ± 0.89	52.90 ± 2.69	54.70 ± 4.10	54.65 ± 1.35	53.45 ± 2.54
Week 2	52.75 ± 3.54	52.40 ± 4.30	56.60 ± 1.50	53.85 ± 4.55	51.20 ± 3.00
Week 3	52.00 ± 2.00	61.00 ± 4.00	62.30 ± 1.80	57.00 ± 3.00	54.80 ± 0.79
Week 4	51.50 ± 1.50	63.50 ± 6.50	62.75 ± 8.05	57.00 ± 2.65	58.15 ± 4.15

S.E.M = Standard error of mean, n = Number of replicate, SBO = Soya bean oil, DMELE = *Datura metel* ethanol leaf extract (500 mg/kg), DM = Dimethoate (30 mg/kg), AT = Atropine (0.02mg/kg).

Table VIII: Serum albumin level (g/dL) in Wistar rats administered dimethoate and treated with Datura metel ethanol leaf extract. (Mean ± S.E.M (n = 3))

Group	SBO	DMELE	DM	DM+DMELE	DM+AT
Week 0	5.10 ± 0.09	5.35 ± 0.15	5.30 ± 0.10	5.25 ± 0.15	5.15 ± 0.25
Week 1	4.95 ± 0.04	4.90 ± 0.30	4.55 ± 0.14	5.05 ± 0.25	5.00 ± 0.09
Week 2	4.95 ± 0.25	4.60 ± 0.09	4.35 ± 0.25	4.75 ± 0.15	4.75 ± 0.25
Week 3	4.95 ± 0.15	4.30 ± 0.30	4.35 ± 0.15	4.80 ± 0.10	4.70 ± 0.10
Week 4	4.90 ± 0.10^{b}	4.25 ± 0.15^{ab}	4.15 ± 0.35^{a}	4.65 ± 0.05^{ab}	4.85 ± 0.05^{ab}

S.E.M = Standard error of mean, n = Number of replicate, SBO = Soya bean oil, DMELE = *Datura metel* ethanol leaf extract (500 mg/kg), DM = Dimethoate (30 mg/kg), AT = Atropine (0.02mg/kg).

Table IX: Serum protein level (g/dL) in Wistar rats administered dimethoate and treated with *Datura metel* ethanol leaf extract

Groups	Mean ± S.E.M (n = 3)				
	SBO	DMELE	DM	DM+DMELE	DM+AT
Week 0	8.10 ± 0.09	7.75 ± 0.25	8.40 ± 0.49	7.85 ± 0.34	7.70 ± 7.70
Week 1	7.80 ± 0.20	7.60 ± 0.09	7.80 ± 0.2	8.05 ± 0.45	7.75 ± 0.25
Week 2	7.95 ± 0.04	7.50 ± 0.19	7.60 ± 0.10	7.60 ± 0.65	7.35 ± 0.55
Week 3	7.75 ± 0.15	7.55 ± 0.14	7.50 ± 0.09	7.80 ± 0.30	7.65 ± 7.65
Week 4	7.85 ± 0.04	7.20 ± 0.10	7.20 ± 0.39	7.60 ± 0.09	7.55 ± 0.14

S.E.M = Standard error of mean, n = Number of replicate, SBO = Soya bean oil, DMELE = *Datura metel* ethanol leaf extract (500 mg/kg), DM = Dimethoate (30 mg/kg), AT = Atropine (0.02mg/kg).

DISCUSSION

In this study, the significant increase in ALT in the serum of Wistar rats DM-treated group suggests the possibility of enzyme leakage from the liver or other tissues into the serum and this may destabilize the liver's integrity (Akanji *et al.* 1993; Shakoori *et al.,* 1994; Konan *et al.,* 2007). The significant increase in serum AST in DM-treated group could be due to tissue damage as reported by Mukinda *et al.* (2010) who stated that increase in serum AST occurs as first sign of cell damage that leads to the out flow of the enzymes into the serum from both the mitochondria and cytoplasm.

Likewise, Abdou *et al.* (2015) reported that increase in serum AST and LDH may indicate hepatic damage probably by the altered cell membrane permeability leading to the leakage of the enzymes from the tissues to the serum. The significant increase in LDH observed in the DM-treated group can beassociated with hepatic injury or damage and might also indicate the presence of RBC hemolysis (Ismail *et al.,* 2014). The ALT, AST and LDH findings in this study are in line with Slimen *et al.,* (2018) but differed in ALP where significant increase was observed. The evaluation of serum biochemical parameters has significant importance on toxicological changes (Debelo *et al.,* 2015). Liver is the major site for metabolism including drugs (Abou, 2016).

When the liver cell membrane is damaged, a variety of enzymes normally located in the cytosol are released into blood stream. The transaminases (ALT, AST and ALP) are well-known enzymes used as good indicators of liver function (Huang *et al.,* 2006; Ismail *et al.,* 2014; Sulaiman *et al.,* 2014) and as biomarkers predicting possible toxicity (Hatayama *et al.,* 2011; Morales *et al.,* 2014) with ALT being a more sensitive indicator of either acute and/or chronic hepatotoxicity than AST (Barth, 1979; Pramyothin *et al.,* 2006). The prevalent use of pesticides in Nigeria and most under-developed countries has led to numerous diseases and dysfunctions that are in consonance with the observed influences in the present study **(**Otitoju and Onwurah, 2005). The non-significant changes in the ALT, AST and LDH levels in the serum of rats of DM+DMELE-group in relation to the control could be due to ameliorative effects of DMELE on DM.

In the current study, the increased concentration of urea and creatinine in the plasma observed in DM-treated group when compared to the control group is suggestive of possible renal impairment or increased rate of the catabolism of protein due to the DM **(**Yakubu *et al.,* 2003). Previous studies have demonstrated that plasma urea and creatinine concentration are important biomarkers of glomerular filtration rate and renal toxicity (Khalil and Granger, 2002; Suryavanshi *et al.,* 2015; Santosh *et al.,* 2016). Any rise in the levels of these parameters indicates a marked renal damage (Soliman *et al.,* 2000; Pagana & PaganaMosby's, 2010; Adewale *et al.,* 2016; Garba *et al.,* 2007). However, extremely low level of urea and creatinine may be suggestive of a severe liver disease (Pagana & PaganaMosby's, 2010).

Although there is no insignificant decrease in TP in the treated groups, the ALB of Wistar rats in DM-treated group in week 4 was significantly lower when compared with the control. Since plasma ALB is synthesized in the liver, the decrease in the ALB of the Wistar rats could be sequel to impairment of liver function or hepatic damage (Anita *et al.* (2012). Albumin is the major plasma protein exclusively synthesized in the liver by hepatocytes. Acute or chronic injury to liver cells alters its synthetic function including production of ALB in acute and subacute liver disease. The change in hepatic function is usually transient due to high power of regeneration of cells via compensatory mechanism. The concentration of ALB is a useful marker of the secretory, synthetic and excretory functions of the liver and kidney (Yakubu *et al.,* 2009).

CONCLUSION

Deviation of the values of the measured biochemical parameters in different treatments compared with control values gave an indication of the toxicity of the tested dose of DM. Whereas, non-significant changes observed in the mixture (DM + DMELE) suggests an ameliorative effect of DMELE on subacute toxicity of DM. Therefore, DMELE appeared to be a potential anti-cholinergic agent against toxic damage induced by DM, especially in animals that are exposed daily to low-doses of the chemical agent.

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