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RESEARCH ARTICLE

ADVERSE EFFECTS OF ANAESTHETIC DRUG COMBINATIONS IN A COMPLETE CYCLE OF ANAESTHESIA IN ALBINO RATS

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ABSTRACT

Background and aim: Drugs used in anaesthesia span through pre-medication, induction, muscle relaxation, maintenance and reversal phases in achieving adequate surgical procedures. Due to lack of information in adverse effects of combined anaesthetic drugs, the study assessed the adverse effects pattern in albino rats to ensure safety in a complete cycle of anaesthesia.

Methods: This study was carried out using sixty-six albino rats that were randomly selected grouped into eleven according to different stages of drug use in anaesthesia. The anaesthetic drugs (Diazepam, Atropine, Neostigmine, Propofol, Atracurium, Fentanyl, Thiopentone, Pentazocine, Midazolam, Suxamethonium, Pethidine, Ketamine, Bupivacaine, Pancuronium and Pethidine) were grouped as in the categories of pre-medication, induction, muscle relaxation, maintenance and reversal stages. They were administered intraperitoneally at 30 minutes interval for a period of twenty-eight days in computed standard doses. The albino rats were sacrificed under chloroform anaesthesia, blood samples collected and assayed for glucose, lipids, liver, renal and haematological indices as toxicity markers. Results were computed and analysed statistically.

Results: Out of the thirty-six toxicity markers assessed, the thirteen parameters that changed significantly were: glucose, total cholesterol, urea, creatinine, white blood cells, lymphocytes, red blood cells, platelets, alkaline phosphatase, aspartate aminotransferase, alanine amino transferase, potassium, and bicarbonate ($P<0.05$). Group 5 rats dosed with Diazepam, Thiopentone, Atracurium, Pentazocine, Neostigmine and Atropine, and Group 6 rats administered with Diazepam, Midazolam, Suxamethonium, Pethidine and Atropine, the toxicity parameters least significantly. However, group 7 rats that received Diazepam, Ketamine, Suxamethonium, Pentazocine and Atropine combinations changed the toxicity parameters most significantly. All the animals in group 8 died after the first two weeks of drug administration.

Conclusion: The results in this study, showed variable adverse effects pattern of anaesthetic agents at various stages of anaesthesia. It is therefore recommended that adequate precaution should be exercised in the selection to ensure safety in a complete cycle of anaesthesia during surgical procedures.

Key words: Anaesthesia, complete cycle, anaesthetic drugs, adverse effects, rats.

INTRODUCTION

Anesthesia usually involves loss of memory and awareness, along with insensitivity to painful stimuli, during a surgical procedure [1,2]. Many drugs aid anesthesiologists in the management and comfort of their patients during the perioperative period. These compounds vary in their chemical, physical characteristics and routes of administration [2-4]. Different agents have been recommended to achieve the various stages of anaesthesia [2,3]. An ideal anaesthetic drug would induce loss of consciousness smoothly and rapidly, while allowing for prompt recovery of cognitive function after its administration is discontinued. The drug may also possess a wide margin of safety devoid of adverse effects [2,3]. No single anaesthetic agent is capable of achieving all of these desirable effects without some adverse effects when used alone or in combination. Even when used alone, some individuals may be on medications that may interfere with anaesthetic drugs in surgical procedure.

The modern practice of anaesthesiology most commonly involves the use of combinations of intravenous and inhaled drugs, taking advantage of their individual favourable properties while minimizing their adverse reactions [1]. A typical anaesthetic agent would involve an intravenous induction followed by an inhalational maintenance [2,5]. The effects of anaesthetic agents spread across different range of agents and wide array of mechanism of action [2,3,6-8]. In the course of a surgical operation various anaesthetic agents have been found useful in achieving an adequate procedure irrespective of the diagnosis. Some of these anaesthetic agents [2,3] may be used alone or as a combination. In the course of usage, the anaesthetist/pharmacologist will want to follow the stepwise procedures to achieve a successful anaesthetic procedure; that is from pre-medication to reversal stage which represent a complete cycle of anaesthesia.

The drawback irrespective of the procedure may be due to synergistic toxic effects the agents may have on the system. The anaesthetic agents are known to be of different classes and different pharmacologic fate, thereby expressing different toxic profile. The situation may be worse if the diagnosed disease worsens the adverse effect of used drugs. These agents may have their inherent adverse effects when used alone but their adverse effect may get worse when they are used in combination. Due to lack of information in adverse effects of combined anaesthetic drugs, the study assessed the adverse effects pattern in albino rats to ensure safety in a complete cycle of anaesthesia.

MATERIALS AND METHODS

Animals: This study was carried out using sixty-six albino rats of either sex weighing between 150 and 300 grams. The animals were obtained from the animal house of the Department of Pharmacology and Toxicology, University of Benin, Benin city, Nigeria after obtaining ethical permission (ADM/E 19/A/VOL VII/1007). The animals were acclimatised for two weeks and fed with standard palletized feed from Ewu Feeds and Flour Mill Limited, Edo State, Nigeria and

tap water *ad libitum*. All other experimental protocols for handling animals were according to the Institutional Animal Ethics Committee (IAEC) and complied with the NIH guidelines.

***Drugs and chemicals*:** The drugs and chemicals purchased and used were branded as: Diazepam 5 mg/ml (Valium®) and Midazolam 5 mg/ml (Hypnovel®) by Roche; Thiopentone 0.05 g/ml, Pancuronium 2 mg/ml, Suxamethonium 50 mg/ml, Neostigmine 2.5 mg/ml and Ketamine 50 mg/ml (Rotex medical Germany); Propofol 10 mg/ml (Fresenius Germany), Bupivacaine 5 mg/ml (Astra Zeneca), Atracurium 10 mg/ml (Lameln pharmaceuticals), Fentanyl 50 µg/ml (Verve humancare laboratory), Pethidine 50 mg/ml and Atropine 60 µg/ml (Martindale pharmaceuticals), Pentazocine 6 mg/ml (Relish pharmaceuticals). The drugs were obtained directly from reputable registered pharmacies in Benin City and Department of Pharmacy, University of Benin Teaching Hospital, Benin City.

***Experimental protocol for administration of anaesthetic drugs*:** The animals were placed in 11 groups of 6 albino rats each, kept in separate standard cages and each rat was treated singly as positive control and as combinations in test groups. Group 1 control animals received distilled water, group 2 animals received Diazepam 5 mg/kg intraperitoneally, group 3 animals received Atropine (0.05 mg/kg) and Neostigmine (200 µg/kg) at interval of 30 minutes after each administration. Furthermore, group 4 animals were dosed with Diazepam (5 mg/kg), Propofol (10 mg/kg), Atracurium (0.2 mg/kg), Fentanyl (0.05 mg/kg), Neostigmine (200 µg/kg) and Atropine (0.05mg/kg) at interval of 30 minutes after each drug administration. Group 5 animals received Diazepam (5 mg/kg), Thiopentone (30 mg/kg), Atracurium (0.2 mg/kg), Pentazocine (10 mg/kg), Neostigmine (200 µg/kg) and Atropine (0.05 mg/kg) at interval of 30 minutes after each drug administration. Group 6 animals were administered with Diazepam (5 mg/kg), Midazolam (5 mg/kg), Suxamethonium (1 mg/kg), Pethidine (12 mg/kg) and Atropine (0.05 mg/kg) at an interval of 30 minutes after each drug administration. Group 7 animals received Diazepam (5 mg/kg), Ketamine (75 mg/kg), Suxamethonium (1 mg/kg), Pentazocine (10 mg/kg) and Atropine (0.05 mg/kg) at an interval of 30 minutes after each drug administration. Group 8 animals received Diazepam (5 mg/kg), Bupivacaine (2 mg/kg), Pancuronium (0.1 mg/kg), Pethidine (12 mg/kg), Neostigmine (200 µg/kg) and Atropine (0.05 mg/kg) at an interval of 30 minutes after each drug administration. Group 9 animals received Pancuromium (0.1 mg/kg), group 10 animals Bupivacaine (2 mg/kg) and group 11 animals Pethidine (12 mg/kg).

A thirty-minute time-lag administration between co-administered drugs was adopted [9] to allow effective bio-availability of every drug administered for most likely adverse effects to be noticed. These processes were repeated daily for 28 days. Thereafter, rats were weighed, sacrificed under chloroform anaesthesia and blood samples assessed for common toxicity markers such as serum glucose, lipids, liver, renal and haematological indices as described [10].

Statistical analysis

The data collected from the various assays were entered into Graph Prism Version 6, San Diego, USA and computed as mean ± SEM. Suitable statistics were applied using Analysis of Variance, Tukey's and Fisher's *post hoc* tests. *P*-values equal to or less than 0.05 was regarded as significant.

Table 1: Experimental design for anaesthetic drugs

Cycle of anaesthesia	G1	G2	G3	G4	G5	G6	G7	G8	G9	G10	G11
Pre-medication	Control Distilled water	Diazepam	Atropine	Diazepam	Diazepam	Diazepam	Diazepam	Diazepam	-	-	-
Induction	Control	-	-	Propofol	Thiopentone	-	Ketamine	Bupivacaine	-	Bupivacaine	-
Muscle relaxation	Control	-	-	Atracurium	Atrcurium	Suxamethonium	Suxamethonium	Pancuronium	Pancuronium		
Maintenance	Control	-	-	Fentanyl	Pentazocine	Midazolam, Pethidine	Pentazocine	Pethidine	-		Pethidine
Reversal	Control	-	Neostigmine	Neostigmine, Atropine	Neostigmine, Atropine	Atropine	Atropine	Neostigmine, Atropine	-		-

Experimental design for the grouping of drugs in pre-medication, induction, muscle relaxation, maintenance and reversal. Group G1: Negative control. G2, G9, G11: Positive control. G3-G8: Test groups in combination.

Table 2: Effects of anaesthetic drugs on renal indices.

Parameters	GROUP1	GROUP2	GROUP3	GROUP4	GROUP5	GROUP6	GROUP7	GROUP9	GROUP 10	GROUP 11
Urea (mg/dl)	48.83±4.69	33.33±2.80	36.67±2.74	44.17±2.02	78.50±3.70*	36.17±0.74	36.87±1.49	34.63±2.67*	37.17±2.97*	30.50±2.26
Na (mMol/L)	149.20±1.57	145.50±1.28	144.30±2.04	144.00±0.51	140.70±1.33	144.00±0.44	145.50±1.17	147.50±0.34	151.50±0.42	142.20±0.32
K (mMol/L)	5.70±1.16	5.50±0.23	6.08±0.43	5.96±0.59	28.53±2.73*	13.32±1.09	9.06±0.79	5.98±0.21	6.10±0.22	5.86±0.19
HCO_3 (mMol/L)	20.83±1.62	21.17±0.79	22.83±1.01	13.83±0.70	12.50±0.88	17.83±0.70	16.67±0.49	32.33±1.90*	16.00±0.57	25.33±1.20
Cl (mMol/L)	108.20±1.49	106.7±1.88	110.30±1.74	109.20±1.72	111.30±2.21	105.50±2.83	100.00±2.62	111.00±1.96	120.00±2.13	107.8±1.86
Cr (mg/dl)	0.75±0.04	0.66±0.04	0.65±0.02	0.63±0.49#	0.66±0.09#	0.70±0.03	0.66±0.03	0.55±0.04#	0.65±0.05	0.61±0.05#

Na: Sodium ion, K: Potassium ion, HCO_3: Bicarbonate ion. Cr: Creatinine.Cl: Chloride ion. Urea: Group 9*, Group 10* Vs Control (P<0.05). Creatinine: Group 4#, Group 5# , Group 9#, Group 11#Vs Control (P<0.05).

Table 3: Effects of anaesthetic drugs on liver function indices

Parameters	GROUP 1	GROUP 2	GROUP 3	GROUP 4	GROUP 5	GROUP 6	GROUP 7	GROUP 9	GROUP 10	GROUP 11
ALP (U/L)	35.00±2.38	64.33±2.89*	59.83±2.89*	58.17±2.24*	61.00±2.42*	54.17±3.6*	58.50±1.68*	58.17±1.37*	59.50±1.47*	60.17±1.52*
AST (U/L)	22.67±1.20	66.17±1.68**	64.50±6.90**	64.67±7.11**	65.50±1.60**	42.33±6.64**	40.17±3.70**	62.33±3.87**	61.17±3.79**	63.50±4.08**
ALT (U/L)	16.00±1.06	30.67±1.66#	30.50±2.95#	28.00±2.50#	39.17±2.22#	19.67±2.49#	14.83±0.87#	24.00±1.70#	24.17±1.77#	28.83±2.06#
TB (mg/dl)	1.30±0.74	0.51±0.03	0.58±0.03	0.63±0.05	0.53±0.421	0.50±0.03	0.55±0.03	0.60±0.05	0.50±0.04	0.53±0.04
TP (mg/dl)	6.43±1.14	3.83±0.022	8.26±0.16	7.50±0.33	8.30±0.24	7.9±0.15	7.40±0.34	7.95±0.14	7.61±0.01	7.80±0.13
ALB (g/dl)	3.75±0.14	3.91±0.03	3.88±0.16	3.96±0.22	4.03±0.24	3.80±0.09	3.85±0.08	4.16±0.26	3.80±0.17	4.10±0.25

ALP: Alkaline Phosphatase, AST: Aspartate aminotransferase, ALT: Alanine aminotransferase). TB: Total Bilirubin, TP: Total Protein, ALB: Albumin. **ALP (U/L)**: Group 2#, Group 3#, Group 4#, Group 5#, Group 6#, Group 7#, Group 9#, Group 10#, Group 11#Vs Control (P<0.05). AST (U/L): Group 2**, Group 3**, Group 4**, Group 5**, Group 6**, Group 7**, Group 9**, Group 10**, Group 11**Vs Control (P<0.05). ALT (U/L): Group 2#, Group 3#, Group 4#, Group 5#, Group 6#, Group 7#, Group 9#, Group 10#, Group 11#Vs Control (P<0.05).

RESULTS

Thirteen out of the thirty-six toxicity markers assessed changed significantly (p<0.05) and compared with the negative control. Haematological markers such as urea, creatinine, potassium and bicarbonate (Table 2) and enzymic markers: alkaline phosphatase (ALP), alanine aminotransferase (ALT) and aspartate aminotransferase (AST) (Table 3) changed significantly (p<0.05).

Similarly, blood glucose and total cholesterol (Table 4), white blood cells (WBC), lymphocytes, red blood cells (RBCs) and platelets (Table 5) changed significantly ($P<0.05$). All the animals in group 8 died after the first two weeks of drug administration.

DISCUSSION

The study has shown some variable changes in reported safety parameters [10]. Some authors have established that selection of an anaesthetic regimen for use in research depends on several factors such as health status and species of animal to be anesthetised, safety considerations, type and duration of the procedure to be performed, recovery time and other research goals [11-14]. There may be changes during acute exposure as observed in most cases of short courses in anaesthetic procedures. This effect can be reversed as the agents have been withdrawn, but may also persist in dose-sensitive subjects. The observed insignificant increases in weight changes can be ascribed to inherent feeding of the animals during the 28 days therapy. However, females' weight tends to increase significantly probably due to hormones and diet [2,3]. Weight increase can persist even after the dosage regimen, especially when the drug metabolites can influence hormonal changes in the subjects.

There seems to be a common phenomenon in significant changes in all the liver enzymes (ALP, AST, ALT) as in Table 3. Significant increase was observed in both single group and combination groups (Table 3). Although administration of some of the drugs in anaesthesia is a short course which represents acute effect if any, but liver damage can be eminent if care is not taken due to inherent effect of the parent drugs or metabolites on long-term exposure. Hepatotoxic effects of some anaesthetic drugs have been documented such as in the use of halothane [2,3]. However, if there were reductions in the expression of the enzymes, it is interpreted as hepato-protective while if there were increase in the expression of the enzymes, it is regarded as hepatotoxic. The elevation of liver enzymes suggests risk in individuals that may have existing liver disease as previously observed [2,10]. Other parameters such as albumin, total bilirubin and total protein that may not have changed significantly should be closely monitored because of possible occurrence of dose or drug sensitivities in some of the animals.

The mechanisms underlying hepatotoxicity potential of the investigated anaesthetic agents may be unclear, but there may be formation of reactive metabolites that either cause direct hepatocellular damage (example, free radicals) or initiate immune-mediated responses [1]. Interestingly, dose-dependent effect has been reported in individuals that are dose-sensitive [2,3]. Some agents may inherently have a good safety margin [2,3], but there can be individual sensitivity to doses considering the principle of pharmacogenomics. The toxic potential observed may be due to reported adverse drug reaction related to induction or inhibition of CYP_{450} enzyme [1,2,3]. There was a significant reduction in creatinine in groups 9 and 11 rats due to Pancuronium and Pethidine (Table 2). It would have been expected that there would be more toxic stress to the kidney in most of the combinations. The reduction in the parameters may be due to on opposing effect [2,3,10] of the agents in the combinations. Therefore, it can be inferred that drug combination in anaesthesia may have little or no effect on renal parameters including creatinine. Interestingly, urea was also found

to change significantly in the combinations with Pancuronium.

Table 4: Effects of anaesthetic drugs on glucose and lipids

Parameters	GROUP 1	GROUP 2	GROUP 3	GROUP 4	GROUP 5	GROUP 6	GROUP 7	GROUP 9	GROUP 10	GROUP 11
Glucose (mg/dl)	121.50±4.99	84.50±4.66	114.70±13.16	94.83±11.53	68.83±2.96*	78.67±3.06*	85.67±8.66*	86.00±7.53*	75.33±6.34*	72.00±6.20*
TC (mg/dl)	101.00±5.16	86.67±3.40	80.00±3.48	78.33±7.14	96.17±4.82$^{\#}$	110.00±6.62	89.50±6.92$^{\#}$	92.00±3.06$^{\#}$	115.30±3.37	83.00±2.78$^{\#}$
TG (mg/dl)	112.00±4.99	135.30±7.66	128.70±8.93	115.30±2.45	115.50±4.86	113.00±26.90	101.00±11.00	84.50±8.01	73.83±7.89	101.70±11.50
HDL (mg/dl)	47.83±3.39	45.33±2.51	45.67±3.21	52.17±3.00	56.33±1.82	51.67±2.92	41.83±1.92	50.00±1.32	50.83±1.44	52.33±1.67
LDL(mg/dl)	30.50±5.64	14.67±2.60	9.16±1.24	29.33±4.52	25.00±4.63	33.00±9.91	37.67±7.008	29.50±2.14	51.67±4.27	25.50±1.98

Glucose: Group 5*, Group 6*, Group7*, Group 9*, Group 10*, Group 11* Vs Control (P<0.05). Lipid profile: Group 5$^{\#}$, Group 9$^{\#}$, Group 11$^{\#}$Vs Control (P<0.05).

Table 5: Effects of anaesthetic drugs on haematological indices

Indices	GROUP 1	GROUP 2	GROUP 3	GROUP 4	GROUP 5	GROUP 6	GROUP 7	GROUP 9	GROUP 10	GROUP 11
WBC×10^{3}µl	6.7±0.68	4.48±0.78	4.78±0.60	9.00±0.64	6.13±0.73	2.55±0.77*	0.98±0.30*	1.20±0.70*	2.83±1.04*	2.06±0.90*
LY×10^3 µl	5.50±0.80	3.46±0.63	6.03±0.95	5.50±0.91	4.73±0.58	2.033±0.58$^{\#}$	1.18±0.21$^{\#}$	0.80±0.11$^{\#}$	2.55±0.56$^{\#}$	2.31±0.48$^{\#}$
MO×10^{3}µl	0.61±0.10	0.71±0.20	0.66±0.08	1.10±0.08	0.86±0.27	0.71±0.11	0.21±0.04	0.60±0.19	0.68±0.23	0.41±0.08
GR×10^3 µl	0.61±0.13	0.8167±0.27	0.63±0.11	1.55±0.28	1.01±0.44	0.28±0.03	0.28±0.03	0.25±0.03	0.93±0.34	2.08±0.02
LY%	79.87±5.89	67.78±6.71	69.92±5.84	71.50±8.02	65.37±8.69	85.63±3.07	79.67±1.66	73.82±7.04	57.02±6.28	41.38±4.32
MO%	10.02±2.61	15.32±2.63	14.73±2.22	11.78±2.04	9.483±3.81	4.28±2.45	7.78±0.39	10.30±1.45	18.73±2.94	18.88±2.84
GR%	10.22±3.31	16.90±4.07	15.35±3.80	22.68±7.37	15.67±4.55	7.667±1.69	14.60±1.55	17.25±3.99	23.83±3.38	39.90±4.56
RBC×10^{6}µl	8.72±0.33	8.99±0.31	8.68±0.20	8.097±0.37	7.497±0.33	8.04±0.36	6.838±0.43$^{\#\#}$	5.48±0.23$^{\#\#}$	6.34±0.24	6.80±0.28$^{\#\#}$
Hgb g/dl	15.52±0.46	16.45±0.28	16.08±0.29	20.17±1.31	14.35±0.90	13.13±0.28	11.80±0.64	9.500±0.46	11.68±0.51	12.33±0.70
HCT%	47.32±1.34	50.17±1.09	49.00±0.80	44.43±1.01	37.82±2.46	38.32±0.94	12.22±0.61	27.70±0.87	33.43±1.27	35.22±1.45
MCV fl	55.08±0.48	56.02±1.84	56.62±1.15	53.00±1.17	53.00±0.81	48.80±2.08	52.35±0.66	50.90±0.45	51.83±0.83	50.40±0.56
MCH pg	32.65±0.32	18.33±0.45	18.48±0.21	20.87±1.07	16.83±0.41	16.75±0.56	17.33±0.39	16.77±0.20	17.73±0.22	17.00±0.30
MCHC g/dl	32.65±0.32	32.80±0.67	32.68±0.42	36.23±1.93	35.27±0.37	33.30±0.28	32.53±0.99	34.05±0.30	33.05±0.53	34.48±0.27
RDW %	16.83±0.53	18.90±0.65	18.32±0.34	17.63±0.48	16.53±0.38	16.53±0.38	15.70±0.27	14.80±0.26	16.85±0.23	14.27±0.25
PLT×10^{3}µl	700.80±25.02	494.50±57.69	466.70±91.43	558.5±60.03$^{\#\#}$	671.30±114.20	739.80±57.96	471.7±34.19$^{\#\#}$	567.30±98.70$^{\#\#}$	533.80±107.10	458.80±89.30$^{\#\#}$
PCT%	0.38±0.02	0.28±0.02	0.27±0.04	0.29±0.03	0.41±0.07	0.425±0.02	0.29±0.01	0.01±0.002	0.30±0.06	0.35±0.06
MPV fl	5.50±0.21	5.91±0.16	6.05±0.23	5.76±0.29	6.46±0.13	5.53±0.14	5.91±0.14	5.70±0.17	5.75±0.18	5.41±0.15
PDW%	30.45±0.55	33.48±1.28	35.15±3.04	33.77±1.67	18.25±0.39	30.88±0.36	32.07±0.62	31.63±0.72	32.22±0.87	31.13±0.69

WBC: White blood cells, LY: Lymphocytes, MO: Monocytes, GR: Granulocytes, RBC: Red blood cells, HGB: Haemoglobin, PCV: Packed cell volume. MCV: Mean cell volume, MCH: Mean cell haemoglobin, MCHC: Mean cell haemoglobin concentration, RDW: Radius diameter weight, PLT: Platelets, PCT: Platelet concentration transmittance, MPV: Mean platelet volume, PDW: Platelet diameter weight, RDW: Red cell distribution weight, MPV: Mean platelet

volume, Mean corpuscular haemoglobin concentration. WBC: Group 6*, Group 7*, Group 9*, Group 10*, Group 11* Vs Control (P<0.05). LY×10^{3}µl: Group 6$^{\#}$, Group 7$^{\#}$, Group 9$^{\#}$, Group 10$^{\#}$, Group 11$^{\#}$ Vs Control (P<0.05). RBC: Group 7$^{\#\#}$, Group 9$^{\#\#}$, Group 11$^{\#\#}$ Vs Control (P<0.05).PLT×10^{3}µl: Group 4$^{\#\#}$, Group 7$^{\#\#}$, Group 9$^{\#\#}$, Group 11$^{\#\#}$ Vs Control (P<0.05).

The reduction due to Pancuronium, Bupivacaine and Pethidine represent good safety profile in urea and creatinine as common markers for the kidney. Meanwhile, reduced renal function has been documented in the use of Pancuronium in other studies [2,3]. Similar effect may have been caused by Thiopentone leading to acute renal failure [2,3] which may be dose or species-dependent. Therefore, the effect may worsen when Thiopentone is combined with Pancuronium. Propofol can cause rare fatal complication called Propofol Infusion Syndrome (PIS) characterised by hyperlipidaemia and enlarged liver [9]. It is however to be noticed that toxicity may be absent due to short duration of the drug. Immediate acute effects may be imminent in cardiovascular system, respiratory and central nervous system effects [2,3]. When drugs are devoid of inherent toxic effects in therapy, high doses can cause acute toxicity, and this can become worse when combined with other agents as seen in some cases in this study. Various techniques have been implemented in the pre-, intra- and postoperative monitoring of surgical patients.

Analysis of whole blood parameters is relevant to risk in reduction of RBCs, as the changes in the haematological parameters have a higher predictive value for human toxicity when data are translated from animal studies [15]. The significant reduction in RBCs in groups 7, 9, and 11 rats (Table 5) can pose more risk to subjects that may be bleeding during surgical procedure, hence caution should be exercised when using any of these combinations. There was a significant decrease in blood glucose levels (BLG) in the group treated with a combination dose of Diazepam, Midazolam, Suxamethonium, Pethidine and Atropine (Table 4). This reduction could be due to splenic sequestration of blood elements. The spleen as an organ is capable of sequestering the RBCs through splenic vascular relaxation.

Many anaesthetic drugs can induce splenic vascular relaxation and cause a decrease in the circulating erythrocytes. Blood sequestration could also occur at different organs such as in skin and skeletal muscles [16]. Midazolam is a benzodiazepine that is metabolised in the liver [1-3]. Infusions of Midazolam have been reported to decrease adrenocorticotropic hormone and cortisol secretion [17]. Benzodiazepines reduce sympathetic stimulation but increase growth hormone secretion, resulting in a decrease in the hyperglycaemic response to surgery. This effect is significant if midazolam is given by continuous infusion at 0.125 mg/kg/h [17]. Pethidine as a synthetic opioid analgesic [1-3,9] has been known to cause central nervous system seizures [1-3] by reducing blood glucose, resulting in hypoglycaemia. There was a significant decrease in BGL in the group treated with Bupivacaine when compared with the control group. In this present study, the anaesthetic agents were observed to be effective in reducing BGL in the animals. However, they can induce hypoglycaemia and glycaemic-dysregulation on prolonged administration. There seems to be glucose reduction in the single and combination groups. This may worsen patients that may have existing hypoglycaemia while those that have existing diabetes will benefit from any of the groups that had shown reduction. Surgery produces a stress response that can be modified by anaesthetic agents. Furthermore, anaesthetics can affect glucose homeostasis peri-operatively in diabetic patients by decreasing catabolic hormone secretion [18].

Results from this study revealed that there was significant decrease in BGL irrespective

of the combinations when treated with Diazepam or Bupivacaine compared with the control group. In this present study, anaesthetic agents seem to be effective in reducing BGL. The body naturally tightly regulates BGL as a part of metabolic homeostasis although this may not be sufficient during surgical operation because of the short time duration of operation as seen in this study. Anaesthetics can also affect glucose homeostasis peri-operatively in existing diabetic patients by decreasing catabolic hormone secretion [18]. This study has revealed significant decrease ($P<0.05$) in BGLs especially in combination groups 4, 5, 6 and 7 when compared with the control group. Even with Pethidine alone, this seems contrary to the documented evidence that opioids could cause hyperglycaemia [2,3].

The outcome in adverse effects can also be due to route of drug administration in this study. Intraperitoneal route may have produced faster onset of action hence such drugs enter blood vessels unlike subcutaneous route. The oral route may have produced delayed effect compared to other routes. Propofol is used in induction and maintenance of anaesthesia as part of total intravenous or balanced anaesthesia techniques and is the agent of choice for ambulatory surgery [1-3]. This dual use in these phases provides significant effect in potentiating the adverse effects associated with drug used at the induction stage and maintenance phase. It is however interesting to note that at any stage in an ideal anaesthetic procedure, there can be drug interaction especially with thiopentone as inducers of Cytochrome P_{450} [1-3,9]. This induction may increase metabolism of the drug and cause harmful effect especially if the metabolites are known to be noxious. The lethality observed in group 8 may be due to dose sensitivity of the animals or an enhanced toxic effect potentiated by the individual drugs as in the principles of drug-drug interactions [1-3,9].

From the foregoing, subjective adverse effects may be common in all the agents as is commonly observed. This study recognised adverse effects that may occur in the assay of blood samples for toxicity markers. Some of these agents are introduced at one stage or the other to achieve a unique goal without predicting the synergistic toxic effect. The purpose of monitoring anaesthetic drugs in clinical medicine is to provide information that may impact decisions during surgeries.

CONCLUSION

Findings in this study had shown that drugs used in a complete cycle of anaesthesia are capable of altering some biochemical and haematological parameters. Therefore, it is important that models (animals/ humans) are closely monitored in the course of anaesthetic procedures to ensure safety. The study highlights the importance of selecting safe anaesthetic drugs and also considering the influence of disease or co-morbid conditions on anaesthetic outcomes. Meanwhile the observed lethality in certain drug combinations suggests the need for further review and possible withdrawal or re-evaluation of these agents in future studies.

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Conflict of interest

The authors wish to declare that there is no conflict of interest of any kind.

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