LASU Journal of Health Sciences
www.lasucom.edu.ng; e-mail: lasu.jhs@lasucom.edu.ng
Volume 3 Issue 1(2020), pages 1 - 8

Topically-administered Betamethasone and Neomycin can cause Hepatotoxicity and Nephrotoxicity In Rats: are these effects reversible?

Olayemi S. O.[1], Yemitan O. K.[2,*], Nwaiwu Obiyo[1], Amagon K. I.[1,3], Idowu Abimbola J.[4], Nwali N. B.[1]

[1]Department of Pharmacology, Therapeutics and Toxicology, College of Medicine, University of Lagos, Lagos, Nigeria. [2]Department of Pharmacology, Therapeutics and Toxicology, Lagos State University College of Medicine, Ikeja, Lagos, Nigeria. [3]Department of Pharmacology, Faculty of Pharmaceutical Sciences, University of Jos, Jos, Nigeria. [4]Department of Physiology, Faculty of Basic Medical Sciences, Lagos State University College of Medicine, Ikeja, Lagos, Nigeria.

[‡]*Corresponding author*
Yemitan O. K.
Department of Pharmacology, Therapeutics and Toxicology, Lagos State University College of Medicine, Ikeja, Lagos, Nigeria.
Email: omoniyi.yemitan@lasucom.edu.ng; Phone: +2348023056011

ABSTRACT

Introduction: Different cream formulations of neomycin and betamethasone are presently very popularly and are purchased over-the-counter to treat various skin diseases in Nigeria. Their use has been postulated to have a possible link to the now common incidences of kidney and liver failure in the country.

Method: Each of 36 juvenile Sprague-Dawley rats, of four groups (n = 9), had a 2 cm diameter circumference shaved at their back and treated by gently rubbing the various preparations, once daily, for 30 days as follows: Group 1: normal rats, no treatment; Group 2: 0.5 g of Neomycin + 0.5 g of petroleum jelly; Group 3: 0.5 g of Betamethasone + 0.5 g of petroleum jelly; Group 4 (control): 0.5 g of petroleum jelly. On day 31, blood was collected retro-orbitally from five animals in each group for assessment of the liver and kidney removed for biochemical assays and oxidative stress indicators. The remaining four rats in each group were left untreated for a further 15 days for reversibility tests.

Results: Results showed significant ($P<0.05$) increases in aspartate aminotransaminase, alanine aminotransaminase, low-density lipoprotein, total protein, triglycerides, creatinine and urea, as well as significant ($P<0.05$) decreases in levels of glutathione, superoxide dismutase and catalase, compared to the control group. Following the 15-day reversibility period, both biochemical and anti oxidant parameters were not restored to normal levels.

Conclusion: Prolonged topical exposure to neomycin and/or betamethasone-containing creams has the potential to cause irreversible hepatic and kidney injury.

Keywords: Antioxidants, Hepatotoxicity, Nephrotoxicity, Topical, Betamethasone, Neomycin.

INTRODUCTION

Drug metabolism which is the major role of the liver and kidney, predisposes them to toxic injury following exposure to various drugs. Drugs and other compounds are converted into products that are more easily excreted and that usually have a lower pharmacologic activity than the portent compound, via hepatic metabolism[1,2]. A metabolite may have higher activity and/or greater toxicity than the original drug. Metabolites of the drugs that are excreted from kidneys may also cause cellular damage leading to kidney dysfunction[3].

Aminoglycoside antibiotics have long been used in therapy to treat bacterial diseases. Despite their wide use in therapy, these drugs exhibit a low therapeutic index, as there is little difference between a therapeutic and toxic dose[4]. All aminoglycoside agents are nephrotoxic, although there is variability with regard to toxic potential, with neomycin being the most toxic and streptomycin the least[5].

Local anaesthetic and corticosteroid combination injections are often used in clinical practice. Corticosteroids and local anaesthetics may have a synergistic effect on chondrocyte death[6]. The results by Braun et al., 2012, suggest that Betamethasone sodium phosphate and Betamethasone acetate (Celestone® Soluspan®) are highly chondrotoxic in the presence of local anaesthetics.

Topical medications are medications applied to body surfaces such as skin or mucous membranes to treat various ailments. Examples include: topical solutions, creams, gels, foams, shake lotions, lotions, creams, ointments, transdermal patches, powder, solids, sponge, tape, vapour, paste and tincture. Topical medications differ from many other types of drugs because mishandling them can lead to nephrotoxicity

and hepatotoxicity in a patient or the individual administering the drug[7].

The rise of cases of nephrotoxicity and hepatotoxicity has raised questions regarding the potential of some topical medications to cause toxicity in the liver and kidney. Concerns exist regarding the surge in kidney and liver failures worldwide, including Nigeria. Many contributory factors have been implicated, including the use of a plethora of topical corticosteroids, antibiotics and antifungal creams, singly or in various combinations to treat various ailments. In Nigeria, some of these creams are used as skin toners, without regard to the risk of toxicity.

The exposure of drugs, ionizing radiations and environmental pro-oxidant pollutants to the body induce formation of free radicals, superoxide anions, hydroxyl radicals, hydrogen peroxide, and other radicals. These free radicals initiate lipid peroxidation, considered to be deleterious to cell membranes and associated with a number of abnormal physiological processes[8, 9].

Vitamin C is a well-known cell protective natural antioxidant, with its protective effects observed in oxygen-dependent pathophysiological conditions[10]. Antioxidants are compounds that protect cells against the damaging effects of reactive oxygen radicals[11], which if not scavenged effectively in time, may damage crucial biomolecules like lipids, membrane proteins and DNAs resulting in abnormalities leading to disease conditions[12]. Antioxidants play an important role in preventing the formation of and scavenging of free radicals and other potentially toxic oxidizing species.

This study therefore was designed to test the toxic effects due to the topical use of Betamethasone and Neomycin creams in rats with reference to their use in humans.

MATERIALS AND METHODS

ANIMALS

Thirty-six young female Sprague-Dawley rats (about 100 g) were obtained from the animal house of University of Ibadan, Nigeria, placed in plastic cages and transported to the Laboratory Animal House of the Department of Pharmacology, Therapeutics and Toxicology, University of Lagos, Nigeria. The animals were maintained under standard environmental conditions (24 - 25°C, 12h/12h light/dark cycle) and were fed on Pfizer-branded standard rodent cubes and water *ad libitum*.

All ethical protocols and regulations on experimentation with laboratory animals were used according to internationally established standards (13) and with the approval of the Ethics Committee, Department of Pharmacology, Therapeutics and Toxicology, University of Lagos, Nigeria

Drugs Used

Betamethasone (Glaxo-SmithKline Pharmaceuticals, UK) and Neomycin (Drugfield Pharmaceutical Limited, Sango-Otta, Nigeria) dermal creams were purchased from Health Plus Pharmacy, Nigeria Limited, Yaba, Lagos, Nigeria.

Experimental Design

The rats were allowed to acclimatize for seven days, after which a 2 cm diameter circumference of fur on the back of the animals was shaved off, as described by[14]. The animals were left for another 24 hours after shaving, then randomly distributed into four groups of nine animals each. Each test drug was administered once daily onto the shaved skin area for 30 days as follows; Group 1: no treatment; Group 2: 0.5 g Neomycin + 0.5g of petroleum jelly; Group 3: 0.5g of Betamethasone + 0.5g of petroleum jelly; Group 4 (control), 0.5g of petroleum jelly only. Each animal was kept in a separate cage after administering the test drug.

Collection of Blood and Organs Samples

The rats were fasted overnight, and on day 31, blood was collected retroorbitally for biochemical tests using a capillary tube into lithium heparin anticoagulant bottles[15]. The rats were anaesthetized with chloroform (BDH Chemicals Limited, Poole, England), then, sacrificed by standard protocol of cardiac perfusion fixation using 10 % buffered formalin, prior to histological analysis[16].

The remaining four rats in each group were left untreated for additional 15 days for reversibility assessment.

Determination of Biochemical Parameters

Liver enzymes such as aspartate aminotransferase (AST), alanine aminotransferase (ALT), alkaline phosphate (ALP) and lipid profile enzymes, cholesterol, High-Density Lipoprotein (HDL), Low-Density Lipoprotein (LDL) and triglycerides (TG) were estimated using commercially available kits based on the method described by[17]. Bilirubin was determined by the method described by[18]. Total protein, albumin, creatinine and urea levels were analysed using the fully automated biochemical analyser (Flexor E, Vita Scientific, Functional, Netherlands).

Determination of Antioxidant Capacity

Superoxide dismutase (SOD) was determined by the method of [19]; Catalase (CAT) by the method of [20]; Reduced Glutathione (GSH) by the method of [21]; Malondialdehyde (MDA) by the method described by [22].

Statistical Analysis

Data are expressed as mean ± S.E.M; and were analysed using one way Analysis of Variance (ANOVA), Bonferroni tests were used for comparisons between groups. Confidence interval with least significant difference (LSD) was placed at 95%. In all cases, a value of $P < 0.05$ was significant. All statistical calculations were performed using SPSS 20.0 software (SPSS Inc., Chicago, IL).

RESULTS

Effect of Topical Administration of Betamethasone and Neomycin Creams on Liver Enzymes in Rats.

A significant (P<0.05) increase was observed in AST and ALT after betamethasone administration. There were no significant changes in the other parameters.

Effect of topical administration of betamethasone and neomycin on concentrations of total bilirubin, creatinine and urea in rats.

An insignificant (P>0.05) increase in bilirubin, creatinine and urea concentrations occurred after topical application of betamethasone, relative to the control. Topical administration of neomycin caused an insignificant (P>0.05) increase in creatinine and urea concentrations only, relative to the control that had no drug administered (table 2).

Effect of topical administration of betamethasone and neomycin on Low density Lipoprotein, Cholesterol, Total protein and Triglycerides in rats.

Compared to the control group, an insignificant (P>0.05) increase was observed in concentrations of LDL, CHOL; an insignificant (P>0.05) decrease in HDL concentration, and a significant (P<0.05) increase in levels of TP and TG, after topical administration of betamethasone (table 3). After administration of neomycin, a significant (P<0.05) increase was observed in concentration of TP, while an insignificant increase occurred in concentrations of CHOL, TG and an insignificant decrease in HDL respectively, relative to the control.

Effect of topical administration of betamethasone and neomycin on antioxidant enzymes in rats.

Topical administration of neomycin and betamethasone resulted in a significant (P<0.05) decrease in concentrations of GSH, SOD and CAT, compared to the control that had no drug administered (table 4). A significant (P<0.05) increase and an insignificant (P>0.05) increase in MDA concentration was observed after topical administration of betamethasone and neomycin respectively, relative to the control.

REVERSIBILITY TESTS

Effect of reversibility test on concentrations of liver enzymes after topical administration of betamethasone and neomycin creams.

An insignificant (P>0.05) increase was observed in AST, ALP, and ALT concentrations after betamethasone administration and an insignificant (P>0.05) increase in AST and ALT concentrations after neomycin administration, relative to the control (table 5). ALB remained significantly higher in the Betamethasone group.

Table 1: Effect of Topical Administration of Betamethasone and Neomycin Creams on Liver Enzymes in Rats

Treatment	Liver Function Parameters			
	AST (µL-1)	ALP (µL-1)	ALT (µL-1)	ALB (mg/dL)
Control	24.00 ± 1.08	21.50 ± 0.87	16.75 ± 0.75	2.98 ± 0.09
Neomycin	25.00 ± 0.41α	22.75 ± 0.95	22.50 ± 0.87α	3.00 ± 0.16
Betamethasone	28.00 ± 0.91α†	23.75 ± 0.85	27.00 ± 1.08*†	3.1 ± 0.07
Petroleum Jelly	23.50 ± 0.65α	17.50 ± 1.32α	3.2 ± 0.25	3.2 ± 0.25

Values are represented as Mean ± SEM; P<0.05; n=5
* depicts significance compared to GROUP 1;
α depicts significance compared to GROUP 3;
† depicts significance compared to GROUP 4;.

Table 2: The effect of topical administration of Betamethasone and Neomycin on Total bilirubin, Creatinine, Urea in rats

Treatment	Renal Function Parameters		
	Bilirubin (mg/dL)	Creatinine (mg/dL)	Urea (mg/dL)
Control	0.53 ± 0.05	0.73 ± 0.05	16.75 ± 0.75
Neomycin	0.53 ± 0.03	0.73 ± 0.10	22.50 ± 0.87α
Betamethasone	0.60 ± 0.04	0.78 ± 0.05	27.00 ± 1.08*†
Petroleum Jelly	0.53 ± 0.03	0.70 ± 0.06	3.2 ± 0.25

Values are represented as Mean ± SEM; P<0.05; n=5
α depicts significance compared to GROUP 3;
BIL= Bilirubin
CR= Creatinine
U= Urea

Table 3: Effects of topical administration of Betamethasone and Neomycin on Low density Lipoprotein, Cholesterol, Total protein and Triglycerides in rats.

Treatment	Lipid Profile Parameters				
	LDL (mg/dL)	CHOL (mg/dL)	TP (g/L)	TG (mg/dL)	HDL (mg/dl)
Control	75.25 ± 2.39	146.75 ± 2.84	6.38 ± 0.13	33.50 ± 1.50	28.25 ± 1.03
Neomycin	72.25 ± 1.65α	153.25 ± 3.86	5.75 ± 0.14*	34.50 ±0.65α	25.50 ± 3.59
Betamethasone	78.50 ± 2.25	147.50 ± 2.06	5.35 ± 0.12*†	48.75 ±1.97*β†	26.00 ± 0.82
Petroleum Jelly	76.25 ± 1.25α	143.25 ±4.11	6.13 ± 0.13 α	32.25 ± 1.60α	27.75 ± 0.85

Values are represented as Mean ± SEM; P<0.05; n=5
* depicts significance compared to GROUP 1;
β depicts significance compared to GROUP 2;
α depicts significance compared to GROUP 3;
† depicts significance compared to GROUP 4;.
LDL= Low density Lipoprotein
CHOL= Cholesterol
TP= Total protein
TG= Triglycerides

Table 4: Shows the effect of topical administration of Betamethasone and Neomycin on Antioxidant Enzymes in rats

Treatment	Antioxidant Parameters			
	MDA (nm/g)	GSH(U/g)SOD	(U/g tissue)	CAT (mmol/g tissue)
Control	1.81 ± 0.01	4.33 ± 0.03	29.32 ± 0.16	0.59 ± 0.00
Neomycin	1.86 ± 0.01α	3.65 ± 0.07*α	23.33 ± 0.40*α	0.51 ± 0.00*α
Betamethasone	1.90 ± 0.01*α†	3.15 ± 0.05*αβ†	20.99 ± 0.36*αβ†	0.44 ± 0.00*αβ†
Petroleum Jelly	1.80 ± 0.01α	4.34 ± 0.03αβ	29.30 ± 0.22αβ	0.58 ± 0.01αβ

Values are represented as Mean ± SEM; P<0.05; n=5
* depicts significance compared to GROUP 1;
β depicts significance compared to GROUP 2;
α depicts significance compared to GROUP 3;
† depicts significance compared to GROUP 4;.

Table 5: Effect of reversibility test on concentrations of liver enzymes after topical administration of betamethasone and neomycin creams

Treatment	Liver Function Parameters			
	AST (µL-1)	ALP (µL-1)	ALT (µL-1)	ALB (mg/dL)
Control	2.98 ± 0.09	25.00 ± 1.08	22.00 ± 0.91	17.00 ± 0.41
Neomycin	3.1 ± 0.07	24.50 ± 0.48	22.75 ± 0.95	18.25 ± 0.25α
Betamethasone	3.2 ± 0.25	25.50 ± 0.65	23.75 ± 0.85	20.75 ± 1.55
Petroleum Jelly	2.95 ± 0.06	24.50 ± 1.04	22.25 ± 0.85	16.75 ± 0.85α

Values are represented as Mean ± SEM; P<0.05; n=4
α depicts significance compared to GROUP 3

Effect of reversibility test on concentrations of total bilirubin, creatinine and urea in rats after topical administration of betamethasone and neomycin creams.

An insignificant (P>0.05) increase in bilirubin, creatinine and urea concentrations occurred after topical application of betamethasone, relative to the control. Topical administration of neomycin caused an insignificant (P>0.05) increase in creatinine and a decrease in BIL and urea concentrations, relative to the control that had no drug administered.

Effect of reversibility test on concentrations of low-density lipoprotein, cholesterol, total protein and triglycerides in rats after topical administration of betamethasone and neomycin creams.

Compared to the control group, an insignificant (P>0.05) increase was observed in concentrations of LDL, CHOL; an insignificant (P>0.05) decrease in HDL concentration, and a significant (P<0.05) increase in levels of TG, after topical administration of betamethasone (table 7). After administration of neomycin, an insignificant (P>0.05) increase was observed in concentration of CHOL and TG, while an insignificant (P>0.05) decrease occurred in concentrations of LDL, TP and HDL, relative to the control.

Effect of reversibility test on concentrations of antioxidant enzymes in rats after topical administration of betamethasone and neomycin creams.

Topical administration of neomycin and betamethasone resulted in an insignificant (P>0.05) decrease in concentrations of GSH, SOD and CAT, compared to the control that had no drug administered (table 8). An insignificant (P>0.05) increase in MDA concentration was observed after topical administration of betamethasone and neomycin respectively, relative to the control (table 8).

Table 6: Effect of reversibility test on concentrations of total bilirubin, creatinine and urea in rats after topical administration of betamethasone and neomycin creams

Treatment	Renal Function Parameters		
	Bilirubin (mg/dL)	Creatinine (mg/dL)	Urea (mg/dL)
Control	0.53 ± 0.06	0.70 ± 0.06	23.50 ± 0.65
Neomycin	0.53 ± 0.05	0.73 ± 0.05	23.00 ± 0.82
Betamethasone	0.58 ± 0.05	0.75 ± 0.05	26.50 ± 1.19
Petroleum Jelly	0.53 ± 0.08	0.68 ± 0.05	23.50 ± 0.45

Values are represented as Mean ± SEM; P<0.05; n=4

Table 7: Effect of reversibility test on concentrations of Low density Lipoprotein, Cholesterol, Total protein and Triglycerides in rats after topical administration of betamethasone and neomycin creams

Treatment	Lipid Profile Parameters				
	LDL (mg/dL)	CHOL (mg/dL)	TP (g/L)	TG (mg/dL)	HDL (mg/dl)
Control	75.25 ± 2.39	146.75 ± 2.84	6.25 ± 0.12	32.50 ± 0.96	28.25 ± 1.03
Neomycin	72.25 ± 1.65α	153.25 ± 3.86	5.88 ± 0.14	33.00 ± 1.08	25.50 ± 3.59
Betamethasone	78.50 ± 2.25	147.50 ± 2.06	5.95 ± 0.13	39.00 ±1.87*β†	26.00 ± 0.82
Petroleum Jelly	76.25 ± 1.11	143.25 ± 4.11	6.15 ± 0.10	32.75 ± 0.48	27.75 ± 0.85

Values are represented as Mean ± SEM; P<0.05; n=4
* depicts significance compared to GROUP 1;
β depicts significance compared to GROUP 2;
† depicts significance compared to GROUP 4;.

Table 8: Effect of reversibility test on concentrations of antioxidant enzymes in rats after topical administration of betamethasone and neomycin creams

Treatment	Antioxidant Parameters			
	MDA (nm/g)	GSH(U/g)	SOD (U/g tissue)	CAT (mmol/g tissue)
Control	1.80 ± 0.02	4.35 ± 0.03	29.32 ± 0.17	0.57 ± 0.01
Neomycin	1.80 ± 0.01	4.31 ± 0.06	23.23 ± 0.10	0.55 ± 0.01
Betamethasone	1.82 ± 0.02	4.32 ± 0.02	28.93 ± 0.20	0.55 ± 0.01
Petroleum Jelly	1.78 ± 0.02	4.34 ± 0.03	29.22 ± 0.11	0.57 ± 0.01

Values are represented as Mean ± SEM; P<0.05; n=5

DISCUSSION

A review of literature shows that an increase in serum Alanine aminotransferase (ALT), alkaline phosphatase and Aspartate aminotransferase (AST) activities may indicate liver tissue damage probably by altered cell membrane permeability leading to the leakage of the enzymes from the tissues to the serum though alanine and aspartate aminotransaminases are considered to be sensitive indicators of hepatocellular damage[23]. This is similar to results from this study, which showed elevation of liver enzymes (AST, ALT, ALP) following exposure to neomycin and betamethasone, compared to the group of animals that had no drug administered. Therefore, there is an indication of their deleterious effects on the liver, despite the fact that they were administered through the dermal route. Since this effect was observed after a 30-day administration, it is logical to state that more damage to the liver cells would occur if these drugs are used for longer periods. This is particularly worrisome as particularly women often use these creams for cosmetic purposes.

Large community-based studies have shown a link between low serum albumin and an increase in morbidity and mortality[24]. Under physiological conditions, albumin may have significant antioxidant potential, as it is involved in the scavenging of oxygen free radicals, which have been implicated in the pathogenesis of injury/disease, as occurs in liver and kidney injury[25]. The increase in albumin in the test groups in this study relative to the control, can be said to be a natural mechanism where albumin synthesis is increased to scavenge the oxygen free radicals produced in the event of liver or kidney injury.

The liver, to make bile, uses bilirubin, a chemical released into blood. Previously, it was believed that increase in bilirubin levels is indicative of liver injury or damage and was regarded as a toxic metabolite in the central nerve system [26]. However, some studies have shown the cytoprotective effects of bilirubin[27,28]. It was reported that serum bilirubin significantly contributes to total antioxidant capacity[29] and showed anti-inflammatory effects as well as acting as scavengers of reactive oxygen species[28,30]. A similar trend was observed in this current study where bilirubin concentration was higher in the test groups administered neomycin and betamethasone compared to the control, which had no treatment. As supported by other studies mentioned above, this increase in bilirubin may be an intrinsic mechanism in order to adequately fight the reactive oxygen species produced as a result of liver and kidney injury. In some clinical studies, a high or increased level of bilirubin reduced the risk of diseases like bilirubin and diabetic nephropathy[31].

Creatinine and urea levels increase as kidney function decreases and taken together, provide a very accurate estimation of how well the kidneys are working [32], though creatinine is a more accurate predictor of kidney damage or injury. This observation was similar to that observed in this study, as creatinine and urea levels increased following dermal betamethasone administration, relative to the group that had no drug administered. Both the liver and the kidney must be functioning properly for the body to maintain a normal level of urea in the blood[32].

Results from this study showed an insignificant ($P>0.05$) increase in LDL, CHOL and an insignificant ($P>0.05$) decrease in HDL following betamethasone administration, compared to the control. TG showed a significant ($P<0.05$) increase following betamethasone administration, relative to the control group. It is instructive to note that increase in TG, CHOL, LDL and a decrease in HDL are indicative of injury/damage to cells of the liver, as observed by (33). Increase in level of cholesterol and low-density lipoprotein have been linked to the development of arteriosclerosis[34], a risk that long-term users of such creams are likely be exposed to. Both neomycin and betamethasone showed a significant ($P<0.05$) decrease in total proteins (TP), relative to the control. This decrease, similar to that observed by [35], points to liver injury or damage.

The anti-oxidant defense system helps in maintaining normal cellular physiology, protecting against oxidative stresses[36,37]. Under such normal situations, levels of SOD, CAT, GSH are high while levels of MDA are low. However, during cellular injury, including those affecting the liver and kidney, levels of SOD, CAT and GSH decrease while MDA level increases. In the liver, in addition, the excessive generation of ROS, with the reduction of antioxidant defense activities, has been known to be closely related with the induction and progression of hepatic cell death[36,38]. This is similar to findings from this study where a significant ($P<0.05$) decrease in concentration of SOD, CAT and GSH was observed after both neomycin and betamethasone dermal application, which indicates decline of antioxidant defense capacity in the test animals. Betamethasone caused a significant ($P<0.05$) increase in MDA levels due to lipid peroxidation and an insignificant ($P>0.05$) increase observed after neomycin application, relative to the control. A similar result has been reported by [39] in their study.

The reversibility tests, during which no drug was administered, were conducted to observe the ability of the liver enzymes and antioxidant enzymes and other parameters to be restored to normal levels in the test animals. In the test groups, concentrations of liver enzymes (AST, ALT and AST) were not restored to levels below the control; rather, the concentrations were higher than the control. A similar trend was also observed in concentrations of creatinine, urea, LDL, CHOL and TG in the test groups (particularly betamethasone), relative to the control. It was also observed that in the test groups, levels of SOD, CAT and GSH did not increase while MDA level did not decrease in the test groups relative to the control. These observations point to the persistence of the injurious drugs in the system or the extent of liver and kidney damage.

CONCLUSION

This study has shown the potential of topical application of betamethasone and neomycin to adversely alter biochemical parameters in the liver and kidneys, as well as affect the antioxidant system in a deleterious manner. Long-term application of these drugs is thus capable of toxicity, leading to hepatic or renal damage.

REFERENCES

1. Poppers PJ. Hepatic drug metabolism and anesthesia. Anaesthesist 1980; 29: 55–58.
2. Tolman KG Hepatotoxicity of non-narcotic analgesics; Am. J. Med. 1998; 105: 13S–19S
3. Singhal PC, Sharma P, Sanwal V, Prasad A, Kapasi A, Ranjan R, Franki N, Reddy K, Gibbons N. Morphine modulates proliferation of kidney fibroblasts. Kidney International 1998; 53: 350–357
4. Begg EJ, Barclay ML, Kirkpatrick CM. The therapeutic monitoring of antimicrobial agents. Br J Clin Pharmacol 2001; 52(1): 35S–43S
5. Edson RS, Terrell CL. The aminoglycosides. Mayo Clin Proc 1991; 66(11):1158–1164.
6. Braun HJ, Wilcox-Fogel N, Kim HJ, Pouliot MA, Harris HS, A Dragoo JL. Local Anesthetics and Corticosteroids: A Chondrotoxic Combination. ORS Annual Meeting. 2012; No. 1829.
7. Zaghi D, Maibach HI. Survey of Safety and efficacy information in drug inserts for topical prescription medications. American Journal of Clinical Dermatology. 2007; 8(1): 43-46.
8. Tirkey N, Pilkhwai S, Kuhad A, Chopra K. Hesperidin, a citrus bioflavanoid, decreases the oxidative stress produced by carbon tetrachloride in rat liver and kidney. BMC Pharmacology 2005; 5(2):1-21.
9. Cabre M, Comps J, Paternain JL, Ferre N, Joven J. Time course of changes in hepatic lipid peroxidation and glutathione metabolism in rats with carbon tetrachloride-induced cirrhosis. Clin Exp Pharmacol Physiol 2000; 27(9):694-9.
10. Jacob RA, Sotoudeh G. Vitamin C function and status in chronic disease. Nutr Clin Care 2002; 5:47-9.
11. Silva MF, Marques A, Chaveiro A. Reactive Oxygen Species: A double-edged sword in reproduction. Open Vet Sci J 2010; 4:127-33.
12. Uddin SN, Akond MA, Mubassara S, Yesmin MN. Antioxidant and Antibacterial activities of Trema cannabina. Middle East J Sci Res 2008; 3:105-8.
13. Derrell, C., Guide for the care and use of laboratory animals. Institute of Laboratory Animal Resources. National Academy Press, Washington DC, USA,1996.
14. Adeyemi O.O.; Yemitan K.O.; Afolabi L. Inhibition of chemically induced inflammation and pain by orally and topically administered leaf extract of Manihot esculenta Crantz in rodents. Journal of Ethnopharmacology 2008; 119(1): 6-11.
15. Onyeike EN, Monanu MO, Okoye CN. Changes in the blood lipid profile of wistar albino rats fed rich cholesterol diet. Annals of Biological Research 2012; 3(11):5186-5191.
16. Porto AN, Borges AH, Semenoff-Segundo A, Semenoff TA, Pedro FLM, Bandeca MC, Cortelli SC. Lipid Profile parameters under the influence of periodontitis associated with chronic stress: An animal model study. Journal of International Oral Health. 2013; 5(4): 8-14.
17. Reitman S, Frankel S. A colorimetric method for determination of serum glutamate oxaloacetate and glutamic pyruvate transaminase. Am. J. Clin. Pathol. 1957; 28: 56-58.
18. Jendrassik L. and Grof P. Colorimetric method of determination of bilirubin. Biochemische Zeitschrift 1938; 297: 81-82.
19. Kakkar P, Das B, Viswanathan PN. A modified spectrophotometric assay of superoxide dismutase. Indian Journal of Biochemistry and Biophysics. 1984; 21: 130-132.
20. Sinha AK. Colorimetric assay of catalase. Analytical Chemistry 1972; 47(2):389-94.
21. Jollow DJ, Mitchell JR, Zampaglione N, Gillettte JR. Bromobezene-induced liver necrosis. Protective role of gluthatione and evidence for 3,4-bromobenzene oxide as the hepatotoxic metabolite. Pharmacology 1974; 11: 151-169.
22. Colado MI, OShea E, Granados R, Misra A, Murray TK, Green AR. A study of the neurotoxic effect of MDMA ('ecstasy') on 5-HT neurones in the brains of mothers and neonates following administration of the drug during pregnancy. British Journal of Pharmacology.1997; 121: 827-833.
23. Umana UE, Timbuak JA, Musa SA, Asala S, Hambolu J, Anuka JA. Acute and Chronic Hepatotoxicity and Nephrotoxicity Study of Orally Administered chloroform extract of Carica papaya Seeds in Adult Wistar Rats. International Journal of Scientific and Research Publications 2013; 3(4)1-8.
24. Klonoff-Cohen H, Barrett-Connor EL, Edelstein SL. Albumin levels as a predictor of mortality in the elderly. J Clin Epidemiol 1992; 45: 207–12.
25. Nicholson JP, Wolmarans MR, Park GR. The Role of albumin in critical illness. Br. J. Anaesth 2000; 85(4): 599-610.
26. Hansen TW. Mechanisms of bilirubin toxicity: clinical implications. Clin Perinatol 2002; 29:765-778.
27. Sedlak TW, Saleh M, Higginson DS, Paul BD, Juluri KR, Snyder SH. Bilirubin and glutathione have complementary antioxidant and cytoprotective roles. Proc Natl Acad Sci U S A 2009;106:5171-5176.
28. Vitek L, Schwertner HA. The heme catabolic pathway and its protective effects on oxidative stress-mediated diseases. Adv Clin Chem 2007; 43: 1-57.
29. Frei B, Stocker R, Ames BN. Antioxidant defenses and lipid peroxidation in human blood plasma.

Proc Natl Acad Sci U S A 1988; 85:9748-9752.
30. Deetman PE, Bakker SJL, Kwakernaak AJ, Navis G, Dullaart RPF. The Relationship of the Anti-Oxidant Bilirubin with Free Thyroxine Is Modified by Insulin Resistance in Euthyroid Subjects. PLoS One. 2014; 9(3): e90886.
31. Han SS, Na KY, Chae DW, Kim YS, Kim S, Chin HJ. High serum bili- rubin is associated with the reduced risk of diabetes mellitus and diabetic nephropathy. Tohoku J Exp Med 2010; 221:133-140.
32. Ajeniyi SA and Solomon RJ. Urea and Creatinine of Clarias Gariepinus in three different Commercial Ponds. Nat Sci 2014; 12(10):124-138.
33. Ahmad D, Gulfraz M, Ahmad MS, Nazir H, Gul H, Asif S. Protective action of Taraxacum officinale on CCL4 induced hepatotoxicity in rats. African Journal Of Pharmacy and Pharmacology 2014; 8(30): 775-780.
34. Razzaq FA, Khan RA, Feroz Z, Afroz S. African Journal of Pharmacy and Pharmacology 2011; 5(7): 943-947.
35. Rajesh SV, Rajkapoor B, Kumar RS, Raju K. Effect of Clausena dentata (Willd.) M. Roem against paracetamol induced hepatotoxicity in rats. Pak. J. Pharm. Sci 2009; (22)1:90-9.
36. Akbulut S, Elbe H, Eris C, Dogan Z, Toprak G, Otan E, Erdemli E, Turkoz Y. Cytoprotective effects of amifostine, ascorbic acid and N-acetylcysteine against methotrexate-induced hepatotoxicity in rats. World J Gastroenterol 2014; 20:10158-65.
37. Cayır K, Karadeniz A, Simşek N, Yıldırım S, Karakuş E, Kara A, Akkoyun HT, Sengül E. Pomegranate seed extract attenuates chemotherapy-induced acute nephrotoxicity and hepatotoxicity in rats. J Med Food 2011;14:1254-62.
38. Hadi NR, Al-Amran FG, Swadi A. Metformin ameliorates methotrexate-induced hepatotoxicity. J Pharmacol Pharmacother 2012; 3:248-53.
39. Awodele O, Tomoye OP, Quashie NB, Amagon KI, Ogunnowo SA. Gentamicin nephrotoxicity: Animal experimental correlate with human pharmacovigilance outcome. Biomedical Journal 2015; 38(20): 125-130.