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Biochemical and Histopathological Toxicity by Multiple Drug Administration

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1. Introduction

With the increase in ways and means to improve health care there has been an increase in miseries of humanity a patient is often presented with several pathological situations that greatly necessitate the need for multiple drug administration and this in turn increases the chances of drug toxicity. Hence there is an immense need to explore such drug combinations that could be given safely to patients with multiple disorders.

Multiple drug administration increases chances of drug interaction, altering the responses of drugs either increased or decreased pharmacological effects, or a new pharmacological response. Generally drug interactions should be avoided, due to the possibility of poor or unexpected outcomes and can be prevented with access to current, comprehensive and reliable information which may improve the safe and cost-effective patient care. Most countries face an augmented load of cardiovascular diseases (CVD) and epilepsy along with chronic non-communicable disorders such as diabetes mellitus. Hence it is essential to recognize the probable toxicities that might occur due to multiple drug administration. Occasionally these toxicities are predictable on the basis of known pharmacology of the drugs used, thus combinations require separate investigations with animal toxicity studies.

1.1 Epilepsy

Epilepsy is the leading neurological disorder in the world categorized by abnormal hyper excitability of the neurons causing seizures with or without loss of consciousness. These seizures are of short-term and an indication of unusual, extreme or synchronous neuronal activity in the brain (Fisher et al., 2005).

Epilepsy represents the 3rd most common neurologic disorder in developed countries after stroke and dementias, encountered in elderly (Lim, 2004). The prevalence of epilepsy is around 0.4 to 0.8 % (Brodie and Dichter, 1996) and its overall occurrence is around 50-70 cases per 100, 000 in developed countries and 100 per 100, 000 in developing countries (Lim, 2004). Epilepsy

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is a significant, but often underappreciated, health problem in Asia (Mac et al., 2007). It is estimated that approximately 50 million people worldwide have epilepsy (Kwan and Brodie, 2000). This figure had recently reduced and it has been estimated that approximately 45 million of population globally have epilepsy (French and Pedley, 2008). In Pakistan its predominance is approximately 1 % (Aziz et al., 1994 and 1997; Khatri et al., 2003). The utmost occurrence rate of 1.25% was found at the age group 20-29 years. The incidence rate gradually dropped, reaching the lowest of 0.49% in the age group of 50-59. Conversely the prevalence rate augmented again reaching to 1.1% at age > 60 years (Aziz et al., 1994).

Etiology of epilepsy is age related, in children, approximately 20% are remote symptomatic, 50% are cryptogenic while 30% are idiopathic. On the other hand, in elderly, approximately 55% are remote symptomatic whereas 45% are idiopathic/ cryptogenic. In elderly causes and risk factors for seizures are significantly variable (LIM, 2004). The cumulative lifetime risks for epilepsy and unprovoked seizure in industrialized countries are 3.1% and 4.1%, respectively (McHugh and Norman, 2008). In most of the cases (62%) the reason is unidentified, stroke (9.0%), head trauma (9.0%), alcohol (6.0%), neurodegenerative disease (4.0%), static encephalopathy (3.5%), brain tumors (3.0%), and infection (2.0%) account for remaining cases. Although cerebrovascular reasons are more widespread in the old age, the reason is yet to be explored in 25% to 40% of patients who are 65 years of age or older (French and Pedley, 2008). Majority of the patients are well controlled on a single antiepileptic drug (Nadkarni et al., 2005). Since the early 1990s, a number of latest antiepileptic drugs have arrived in the market that proposed considerable benefits in terms of their favorable pharmacokinetics, enhanced tolerability and decrease probability for drug-drug interactions (Bialer and White, 2010). Every new drug offers a special profile of pharmacokinetics, undesirable effects, and mechanisms of action, making best utilization of these agents even more difficult (LaRoche and Helmers, 2004). Seizures can be managed in patients with epilepsy by means of conventional antiepileptic drugs, but regardless of optimal therapy 25% to 30% of patients continue to have seizures and others have undesirable side effects. Hence there is a need for additional drugs as well as new approaches for preventing epilepsy (Dichter and Brodie, 1996; Bialer and White, 2010).

1.2 Hypertension

Hypertension is a widespread human disease that badly affects approximately 1 billion people globally (Varon and Marik, 2003; Dickson and Sigmond, 2006; Chobanian, 2008). Unfortunately, regardless of current progresses in understanding and treating hypertension, its occurrence keeps on increasing. Worldwide 26% of the adult population suffers from hypertension (Dickson and Sigmond, 2006) and its occurrence is projected to boost up to 60% by 2025, when a total of 1.56 billion people may be exaggerated. Pakistan ranks at number sixth in terms of its population (165million in 2007) which is constantly increasing at a rate about 1.83% per year, national heath surveys reveals that 33% of Pakistan's population beyond the age of 45 has hypertension (Wasay and Jabbar, 2009). Hypertension is the foremost threat for cardiovascular disease (Lardinois, 1995; Peralta et al., 2007; Chobanian, 2008) responsible for one half of the coronary heart disease and about two third of the cerebrovascular disease load (Cutler et al., 2008) and is accountable for the most of deaths globally (Adrogue and Madias, 2007), even moderate increase in arterial blood pressure results in reduced life span.

1.3 Diabetes

Diabetes mellitus and hypertension are widespread that exist together at a larger rate than the individual one (Sowers and Zemel, 1990; Epstein and Sowers, 1992; Tenenbaum et al., 1999; Zanella et al., 2001). The occurrence of hypertension in the diabetic individual noticeably enhances the threat and hastens the course of cardiac disease, peripheral vascular disease, stroke, retinopathy, and nephropathy (Epstein and Sowers, 1992; Zanella et al., 2001). The occurrence of simultaneous hypertension and diabetes appears to be growing in developed nations because populations are aging and both hypertension and non-insulin dependent diabetes mellitus occurrence increases as the age progresses (Sowers and Epstein, 1995). People with diabetes faces two to four times augmented risk of CVD in contrast to the general population, simultaneous hypertension triples the already high risk of coronary artery disease, doubles total mortality and stroke risk, and may be accountable for up to 75% of all CVD in people with diabetes (Stults and Jones, 2006).

Rates of diabetes are increasing around the world (Kassab et al., 2001) which now becomes one of the major public health challenges for the 21st century. The increase occurrence in diabetes is because of aging population, obesity and stressing life style. Poverty has been under recognized as a contributor to prevalence of diabetes but it is strongly associated with the unhealthy alimentary habits (Krier et al., 1999; Riste et al., 2001). The incidence of diabetes get higher in the last decade because of factors that are strongly related to the life style as is inactivity and population aging (Muchmore et al., 1994; Keen, 1998). Studies show that type 2 diabetes affects 3% to 5% of the population in some countries and type 1 moves towards the younger ages (Dixon, 2002; Petkova et al., 2006).

The increase rate of diabetes will be noticeably higher in developing countries, between 1995 and 2025, the number of persons with diabetes is predictable to enhance by 170% in the developing world, in contrast with 42% in developed nations. Hence, by the year 2025 above 75% of the people with diabetes will exist in developing countries (Nicolucci et al., 2006). A national health survey of Pakistan reveals that 25% of patients above 45 years have diabetes mellitus and Pakistan ranks number six globally in terms of prevalence of diabetes. It was projected that in 2000 there were 5.2 million diabetic patients and this will increase to 13.9 million by 2020, leading Pakistan to 4th most populous country for diabetes mellitus (Wasay and Jabbar, 2009). However a survey conducted in 2010 by Hayat and Shaikh reveals that Pakistan ranks number seven on diabetes prevalence and figures show that about 6.9 million people have diabetes. The International Diabetes Federation predicts that this number will rise to 11.5 million by 2025 if effective procedures are not taken to control the disease (Jawad, 2003).

The occurrence of diabetes is continually growing and rising at a distressing rate, and it is projected that, unless successful prevention and control measures are put into practice, this disease will soon involve 300 million persons worldwide (Sowers, 2004). Globally more than 170 million people have diabetes, and this figure is expected to be more than double by the year 2030, if existing trends continue (Boden and Taggart, 2009, Hoque et al., 2009).

1.4 Arrhythmia

Hypertension is usually linked with arrhythmias in patients with and without simultaneous CVD. There are studies which show the possible links between hypertension and atrial and

ventricular arrhythmias, though the principal pathophysiological mechanism remains unclear (Yiu and Tse, 2008). The prevalence and risk factors for arrhythmias vary among men and women (Wolbrette et al., 2002). The most prevalent arrhythmia seen in clinical practice is atrial fibrillation which currently influences more than 2 million Americans, with an expected rise to 10 million by the year 2050 (Zimetbaum, 2007).

A patient is often presented with several other pathological states along with epilepsy; such as hypertension, arrhythmias, and diabetes, therefore it is essential to discover the drug-drug interaction upon simultaneous use of anti-epileptic with antihypertensive, antiarrhythmic and antidiabetic. A well reported example is the increase in serum phenytoin levels when used concomitantly with amiodarone and therefore resulting in phenytoin toxicity (Lesko, 1989; Nolan et al., 1990) thus there is an massive need to assess the toxicities of multiple drug administration and to explore relatively safe combination for individuals with multiple disorders, not to predict but rather to warn the users and prescribers, of the possible dangers, to discourage the use of combination which have high cumulative toxicities in animals and to suggest more useful combination in countries where drug regulatory control is very poor.

2. Biochemical testing and histopathological examination of liver toxicities

Serum biochemical parameters can provide important and useful information in assessing not only the extent and severity of liver damage, but also the type of liver damage (Ramaiah, 2007). Histopathological assessments also take part in the diagnosis of liver disease; moreover evaluation of morphological changes may provide additional information that may be useful for clinical management for example, grading of inflammatory activity and staging of fibrosis in chronic viral hepatitis, and the distinction between simple steatosis and steatohepatitis in alcoholic and non-alcoholic fatty liver disease (Hubscher, 2006).

Liver function tests (LFT) are helpful screening tools to detect hepatic dysfunction (Kim, 2008; Thapa and Walia, 2007; Astegiano et al., 2004). Since liver performs a variety of functions, no single test is sufficient enough to provide complete estimate of liver functions (Kim, 2008; Astegiano et al., 2004).

Table 1A and 1B reveals the comparison of γ–glutamyl transferase (γ-GT), alkaline phosphatase (ALP), alanine transaminases (ALT), total bilirubin (TBR) and direct bilirubin (DBR) levels between control animals and animals kept on individual drugs and their combinations for a period of 60 days and then after drug free interval of 15 days in normal therapeutic doses. The administration of amiodarone (4.285 mg/kg) in rabbits shows highly significant elevation in the levels of serum γ–GT, ALP, ALT and DBR (Feroz et al., 2011a). There are studies in which long-term administration of amiodarone was associated with fatal hepatotoxicity (Richer and Robert, 1995; Usdin et al., 1996; Mendez et al., 1999) although most hepatic adverse effects were transient and reversible; however deaths have also been reported from amiodarone-induced hepatotoxicity (Richer and Robert, 1995). Microscopic examination of the hepatic tissue has shown mild diffuse cellular swelling in hepatocytes (Fig 1B). Moreover the administration of losartan potassium (0.892 mg/kg) and verapamil (1.714 mg/kg) revealed highly significant elevation in serum ALP, elevations in serum ALP initiate predominantly from liver and bone (Renner and Dallenbach, 1992). There was also a significant elevation in TBR in animals kept on verapamil alone (Feroz et al., 2011a). However

no significant changes have been found in animals kept on glibenclamide (0.125 mg/kg), oxcarbazepine (18.5 mg/kg) and captopril (0.512 mg/Kg) alone.

Data from animal's studies also shows that the administration of amiodarone-glibenclamide-losartanpotassium- oxcarbazepine (AGLO) combination causes highly significant elevation in serum ALP. The transaminases, ALP and γ -GT are most widely used as indicators of hepatobiliary disease (Renner and Dallenbach, 1992). Increase in ALP might be due to cholestasis (Giannini et al., 2005) or it may also suggest a biliary tract disorder (Herlong, 1994). This explains that rise in ALP might be due to chloestatic diseases or partial obstruction of bile ducts or primary biliary cirrhosis (Pratt and Kaplan, 2000). Microscopic examination of hepatic tissue has shown moderate portal inflammation (Fig 1C). There was also a significant decreased in TBR in animals kept on AGLO combination (Feroz et al., 2011a).

The administration of amiodarone-glibenclamide-verapamil-oxcarbazepine (AGVO) combination in animals shows highly significant elevation in γ -GT. Its level is elevated in a number of pathological conditions such as pancreatic disease, myocardial infarction, renal failure, chronic obstructive pulmonary disease, diabetes, and alcoholism. Measurement of serum γ -GT offers the presence or absence of hepatobiliary disease. Microscopic examination of hepatic tissue has shown congestion and mild mononuclear inflammatory infiltrate (Fig 1D). There was also a significant decreased in TBR in animals kept on AGVO combination (Feroz et al., 2011a). There has been a highly significant and significant decreased in ALT and TBR in animals kept on amiodarone-glibenclamide-captopril-oxcarbazepine (AGCO) combination (Feroz et al., 2011a). Aminotransferase levels are sensitive indicators of liver-cell injury and are useful in identifying the hepatocellular disease (Pratt and Kaplan, 2000). Abnormal AST and ALT point to a hepatocyte disorder (Herlong, 1994). However microscopic examination shows congestion only (Fig 1E) illustrating no remarkable changes in the hepatic tissue of these animals (Feroz et al., 2011a).

Parameters/ Groups	**γ-GT (μ/l)**	**ALP (μ/l)**	**ALT (μ/l)**	**TBR** (mg/dl)	**DBR** (mg/dl)
Control	11.28±1.21	55.51±5.92	83.16±5.21	0.32±0.03	0.15±0.01
Amiodarone	19.41±1.43**	88.34±5.64**	96.60±1.52*	0.30±0.02	0.32±0.05**
Glibenclamide	11.02±0.75	62.82±2.06	68.50±4.72	0.23±0.03	0.20±0.02
Los. Pot	8.82±0.80	79.0±3.25**	81.40±7.98	0.21±0.05	0.14±0.02
Oxcarbazepine	14.46±1.41	53.47±1.26	79.26±2.11	0.25±0.01	0.17±0.02
Verapamil	10.77±1.37	80.44±6.89**	80.07±3.67	0.53±0.12*	0.13±0.01
Captopril	13.58±0.55	57.37±2.10	73.94±3.31	0.32±0.06	0.15±0.01
AGLO	11.21±1.20	79.21±5.11**	76.55±4.16	0.13±0.01*	0.18±0.03
AGVO	16.78±0.95**	66.62±7.77	70.76±4.25	0.13±0.03*	0.21±0.04
AGCO	11.95±1.48	47.75±4.03	56.74±4.69**	0.14±0.04*	0.21±0.04

n=9
Mean ± S.E.M
*p < 0.05 significant with respect to control
**p <0.005 highly significant with respect to control

Table 1A.Comparison of hepatic parameters following 60 days administration of individual drugs and their combinations {Adopted from Feroz et al., 2011(a)}.

Parameters/ Groups	**γ-GT (μ/l)**	**ALP (μ/l)**	**ALT (μ/l)**	**TBR** (mg/dl)	**DBR** (mg/dl)
Control	11.30±1.21	55.30±5.90	82.70±5.20	0.31±0.03	0.15±0.01
Amiodarone	19.16±1.42**	88.0±5.70**	96.56±1.50*	0.26±0.02	0.30±0.04**
Glibenclamide	10.58±0.82	62.42±2.10	67.80±4.80	0.22±0.03	0.16±0.01
Los. Pot	8.51± 0.78	79.63±3.10**	80.80±8.0	0.17±0.05	0.13±0.02
Oxcarbazepine	14.17±1.40	53.96±1.30	78.72±2.11	0.23±0.01	0.16±0.01
Verapamil	10.39±1.30	81.0±6.80**	79.4±3.60	0.50±0.12*	0.12±0.01
Captopril	13.17±0.51	56.93±2.20	73.39±3.30	0.30±0.06	0.14±0.01
AGLO	10.79±1.10	79.0±5.10**	76.10±4.20	0.12±0.01*	0.17±0.03
AGVO	16.42±0.92**	66.0±7.70	70.51±4.22	0.11±0.02*	0.18±0.03
AGCO	11.43±1.50	46.90±4.0	54.0±5.10**	0.13±0.04*	0.18±0.03

n=9
Mean ± S.E.M
*p < 0.05 significant with respect to control
**p <0.005 highly significant with respect to control

Table 1B. Comparison of hepatic parameters following drug-free interval of 15 days of individual drugs and their combinations {Adopted from Feroz et al., 2011(a)}.

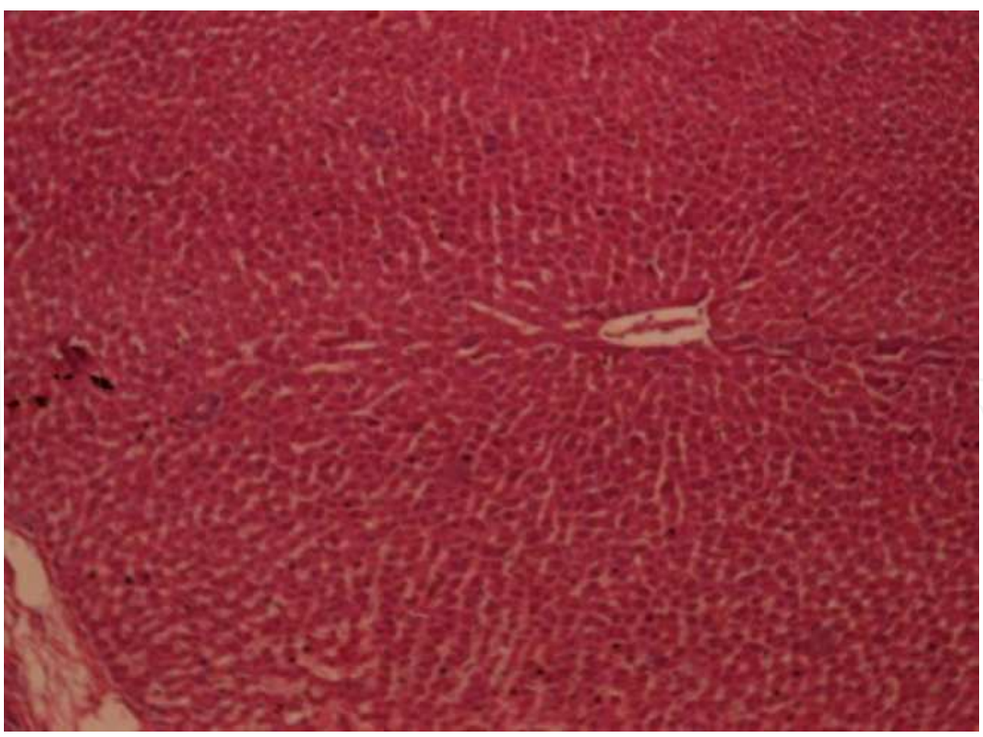

Fig. 1A. Hepatic tissue showing no microscopic change {Adopted from Feroz et al., 2011(a)}.

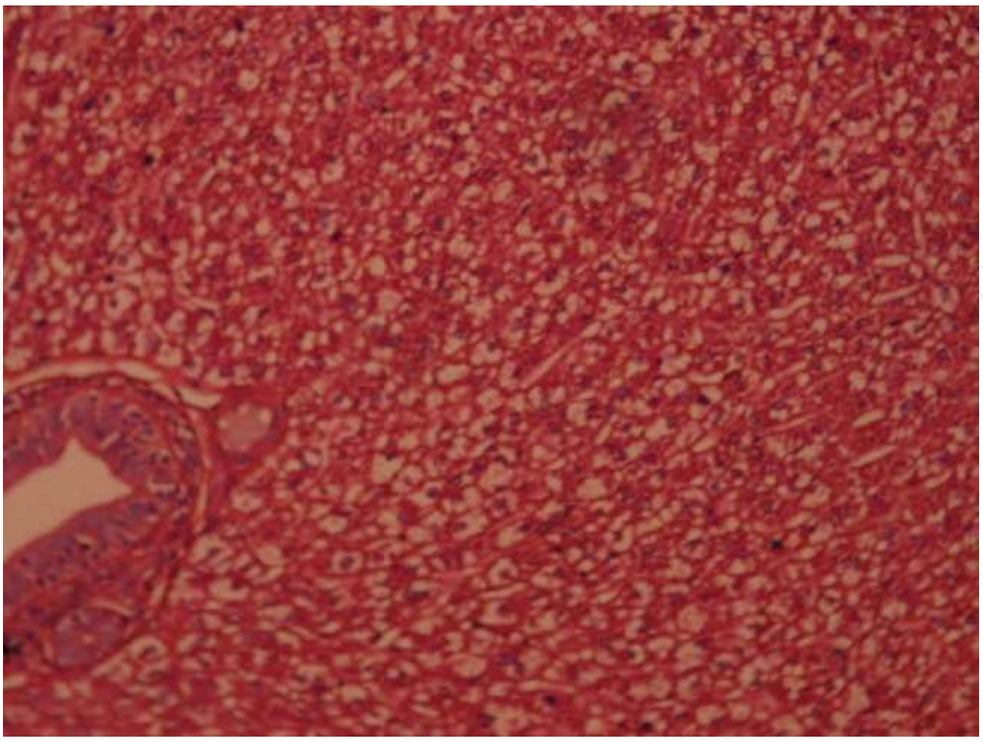

Fig. 1B. Hepatic tissue showing cellular swelling {Adopted from Feroz et al., 2011(a)}.

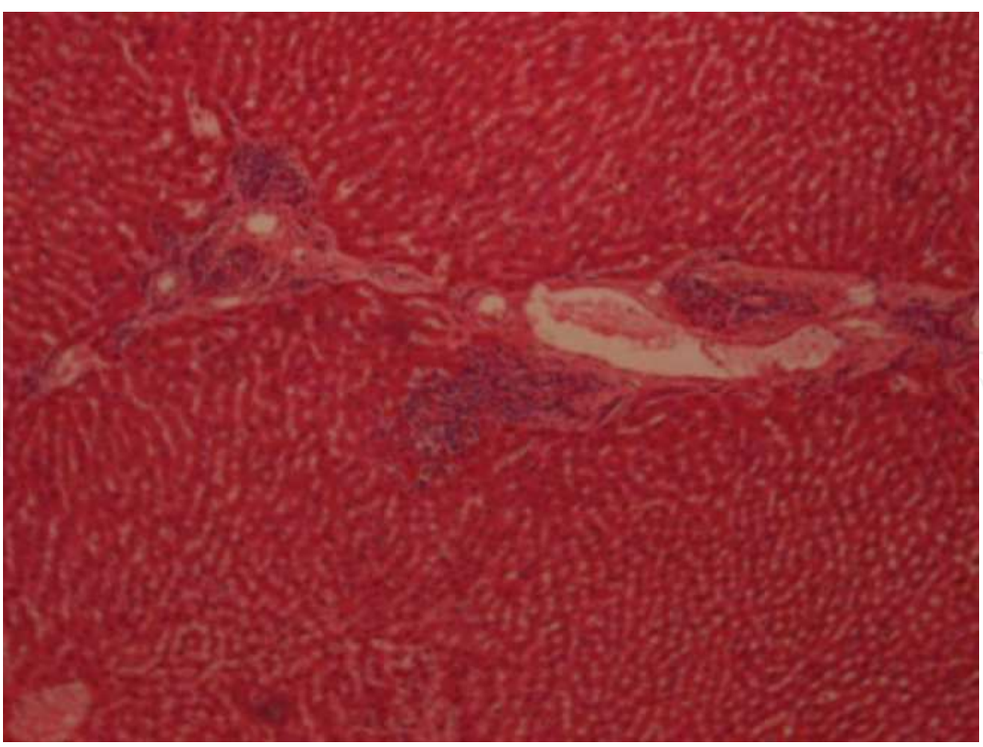

Fig. 1C. Hepatic tissue showing moderate portal inflammation {Adopted from Feroz et al., 2011(a)}.

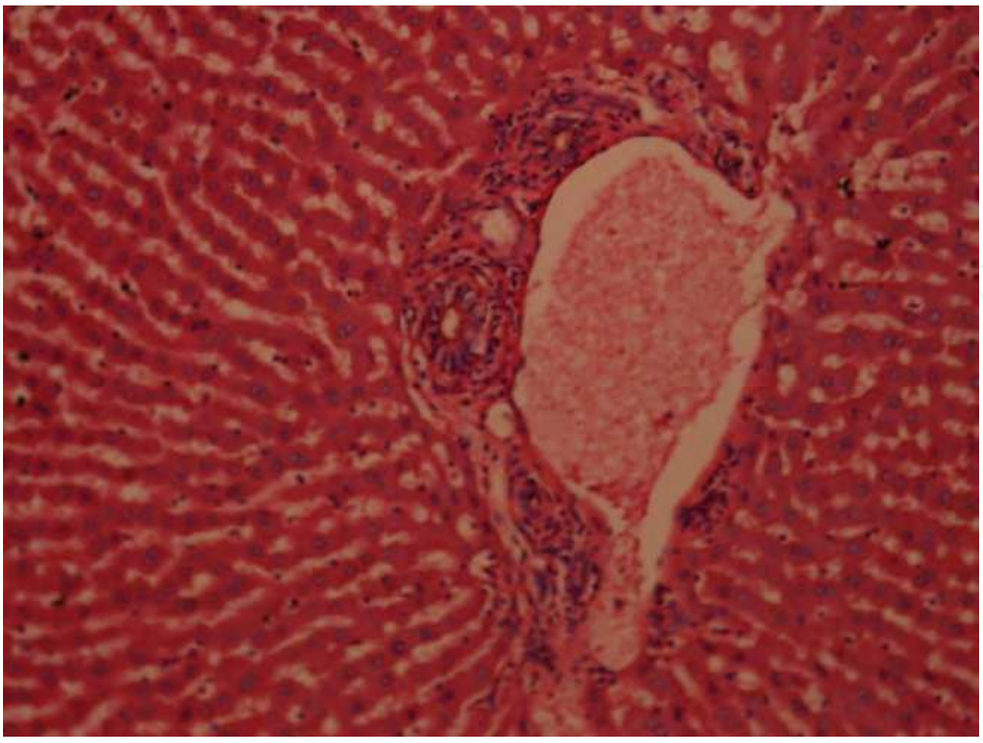

Fig. 1D. Hepatic tissue showing congestion and mild mononuclear inflammatory infiltrate {Adopted from Feroz et al., 2011(a)}.

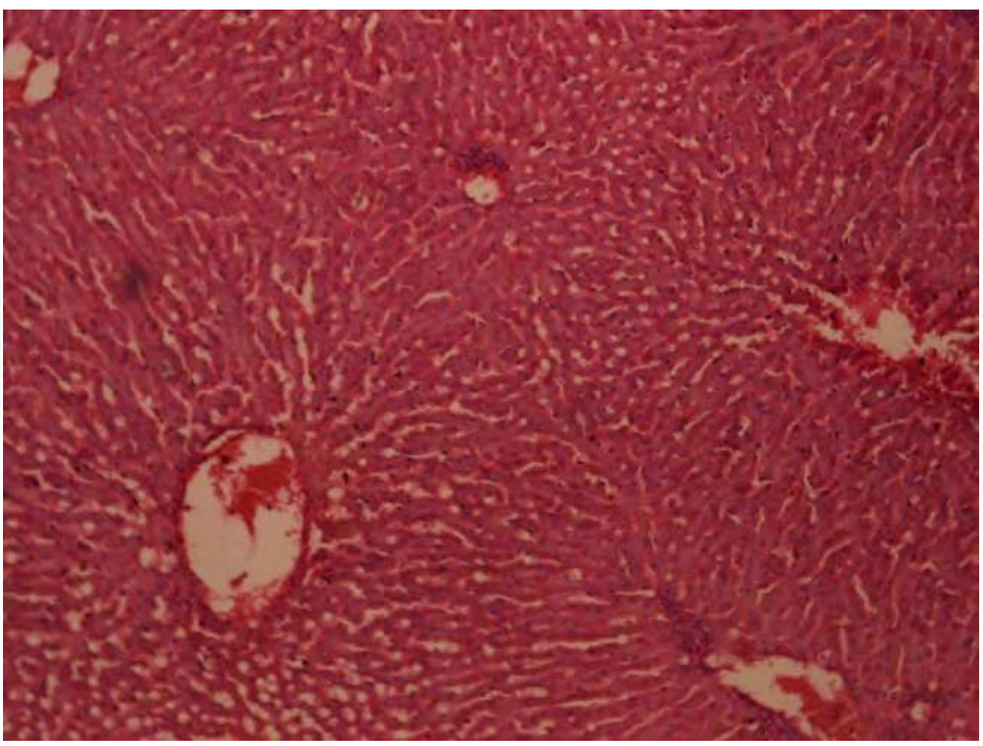

Fig. 1E. Hepatic tissue showing congestion {Adopted from Feroz et al., 2011(a)}.

3. Biochemical testing and histopathological examination of renal toxicities

Drugs are a frequent cause of acute kidney injury particularly in patients older than 60 years and have a higher occurrence of diabetes and CVD (Naughton, 2008). Renal function test are important in assessing the amount and severity of renal damage.

Table 2A and 2B reveals the comparison of urea and creatinine levels between control animals and animals kept on individual drugs and their combinations for a period of 60 days and then after drug free interval of 15 days in normal therapeutic doses. The administration of amiodarone in animals causes increase in the levels of urea and creatinine, whereas animals received verapamil showed significant increase in the level of urea only. However biochemical changes do not correlate to histopathological changes in renal tissue (Fig 2A) hence it is not an indication of renal damage (Feroz et al., 2010). Animals received glibenclamide showed no significant changes at biochemical level; however microscopic examination of renal tissue reveals mild tubulointerestial nephritis (Fig 2B), which together with insignificant rise in serum urea might be an indicative of developing renal damage. Significant elevation in serum urea level in animals received amiodarone and verapamil alone after drug free interval might be due to slow excretion rate of amiodarone and verapamil from the body.

Animals received AGVO combination showed highly significant rise in urea level though it was reversed after the drug-free interval, while animals kept on AGCO combination showed highly significant elevation of serum urea and creatinine. Increased creatinine level suggests decreased creatinine clearance which is a reliable indicator of decreased glomerular filtration rate due to renal damage. However after dug-free interval urea level remained highly significant while creatinine level was changed from highly significant to significant (Feroz et al., 2010).

Parameters/ Groups	**Urea (mg/dl)**	**Creatinine (mg/dl)**
Control	52.12±4.37	0.64±0.02
Amiodarone	169.65±5.73**	1.12±0.07**
Glibenclamide	52.80±2.61	0.70±0.05
Los. Pot	55.05±1.50	0.64±0.02
Oxcarbazepine	49.27±1.40	0.58±0.07
Verapamil	68.14±1.62*	0.71±0.02
Captopril	62.0±1.83	0.60±0.02
AGLO	55.28±2.44	0.64±0.07
AGVO	74.86±6.57**	0.71±0.05
AGCO	148.54±6.81**	1.20±0.05**

n=9
Mean ± S.E.M
*p < 0.05 significant with respect to control
**p <0.005 highly significant with respect to control

Table 2A. Comparison of renal parameters following 60 days administration of individual drugs and their combinations (Adopted from Feroz et al., 2010).

Parameters/ Groups	Urea (mg/dl)	Creatinine (mg/dl)
Control	51.97±4.37	0.63±0.02
Amiodarone	68.15±1.30*	0.70±0.03
Glibenclamide	52.40±2.59	0.66±0.03
Los. Pot	54.61±1.41	0.73±0.05
Oxcarbazepine	49.0±1.41	0.54±0.03
Verapamil	61.88±1.46*	0.64±0.03
Captopril	60.77±1.57	0.67±0.02
AGLO	54.66±2.45	0.68±0.07
AGVO	59.20±4.48	0.63±0.05
AGCO	120.65±6.42**	0.75±0.03*

n=9
Mean ± S.E.M
*p < 0.05 significant with respect to control
**p <0.005 highly significant with respect to control

Table 2B. Comparison of renal parameters following drug-free interval of 15 days of individual drugs and their combinations (Adopted from Feroz et al., 2010).

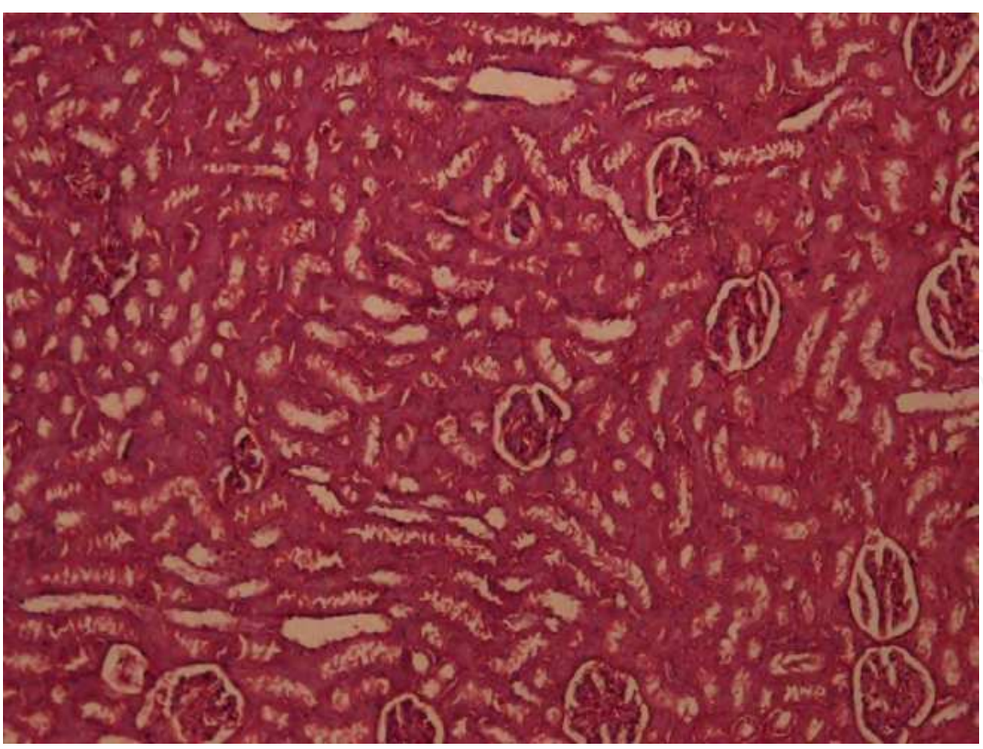

Fig. 2A. Renal tissue showing no microscopic change (Adopted from Feroz et al., 2010).

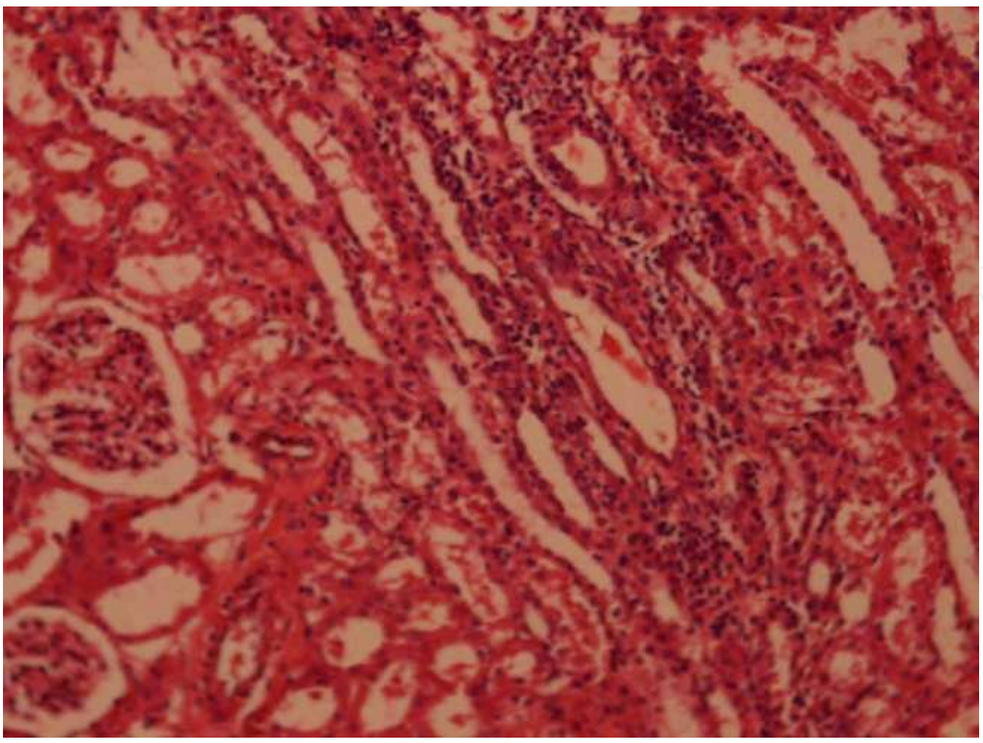

Fig. 2B. Renal tissue showing mild tubulointerestial nephritis (Adopted from Feroz et al., 2010).

4. Biochemical testing and histopathological examination of cardiac toxicities

Cardiac enzymes are proteins that escape out of injured myocardial cells resulting in elevated levels in blood. Table 3A and 3B reveals the comparison of creatinine kinase (CK) and aspartate transaminases (AST) levels between control animals and animals kept on individual drugs and their combinations for a period of 60 days and then after drug free interval of 15 days in normal therapeutic doses. Animals received amiodarone, losartan potassium, oxcarbazepine and captopril alone revealed highly significant elevation in the level of CK but these changes do not correlate with histological changes (Fig 3A) (Feroz et al., 2010). However animals kept on oxcarbazepine alone revealed significant elevation in CK level even after drug free interval which might be an indication of developing neuroleptic malignant syndrome (Pelonero et al., 1998).

The administration of AGLO combination in animals causes significant elevation in CK and AST levels, moreover inflammatory changes in the cardiac tissues (Fig 3B) suggest possible cardiac injury (Feroz et al., 2010). There are studies which suggest that raise in CK level increases the risk of myocardial infarction (Smith et al., 1976; Kumar et al., 2003; Watanabe et al., 2009). Thus simultaneous elevation of AST along with CK and histological changes might be indicative of severe myocardial cellular damage (Kratz et al., 2002). There was significant decrease in CK level in animal received AGCO combination at the end of dosing and after drug-free interval which may be due to reduced muscle mass, wasting or cachexia (Feroz et al., 2010).

Parameters/ Groups	CK (μ/l)	AST (μ/l)
Control	311.35±12.57	54.07±1.03
Amiodarone	567.42±29.61**	54.81±2.37
Glibenclamide	321.26±13.48	48.63±4.69
Los. Pot	400.84±4.24**	51.36±0.99
Oxcarbazepine	392.61±14.86**	56.05±1.99
Verapamil	307.28±6.49	49.42±3.01
Captopril	590.12±12.59**	57.13±0.80
AGLO	393.91±21.54**	60.66±1.93*
AGVO	330.11±38.41	53.05±1.48
AGCO	275.40±7.30*	50.87±1.29

n=9
Mean ± S.E.M
*p < 0.05 significant with respect to control
**p <0.005 highly significant with respect to control

Table 3A. Comparison of cardiac parameters following 60 days administration of individual drugs and their combinations (Adopted from Feroz et al., 2010).

Parameters/ Groups	CK (μ/l)	AST (μ/l)
Control	311.01±12.59	53.78±1.02
Amiodarone	304.16±9.45	54.63±2.32
Glibenclamide	319.67±12.81	53.24±5.45
Los. Pot	310.81±3.57	51.33±0.94
Oxcarbazepine	354.12±15.38*	56.03±1.93
Verapamil	305.35±6.63	49.28±2.96
Captopril	346.63±17.37	56.42±0.73
AGLO	270.56±13.33*	56.57±0.88
AGVO	307.34±23.83	53.36±1.65
AGCO	271.31±6.70*	50.75±1.29

n=9
Mean ± S.E.M
*p < 0.05 significant with respect to control
**p <0.005 highly significant with respect to control

Table 3B. Comparison of cardiac parameters following drug-free interval of 15 days of individual drugs and their combinations (Adopted from Feroz et al., 2010).

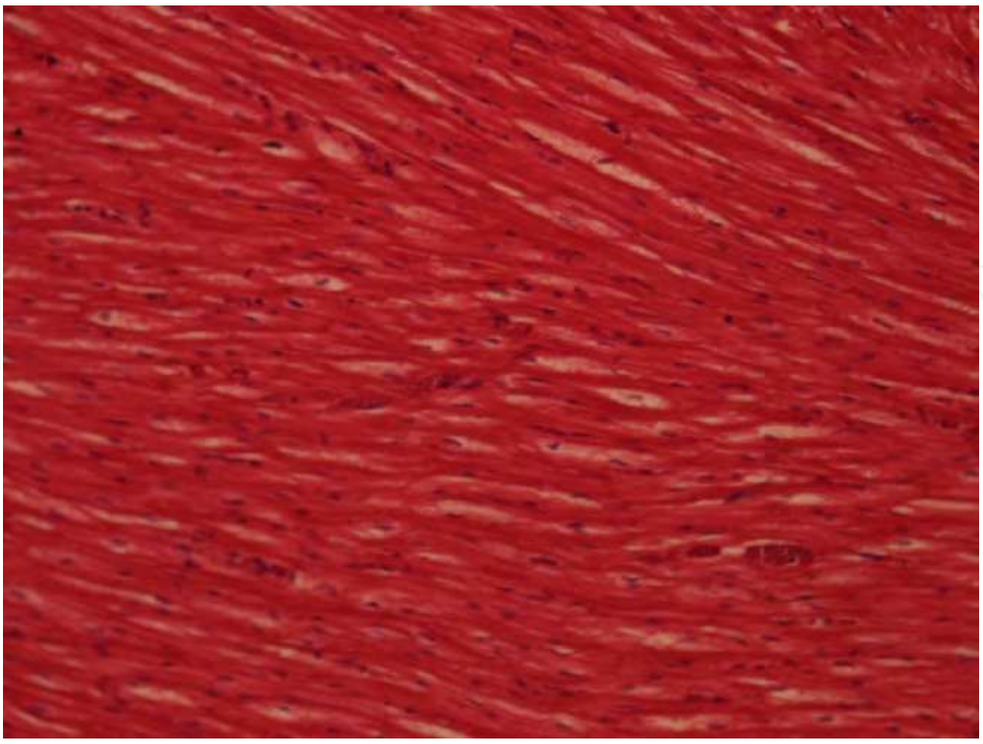

Fig. 3A. Cardiac tissue showing no microscopic change (Adopted from Feroz et al., 2010).

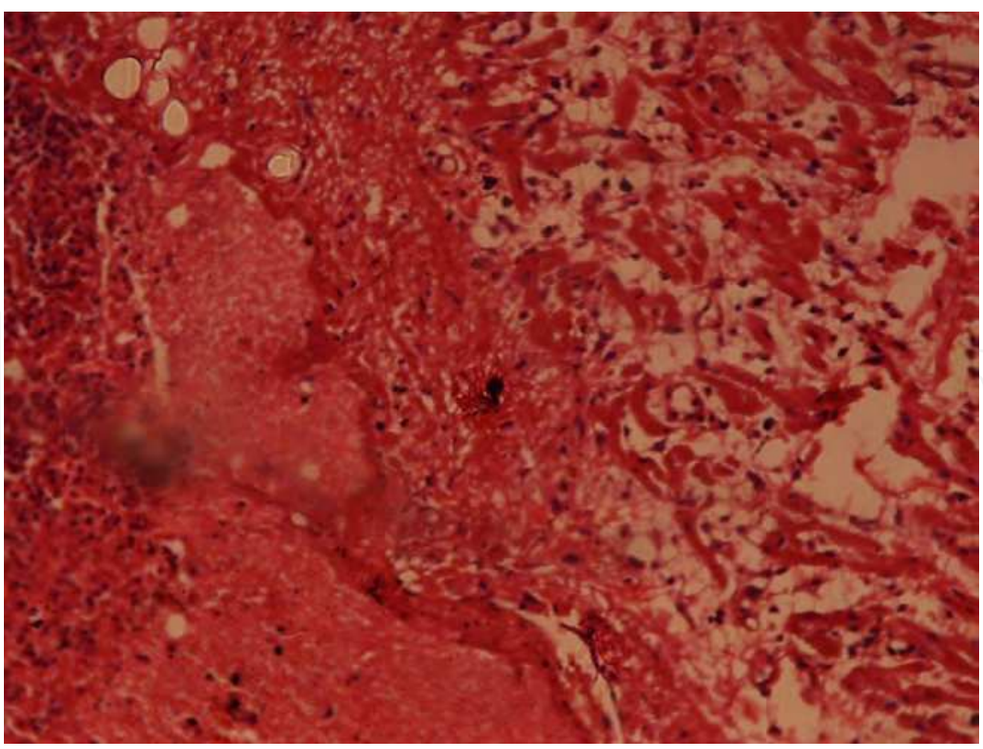

Fig. 3B. Cardiac tissue showing focal pericardial inflammation (Adopted from Feroz et al., 2010).

5. Biochemical testing of lipid profile

Cholesterol and triglycerides are the most important plasma lipids, crucial for formation of cell membrane, synthesis of hormones and offer a source of free fatty acids (Dietschy, 1998). Table 4A and 4B reveals the comparison of cholesterol, triglyceride, HDL-C (high density lipoprotein cholesterol) and LDL-C (low density lipoprotein cholesterol) levels between control animals and animals kept on individual drugs and their combinations for a period of 60 days and then after drug free interval of 15 days in normal therapeutic doses. The administration of losartan potassium and captopril in animals showed highly significant decrease in the level of triglyceride, whereas animals kept on AGLO and AGCO combinations showed highly significant increase in cholesterol at the end of dosing which remained significant even after drug-free interval. There was also highly significant increase in LDL-C level in animals kept on AGLO and AGCO combinations which remained significant even after drug-free interval (Feroz et al., 2011b). Elevated levels of cholesterol and LDL-C are undoubtedly associated with enhanced threat of coronary heart disease (Brown, 1984) and cerebrovascular morbidity and mortality. There has been a correlation among increased LDL-C and atherosclerosis. Since LDL-C gets deposited in the walls of the blood vessel forming atherosclerotic plaque. There are studies which recommend that pathological process could be inverted by dropping the serum LDL-C level (Ross, 1993). There was also significant increase in HDL-C in animals kept on oxcarbazepine alone and AGCO in combination at the end of dosing and following drug-free interval; however reason of elevated HDL-C is yet to be explored (Feroz et al., 2011b).

Parameters/ Groups	**Cholesterol (mg/dl)**	**Triglyceride (mg/dl)**	**HDL-C (mg/dl)**	**LDL-C (mg/dl)**
Control	91.84±2.65	102.65±2.45	3.10±0.13	28.15±1.90
Amiodarone	88.80±0.62	100.51±2.97	3.07±0.04	27.04±1.13
Glibenclamide	93.37±3.07	106.77±4.71	3.28±0.06	23.25±2.08
Los. Pot	92.77±0.57	91.94±2.86*	3.15±0.07	34.87±1.70
Oxcarbazepine	95.08±1.26	97.08±1.45	3.37±0.05*	29.38±2.03
Verapamil	96.90±1.13	108.53±3.32	3.31±0.08	25.24±1.95
Captopril	96.06±2.89	93.40±4.47*	3.32±0.07	34.18±2.26
AGLO	105.15±3.94**	100.20±2.90	2.93±0.05	46.35±3.50**
AGVO	95.93±1.21	104.46±1.31	3.27±0.04	27.41±1.80
AGCO	173.53±4.22**	95.35±3.17	3.40±0.08**	108.83±6.13**

n=9
Mean ± S.E.M
*p < 0.05 significant with respect to control
**p <0.005 highly significant with respect to control

Table 4A. Comparison of lipid profile following 60 days administration of individual drugs and their combinations {Adopted from Feroz et al., 2011(b)}.

Parameters/ Groups	Cholesterol (mg/dl)	Triglyceride (mg/dl)	HDL-C (mg/dl)	LDL-C (mg/dl)
Control	91.61±2.52	102.12±2.46	2.98±0.12	30.56±1.68
Amiodarone	88.18±0.75	100.26±2.93	2.96±0.04	28.81±1.25
Glibenclamide	93.80±3.10	106.7±4.59	3.16±0.05	26.38±2.06
Los. Pot	92.66±0.65	95.72±1.94	3.04±0.06	34.43±1.22
Oxcarbazepine	94.82±1.33	96.91±1.52	3.26±0.05**	31.38±1.96
Verapamil	96.01±1.27	104.27±1.81	3.18±0.07	29.60±1.90
Captopril	95.37±2.87	95.64±3.69	3.21±0.07	36.05±1.65
AGLO	104.31±3.91**	97.97±2.48	2.83±0.05	48.71±3.31**
AGVO	94.98±1.33	102.40±2.11	3.13±0.04	30.85±1.62
AGCO	99.73±1.18*	94.72±3.13	3.26±0.05**	37.88±2.70**

n=9
Mean ± S.E.M
*p < 0.05 significant with respect to control
**p <0.005 highly significant with respect to control

Table 4B. Comparison of lipid profile following drug-free interval of 15 days of individual drugs and their combinations {Adopted from Feroz et al., 2011(b)}.

6. Biochemical testing of glucose

Table 5A and 5B reveals the comparison of glucose level between control animals and animals kept on individual drugs and their combinations for a period of 60 days and then after drug free interval of 15 days in normal therapeutic doses. AGCO combination showed significant increase in glucose level in rabbits at the completion of dosing period of 60 days and following drug-free interval (Feroz et al., 2011b). Elevated blood glucose level may be due to elevation in the level of cholesterol and LDL-C, because diabetes mellitus is a group of heterogenous, autoimmune, hormonal and metabolic disorders, frequently occurs along with hypertension, hyperlipidemia and obesity (Mahomed and Ojewole, 2003), which also augmented the possibility of coronary heart disease (Howard et al., 2000), however threat of CVD fatality in diabetic persons may be as high as that in non-diabetic persons with prior myocardial infarction (Haffner et al., 1998). There was also a significant elevation in glucose level in animals kept on captopril and oxcarbazepine alone, however it has to be elucidated. Conversely animal kept on glibenclamide alone revealed highly significant decrease in glucose level because the major mechanism of action of glibenclamide is the stimulation of insulin release and the inhibition of glucagon secretion; conversely it was inverted following drug-free interval (Feroz et al., 2011b).

Parameter/ Groups	Glucose (mg/dl)
Control	122.20±7.60
Amiodarone	111.84±3.30
Glibenclamide	75.20±5.79**
Los. Pot	119.67±4.0
Oxcarbazepine	142.17±4.54**
Verapamil	123.33±2.31
Captopril	146.06±4.72**
AGLO	102.50±6.12
AGVO	111.88±3.11
AGCO	145.44±2.93**

n=9
Mean ± S.E.M
*p < 0.05 significant with respect to control
**p <0.005 highly significant with respect to control

Table 5A. Comparison of glucose following 60 days administration of individual drugs and their combinations {Adopted from Feroz et al., 2011(b)}

Parameter/ Groups	Glucose (mg/dl)
Control	123.40±7.40
Amiodarone	113.76±3.10
Glibenclamide	104.50±5.40
Los. Pot	122.30±4.30
Oxcarbazepine	141.50±4.70
Verapamil	124.39±2.30
Captopril	144.30±4.30*
AGLO	102.60±6.10
AGVO	112.11±3.10
AGCO	137.90±3.60*

n=9
Mean ± S.E.M
*p < 0.05 significant with respect to control
**p <0.005 highly significant with respect to control

Table 5B. Comparison of glucose following drug-free interval of 15 days of individual drugs and their combinations {Adopted from Feroz et al., 2011(b)}.

7. Biochemical testing of electrolytes

Table 6A and 6B reveals the comparison of sodium, potassium and calcium concentrations between control animals and animals kept on individual drugs and their combinations for a period of 60 days and then after drug free interval of 15 days in normal therapeutic doses. The balance of electrolytes in our bodies is essential for normal cellular function, since it promotes fluid balance, maintain blood volume, facilitate fluid absorption and generate

impulses. Significant alterations may occur in electrolytes following multiple drug administration. There has been significant decrease in concentration of sodium in animals received amiodarone (Feroz et al., 2009), which has potential for significant morbidity and mortality (Goh, 2004). However this decrease became insignificant following drug-free interval. Similarly there was highly significant decrease in serum calcium in animal received glibenclamide, losartan potassium, verapamil, oxcarbazepine, captopril and combination of these drugs (Feroz et al., 2009). Calcium is essentially required for development and maintenance of bones, not only regulate nerve function, but also contributes to the contraction of the muscles and heart. There are studies which suggest that amiodarone induces vitamin D deficiency in individuals not exposed to sunlight (Campbell and Allain, 2006). Vitamin D is essentially required for absorption of calcium; hence in the study by Feroz et al 2009 hypocalcaemia in animals on amiodarone alone or in combination might be due to the deficiency of vitamin D (Cooper and Gittoes, 2008). However reason for hypocalcaemia in other animal groups is yet to be explored. There was highly significant increase in calcium after drug free interval in animals received AGVO combination, this increase in calcium might be due to increase bone resorption, or gastrointestinal absorption or decreased elimination by the kidneys (Strewler, 2000). Hypercalcemia is always a concern, because elevated concentrations can result in renal failure, mineralization of the other soft tissues, cardiac arrhythmia and dysfunction (Sakals et al., 2006). Animals received AGCO combination also showed decrease in potassium level at the end of dosing as well as following drug-free interval. A decreased serum potassium concentration points to disturbance in normal homeostasis which might be an indication of muscle necrosis. However potassium level in animals received AGVO combination was significantly increase after drug-free interval. Hyperkalemia because of drugs most frequently occurs from impaired renal potassium excretion. On the other hand, disturbed cellular uptake of a potassium load as well as unnecessary intake or infusion of potassium-containing substances may also induce hyperkalemia. Therefore prescribing physicians must be conscious about medications that can precipitate hyperkalemia (Perazella, 2000).

Parameters/ Groups	**Sodium (μg/ml)**	**Potassium (μg/ml)**	**Calcium (μg/ml)**
Control	178.50±5.40	5.96±0.38	16.80±1.30
Amiodarone	156.16±3.40*	5.46±0.66	11.34±0.23**
Glibenclamide	187.80±8.90	6.44±0.61	11.04±0.91**
Los. Pot	171.92±2.80	5.36±0.34	11.70±0.33**
Oxcarbazepine	182.70±6.10	5.40±0.24	10.58±0.33**
Verapamil	181.80±9.40	6.04±0.43	11.72±0.52**
Captopril	182.40 11.0	5.50±0.37	10.78±0.52**
AGLO	185.60±11.0	5.26±0.14	10.30±0.51**
AGVO	184.40±5.90	5.66±0.30	12.50±0.59**
AGCO	173.30±7.0	4.54±0.32*	8.92±0.80**

n=5
Mean ± S.E.M
*p < 0.05 significant with respect to control
**p <0.005 highly significant with respect to control

Table 6A. Comparison of sodium, potassium and calcium following 60 days administration of individual drugs and their combinations (Adopted from Feroz et al., 2009).

Parameters/ Groups	Sodium (µg/ml)	Potassium (µg/ml)	Calcium (µg/ml)
Control	178.70±5.80	5.90±0.37	16.80±1.25
Amiodarone	162.72±2.30	5.40±0.65	15.70±1.20
Glibenclamide	181.46±2.90	6.38±0.59	17.04±0.91
Los. Pot	172.50±2.80	5.28±0.31	17.70±0.33
Oxcarbazepine	182.90±6.0	5.26±0.24	16.58±0.33
Verapamil	181.40±9.40	6.0±0.44	17.72±0.52
Captopril	180.60±10.0	5.48±0.33	16.72±0.49
AGLO	175.70±7.40	5.22±0.10	16.30±0.49
AGVO	175.56±3.10	7.0±0.07*	20.38±0.28**
AGCO	170.40±5.90	3.94±0.15**	13.16±0.87**

n=5
Mean ± S.E.M
*p < 0.05 significant with respect to control
**p <0.005 highly significant with respect to control

Table 6B. Comparison of sodium, potassium and calcium following drug-free interval of 15 days of individual drugs and their combinations (Adopted from Feroz et al., 2009).

8. Hematological testing

Table 7A and 7B reveals the comparison of hemoglobin concentration, platelet, leucocytes and erythrocytes count between control animals and animals kept on individual drugs and their combinations for a period of 60 days and then after drug free interval of 15 days in normal therapeutic doses. Changes in hematological parameters such as erythrocytes, leucocytes and platelet count and hemoglobin had always a serious concern following administration of drugs individually as well as in combination. There has been significant increase in platelet count in animal group received captopril and oxcarbazepine alone (Feroz et al., 2011a). Increased in platelet might be due to inflammatory disorder or iron deficiency anemia (Schafer, 2004), however there was also a significant increase in leucocytes count in animal group kept on oxcarbazepine alone, on the other hand animal group received amiodarone alone showed significant decrease in leucocytes count which might be due to disturbance in immune system, where as platelet count was not changed significantly, though amiodarone is known to produce thrombocytopenia (Weinberger et al., 1987).

Study conducted by Feroz et al 2011a revealed more severe hematological changes in animals received drugs in combination throughout the experimental period in comparison to animals received the drugs individually. Concurrent administration of AGLO combination showed a significant increased in leucocytes count which might be an indicator of an infection, inflammation, or allergy. On the other hand concurrent administration of AGVO combination showed highly significant increase in erythrocytes count while the other hematological parameters were not altered significantly.

There was significant increase and decrease in leucocytes and platelet count respectively in animals kept on AGCO combination. Decrease in platelet count may be due to insufficient production of platelet in bone marrow, a variety of reasons such as leukemia, lymphomas and several bone marrow disorders may have this effect on platelet count (McMillan, 2007).

Spleen enlargement may also decrease platelet count, or it may probably due to folic acid deficiency (Mant et al., 1979).

Parameters/ Groups	Hemoglobin (mg/dl)	Platelet ($x10^5$/c.mm)	Leucocytes ($x10^3$/c.mm)	Erythrocytes ($x10^6$/c.mm)
Control	10.62±0.23	412±39	6.26±0.35	6.14±0.16
Amiodarone	11.04±0.37	539±56	3.73±0.44*	6.12±0.26
Glibenclamide	9.90±0.28	433±62	5.61±0.57	5.83±0.19
Los. Pot	10.53±0.72	417±40	5.10±0.69	6.05±0.44
Oxcarbazepine	12.33±1.03	561±50*	8.99±0.78*	6.91±0.67
Verapamil	9.13±0.62	396±48	5.47±0.55	5.25±0.31
Captopril	10.06±0.66	559±66*	4.97±0.63	5.66±0.49
AGLO	10.54±0.69	310±17	8.43±1.24*	5.38±0.23
AGVO	12.01±0.93	320±35	7.74±0.88	9.65±1.22**
AGCO	9.91±0.53	279±16*	8.75±1.01*	6.23±0.36

n=9
Mean ± S.E.M
*p < 0.05 significant with respect to control
**p <0.005 highly significant with respect to control

Table 7A. Comparison of hematological parameters following 60 days administration of individual drugs and their combinations {Adopted from Feroz et al., 2011(a)}.

Parameters/ Groups	Hemoglobin (mg/dl)	Platelet ($x10^5$/c.mm)	Leucocytes ($x10^3$/c.mm)	Erythrocytes ($x10^6$/c.mm)
Control	10.58±0.24	416±40	6.26±0.36	6.03±0.20
Amiodarone	10.84±0.33	541±55	3.66±0.45*	6.18±0.27
Glibenclamide	9.86±0.25	445±57	5.47±0.59	5.85±0.20
Los. Pot	10.44±0.76	429±37	5.01±0.69	6.09±0.45
Oxcarbazepine	12.23±1.10	549±43*	8.90±0.77*	6.98±0.66
Verapamil	9.03±0.59	385±46	5.43±0.56	5.24±0.31
Captopril	10.02±0.64	558±66*	4.93±0.63	5.66±0.49
AGLO	10.42±0.72	324±21	8.40±1.22*	5.38±0.24
AGVO	12.03±0.87	317±34	7.63±0.87	9.65±1.22*
AGCO	9.73±0.54	281±16*	8.62±0.99*	6.27±0.37

n=9
Mean ± S.E.M
*p < 0.05 significant with respect to control
**p <0.005 highly significant with respect to control

Table 7B. Comparison of hematological parameters following drug-free interval of 15 days of individual drugs and their combinations {Adopted from Feroz et al., 2011(a)}.

9. Conclusion

The problems associated with drug therapy are a significant challenge to health care providers, especially in developing countries where health care system is poor. Minimizing

the risk for drug interactions is the desirable aim in drug therapy, since interactions can leads to significant morbidity, mortality and patient quality of life. Individuals taking multiple medications are at increased threat of adverse drug reactions; hence when ever multiple drugs are to be administered in case of multiple disorders such as epilepsy, hypertension, diabetes mellitus and arrhythmias drug treatment should be monitored to avoid adverse effects of the drugs. Studies conducted by Feroz et al not only provides valuable information pertaining to gross toxicities, microscopic changes and toxic effects on hepatic, renal, cardiac, lipid profile, glucose, electrolytes and hematological parameters but also give clues about the drug combination having higher incidence of cumulative toxicities.

These studies in general has revealed that animals received AGCO combination comparatively showed higher toxicities with marked decrease in ALT, TBR, CK, potassium, calcium and platelet count and increase in urea, creatinine, cholesterol, LDL-C, glucose and leucocytes count. However further studies on more animals and human beings are necessary to defend the utilization of multiple drugs.

These studies provides detailed evaluation of dug interaction and adverse effect of cumulative drug therapy; such observations are of undisputed importance but it should not be disregarded that pathway of drug metabolism in man may be quite dissimilar from that which has been determined in many species of laboratory animal, hence trial in man is the only valid way of establish drug interactions, before reaching to any final conclusion. However the risk of adverse drug reactions and drug interactions can be reduced by forming drug information centers, continuous medical education and incorporation of adverse drug reaction reporting into the clinical activities of the physicians (Oshikoya and Awobusuyi, 2009).

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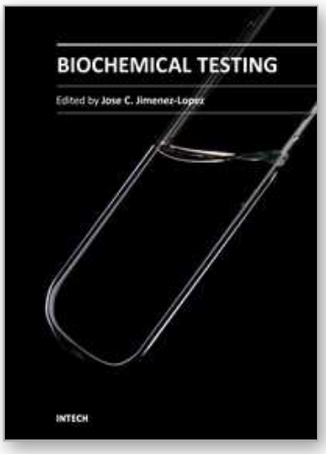

Biochemical Testing

Edited by Dr. Jose C. Jimenez-Lopez

ISBN 978-953-51-0249-6
Hard cover, 216 pages
Publisher InTech
Published online 07, March, 2012
Published in print edition March, 2012

Biochemical testing necessitates the determination of different parameters, and the identification of the main biological chemical compounds, by using molecular and biochemical tools. The purpose of this book is to introduce a variety of methods and tools to isolate and identify unknown bacteria through biochemical and molecular differences, based on characteristic gene sequences. Furthermore, molecular tools involving DNA sequencing, and biochemical tools based in enzymatic reactions and proteins reactivity, will serve to identify genetically modified organisms in agriculture, as well as for food preservation and healthcare, and improvement through natural products utilization, vaccination and prophylactic treatments, and drugs testing in medical trials.

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