WWW.IJRPR.COM

International Journal of Research Publication and Reviews

Journal homepage: www.ijrpr.com ISSN 2582-7421

Evaluation of Hepatotoxic Effect of the Chronic Use of Metronidazole in Wister Albino Rats.

[1]Aroh, Samuel Chinekwu; [2]Winnifred Njideka Adiri, [3]Promise Udoka Asogwa, [4]Bruno Basil, [5]Okereke Chiamaka Edith, [6]Ugwuanyi Joy Oluchukwu

[1,2,3,4,5,6] University of Nigeria

ABSTRACT

Metronidazole is a widely used antibiotic known for its efficacy against anaerobic bacterial and protozoal infections. Despite its therapeutic benefits, concerns have been raised regarding the potential hepatotoxic effects associated with its long-term use. The liver, being the primary site of drug metabolism, is particularly vulnerable to toxic insults from chronic drug exposure. This study aims to evaluate the hepatotoxic effects of chronic metronidazole use in Wistar albino rats, focusing on biochemical, histopathological, and molecular parametersThis study was design with six groups (control: received normal saline, group 1: received 50mg/kg of metronidazole, group 2: received 40mg/kg of metronidazole, group 3: received 30mg/kg of metronidazole, group 4: received 20mg/kg of metronidazole and group 5: received 10mg/kg of metronidazole) per bwt. for 35 days of study. Biochemical markers of liver function, including alanine aminotransferase (ALT), aspartate aminotransferase (AST), and alkaline phosphatase (ALP), was measured. Histopathological analysis of liver tissues was conducted to identify any structural alterations. The result showed a significantly ($P<0.05$) increased serum levels of aspartate aminotransferase, alanine aminotransferase, alkaline phosphatase, total and direct bilirubin and conjugated bilirubin. At the same time, there were decreased activities of serum levels of aspartate aminotransferase, alanine aminotransferase, alkaline phosphatase, total and direct bilirubin and conjugated bilirubin as the dosage concentrations decreases compared with the untreated control group. Administration graded dosage of 50 and 40 mg/kg body weight significantly ($P<0.05$) increased the liver damage marker enzymes, total and conjugated bilirubin, aspartate aminotransferase, alanine aminotransferase, and alkaline phosphatase in serum when compared with group that received low dosage of 10mg/kg body weight and the normal control. These results showed that administration of higher dosage of metronidazole and its long term usage exhibits a potent hepatotoxicity damage in rats. These findings will provide valuable insights into the safety profile of metronidazole, potentially influencing clinical guidelines and therapeutic practices.

INTRODUCTION

Metronidazole is a widely used antimicrobial agent effective against anaerobic bacteria and protozoa. It is commonly prescribed for conditions such as bacterial vaginosis, trichomoniasis, and gastrointestinal infections caused by Helicobacter pylori. Despite its therapeutic efficacy, there are concerns about the potential adverse effects associated with chronic use, particularly hepatotoxicity. Hepatotoxicity refers to liver damage caused by chemical substances and is a significant concern due to the liver's central role in metabolism and detoxification. Understanding the hepatotoxic effects of chronic metronidazole use is crucial for ensuring safe long-term treatment, especially in patients requiring prolonged therapy. Metronidazole is considered to have broad toxicological prospects compared to most xenobiotics due to its biotransformation in the liver through oxidation, hydroxylation, and conjugation of metronidazole glucuronide Moreover, a cumulative number of studies on animals and humans indicated an association of metronidazole with the disturbance of alanine aminotransaminase (ALT), aspartate aminotransaminase (AST), alkaline phosphatase (ALP), total cholesterol (TC), and triglyceride (TG), which are factors involved in hepatotoxicity . Metronidazole also enhances steatosis-related early-stage hepatocarcinogenesis and induces liver tumors through increased hepatic neoplasms(AbdRabou M et al. 2023).

Hepatotoxicity refers to liver dysfunction or liver damage that is associated with an overload of drugs or xenobiotics (Navarro and Senior, 2006). The chemicals that cause liver injury are called hepatotoxins or hepatotoxicants. Hepatotoxicants are exogenous compounds of clinical relevance and may include overdoses of certain medicinal drugs, industrial chemicals, natural chemicals like microcystins, herbal remedies and dietary supplements (Willett *et al.*, 2004; Papay *et al.*, 2006). Certain drugs may cause liver injury when introduced even within the therapeutic ranges. Hepatotoxicity may result not only from direct toxicity of the primary compound but also from a reactive metabolite or from an immunologically-mediated response affecting hepatocytes, biliary epithelial cells and/or liver vasculature (Saukkonen *et al.*, 2006; Deng *et al.*, 2009). The hepatotoxic response elicited by a chemical agent depends on the concentration of the toxicant which may be either parent compound or toxic metabolite, differential expression of enzymes and concentration gradient of cofactors in blood across the acinus (Bleibel *et al.*, 2006).

Metronidazole, a nitroimidazole antibiotic, is extensively used in clinical practice for the treatment of various infections, including those caused by anaerobic bacteria and certain protozoa. Due to its broad spectrum of activity and high efficacy, metronidazole is often prescribed for both acute and chronic infections. However, there is increasing evidence that suggests long-term use of metronidazole may be associated with hepatotoxic effects. The liver, which plays a central role in drug metabolism and detoxification, is particularly susceptible to damage from prolonged drug exposure (Papay *et al.*, 2006).

Several studies have reported instances of elevated liver enzymes and hepatocellular damage in patients undergoing prolonged metronidazole therapy. Despite these reports, the mechanisms underlying metronidazole-induced hepatotoxicity remain poorly understood, and comprehensive preclinical studies are lacking. Understanding the potential hepatotoxic effects of chronic metronidazole use is essential for developing safer therapeutic protocols and preventing liver damage in patients requiring long-term treatment (Plumb, 2005; Roach, 2007).

The liver, as the primary organ for drug metabolism, is particularly vulnerable to damage from chronic exposure to xenobiotics, including pharmaceuticals like metronidazole. Chronic drug-induced liver injury can result in a range of pathological conditions, from mild elevation in liver enzymes to severe liver dysfunction and hepatocellular necrosis. Despite clinical observations of metronidazole-induced liver toxicity, there is limited experimental data on its effects following long-term use (Oda, 2012; Chukwu *et al.*, 2015 a,b; Oyedeji *et al.*, 2015; Chandak *et al.*, 2016).

Drug-induced liver toxicity is a common cause of liver injury. It accounts for approximately one-half of the cases of acute liver failure and mimics all forms of acute and chronic liver disease (Kaplowitz, 2001). There is no scientific investigation on the hepatotoxicity of metronidazole the prolong users (chronic) for infection treatment. Therefore, this study seeks to evaluate the hepatotoxic effects of chronic metronidazole use in Wistar albino rats. By examining biochemical markers of liver function, histopathological changes, and molecular mechanisms of liver injury, this research aims to provide a comprehensive understanding of the potential risks associated with prolonged metronidazole therapy.

STATEMENT OF THE PROBLEM

Uncertainty in Long-term Safety: While metronidazole is effective for treating various infections, there is limited information on its long-term safety profile, particularly concerning hepatotoxicity. Chronic use of metronidazole may pose a risk of liver damage, which is not well-documented (Reynolds et al., 2015; Bergan, 2015; Frey and Löscher, 2016).

While metronidazole is widely used and generally regarded as safe for short-term therapy, there is a significant gap in understanding its safety profile during chronic use. Reports of liver enzyme abnormalities and liver damage in patients undergoing prolonged metronidazole treatment raise concerns about its hepatotoxic potential. However, current knowledge is primarily based on case reports and limited clinical observations, lacking robust experimental evidence to elucidate the mechanisms of metronidazole-induced hepatotoxicity.

Without a thorough understanding of these mechanisms, clinicians are challenged in assessing the risks and benefits of prolonged metronidazole use, particularly in patients requiring long-term therapy. This gap in knowledge highlights the need for detailed preclinical studies to evaluate the hepatotoxic effects of chronic metronidazole administration and to inform safer clinical practices.

Few reports are available concerning the hepatotoxic (Caylor and Cassimatis, 2001; Wright *et al.*, 2003; Ligha and Paul, 2011) and nephrotoxic (Ligha and Paul, 2011) effects of nitromidazole derivatives. The genotoxic effect of MTZ was studied by Mudry *et al.*, (2013) and EL-Nahas and EL- Ashmawy (2015). Although MTZ is considered safe and is widely used in the human and veterinarian populations, it is necessary to clarify the potential biological risks in the use of MTZ. Moreover, histopathological studies concerning MTZ toxicity are scarce. Therefore, the aim of the present study is to assess the hepatotoxicity effect of metronidazole in Wister albino rat.

AIM OF THE STUDY

The aim of this study is to evaluate the hepatotoxicity effect of the chronic use of metronidazole in Wister albino rats.

OBJECTIVES OF THE STUDY

The specific objectives of this study is to:

1. Determine the hepatotoxicity effect of the chronic use of metronidazole in Wister albino rats.
2. To measure biochemical markers of liver function (AST, ALT, ALP, CB, and TB) in Wistar albino rats after chronic metronidazole administration.
3. To assess histopathological changes in liver tissue of Wistar albino rats exposed to chronic metronidazole use.
4. To compare hepatotoxic effects at different dosages of metronidazole over extended periods.

SIGNIFICANCE OF THE STUDY

Liver plays a central role in biotransformation and disposition of xenobiotics (Navarro and Senior, 2006). The liver may be exposed to large concentrations of exogenous substances and their metabolites as a result of prolong usage. The activities of enzymes are influenced by various *endogenous* factors and exogenous drugs or chemicals (Deng *et al.*, 2009). Many substances can influence the cytochrome P450 enzyme mechanism (Kedderis, 1996). Certain substances may share the same cytochrome P450 specificity, thus competitively block their biotransformation activity and lead to accumulation of drugs metabolized by the enzyme.

Enhanced Understanding of Drug Safety, this study will provide valuable insights into the potential hepatotoxic effects of chronic metronidazole use, contributing to a more comprehensive understanding of its safety profile.

Informing Clinical Guidelines, the findings could influence clinical guidelines and recommendations for the use of metronidazole, particularly in settings requiring long-term therapy.

Mechanistic Insights, by elucidating the biochemical and molecular mechanisms underlying metronidazole-induced hepatotoxicity, the research could guide the development of targeted strategies to mitigate these effects.

Implications for Drug Development, understanding the hepatotoxic potential of metronidazole could inform the development of safer analogs or alternative therapies with reduced liver toxicity.

MATERIA AND METHOD

REAGENTS/CHEMICAL

The chemicals and reagents used for this study were of analytical grades. Metronidazole under trade name of Flagyl tablets 400 mg with an expiration date of 2026 was purchased from local pharmacy, Enugu state, Nigeria, Kits for biochemical assaying of ALT, AST and total bilirubin. Reduced glutathione (GSH), KH_2PO_4, K_2HPO_4, KCl, NaCl, and NAC was obtained from decare Chemicals Company (Nsukka, Enugu, Nigeria).

EXPERIMENTAL DESIGN/PROTOCOL

Eighteen (48) male Wister rats weighing between 140 and 180 g Wister rats was randomly divided into six groups, three rats per a group.

Table 3.1 Experimental design

Groups	No. of animals	TREATMENT	Duration	Sacrificed
Group 1	8	50mg/kg metronidazole per b.wt. of rat twice daily	34 days	35th day
Group 2	8	40mg/kg metronidazole per b.wt. of rat twice daily	34 days	35th day
Group 3	8	30mg/kg metronidazole per b.wt. of rat twice daily	34 days	35th day
Group 4	8	20 mg/kg metronidazole per b.wt. of rat twice daily	34 days	35^{th} day
Group 5	8	10mg/kg metronidazole per b.wt. of rat twice daily	34 days	35^{th} day
CONTROL	8	Normal saline	34 days	35th day

SERUM ENZYMES ASSAY

Alanine Amino Transferase (ALT) activity: The ALT activity was assayed using the method of Reitman and Frankel (2007) as outlined in Randox test kit (USA).

Principle: The ALT activity was assayed by monitoring the concentration of pyruvate hydrazone formed with 2, 4-dinitrophenylhydrazine. The colour intensity is measured against the blank at 540 nm.

Aspartate Amino Transferase (AST) activity: The *in-vivo* activity of AST was assayed by Reitman and Frankel (2007) using Randox test kit (USA).

Principle: The AST activity was generally assayed by monitoring the concentration of oxaloacetate hydrazone formed with 2, 4-dinitrophenylhydrazine. The colour intensity is measured against the blank at 546 nm.

Alkaline phosphatase (ALP) activity: The ALP activity was assayed using the method described by Klein *et al*. (2010).

Principle: The principle of this assay is based on the reaction involving serum ALP and a colourless substrate of phenolphthalein monophosphate, giving rise to phosphoric acid and phenolphthalein at alkaline pH values, turns pink that can be determined photometrically.

Total Bilirubin Test: The total bilirubin activity was assayed using the method described by Klein *et al*. (2010).

Principle: Bilirubin reacts with diazotized sulfanilic acid to produce azobilirubin, which has an absorbance maximally at 560nm in the dimethyl sulfoxide (DMSO) solvent. The intensity of the colour produced is directly proportional to the amount of total bilirubin concentration present in the sample.

Conjugated Bilirubin Test: The conjugated bilirubin activity was assayed using the method of Reitman and Frankel (2007) as outlined in Randox test kit (USA).

Principle: Bilirubin reacts with diazotized sulfanilic acid to produce azobilirubin, which has an absorbance maximum at 560nm in the aqueous solution. The intensity of the colour produced is directly proportional to the amount of direct bilirubin concentration present in the sample.

Statistical analysis: Data was expressed as Mean±standard deviation statistically using one-way analysis of variance (ANOVA). Acceptable value of $p<0.05$ was considered to be statistically significant. The Statistical Products and Service Solutions (SPSS) software version 20 was used for this analysis.

RESULT

Data presented in figure 1-5 shows the study of serum liver enzymes levels of metronidazole induced hepatotoxicity rat and liver damage treated groups as compared to control group.

Effect of metronidazole on total bilirubin serum enzymes

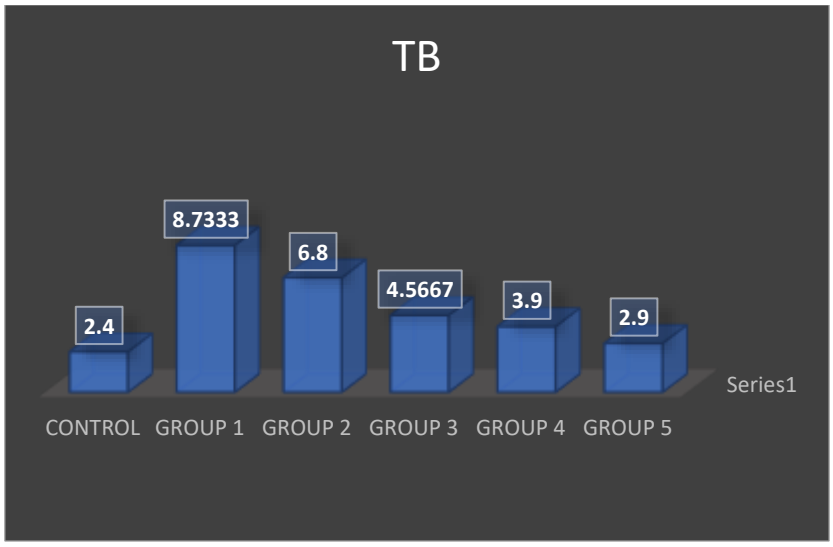

Figure 1: Effect of metronidazole on total bilirubin (TB) serum enzymes

The result in the figure 1 shows that all the groups administered graded dose of metronidazole reveals a significant ($P\leq0.05$) increase in total bilirubin in group one with continuous administration of metronidazole when compared to the control. However, it is observed that group one which received the highest conc. of 50mg/kg metronidazole per body weight of the rats increased significantly ($P\leq0.05$) when compared to the other groups.

There was a statistically significant ($P<0.05$) increase in TB activity in all the groups used in this study but significantly decreased as dosage of administration decreased.

Effect of metronidazole on conjugated bilirubin

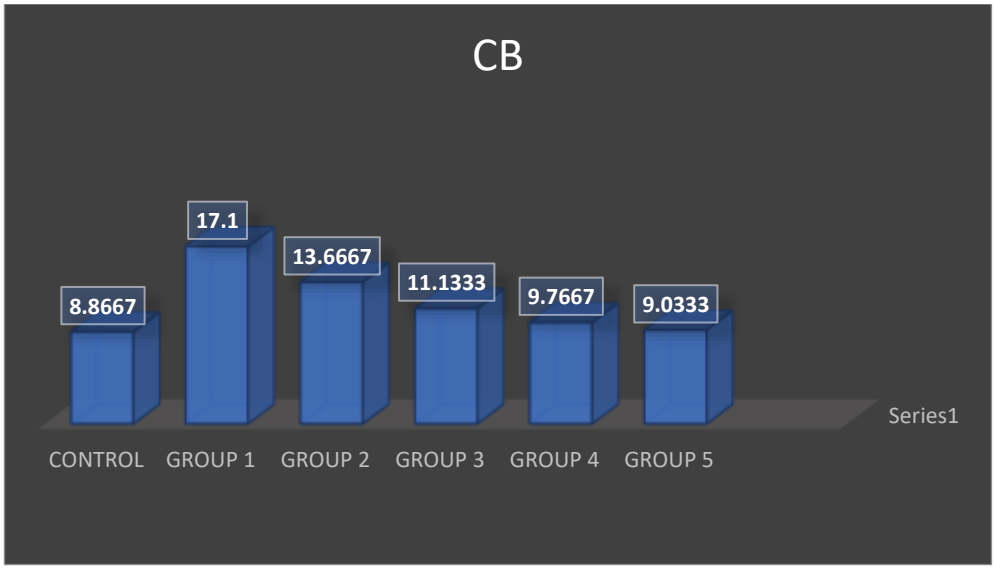

Figure 2: **Effect of metronidazole on conjugated bilirubin**

The data in figure 2 showed that group 1, 2, 3, 4 and 5 had significant (P≤0.05) increase in CB with continuous administration of graded dosage of metronidazole. However, group one which received a highest on body weight of rats showed a high significant (P≤0.05) increase in CB when compared to the control.

There was a statistically significant (P<0.05) increase in CB activity in all the groups used in this study but significantly decreased as dosage of administration decreased.

Effect of metronidazole on Aspartate Amino Transferase (AST) serum enzymes

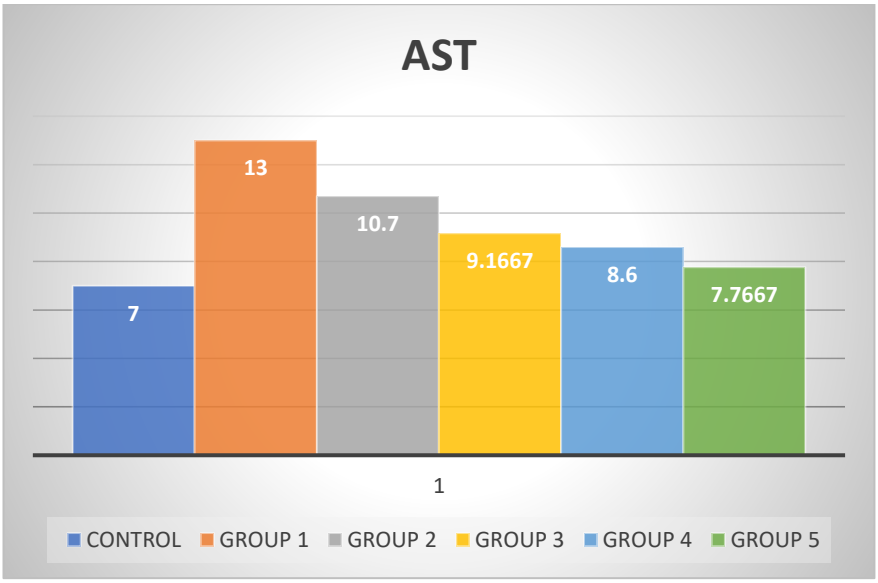

Figure 3 **Effect of metronidazole on Aspartate Amino Transferase (AST) serum enzymes**

The result in figure 3 shows that group 1, 2, 3, 4 and 5 which received different concentrations of metronidazole had significantly (P≤0.05) increase in AST with respect to the dosage concentration. However, group one which receives highest dose of 50mg/kg metronidazole showed a highly significant (P≤0.05).

There was a statistically significant (P<0.05) increase in AST activity in all the groups used in this study but significantly decreased as dosage of administration decreased.

Chronic effect of metronidazole on alkaline phosphatase (ALT) serum enzymes

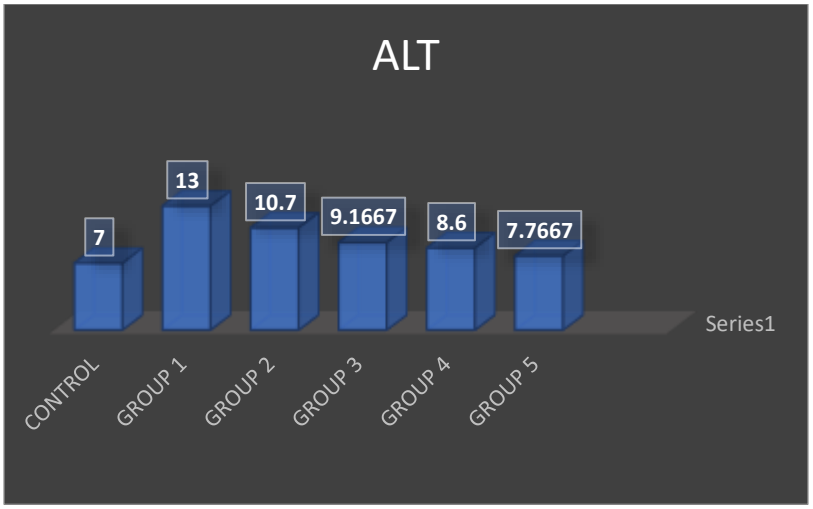

Figure 4: Effect of metronidazole on alkaline phosphatase (ALT) serum enzymes

The results as observed in figure 4 showed that group 1, 2 3, 4 and 5 which received graded dosage of metronidazole had a significant (P≤0.05) increase in liver ALT when compared to that of the normal control. However, group 1 and 2 administered 50 and 40mg/kg of metronidazole showed high significant (P≤0.05) increase in ALT serum level when compared to the normal control.

There was a high statistically significant (P<0.05) increase in ALT activity in group 3, 4 and 5 used in this study but significantly decrease in group 1 and 2 after metronidazole administration.

Chronic effect of metronidazole on alkaline phosphatase (ALP) serum enzyme

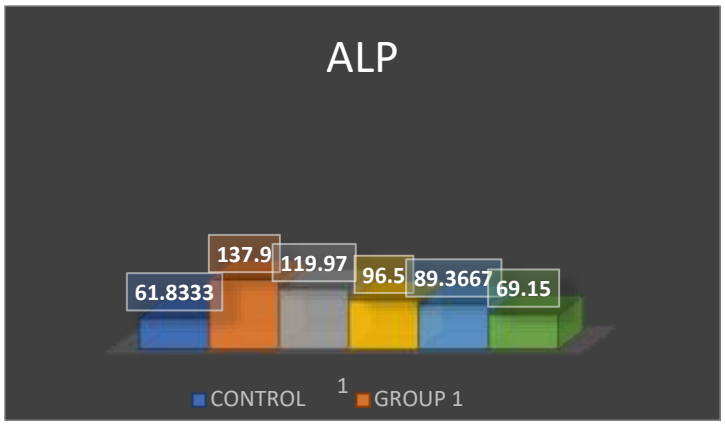

Figure 5: **Effect of metronidazole on alkaline phosphatase (ALP) serum enzymes**

The data in figure 5 shows that all the groups administered with graded doses of metronidazole had significant (P≤0.05) increase in liver enzyme ALP in group 1, 2, 3, 4 and 5 when compare to control. Group 1 and 2 which received highest doses of 50mg/kg and 40mg/kg of metronidazole which showed high significant (P≤0.05) increase when compared to control. However, comparisons between the groups shoed that except, group 5, all the other groups administered varying concentrations of the metronidazole showed high significant (P≤0.05) increase compared to the control.

There was a statistically significant (P<0.05) increase in ALP activity in group 3, 4 and 5 used in this study but significantly decreases in group 1

Discussion

The results obtained from evaluation of hepatotoxicity effect of the chronic use of metronidazole in Wister albino rats, findings from examined liver showed a highly significant differences between the control group and the groups administrated with metronidazole. This was agreed with the results obtained by (Asiedu-Gyekye *et al*., 2014), the biochemical parameters for liver function (ALT, AST) level of control rats and the rats administrated with acetaminophen were in close agreement. Metronidazole treated groups exhibited varying hepatic alterations in liver compared with those of control groups. Al-Dabagh and Mohammad (2008) suggested that liver are considered as potential toxicity targets for drugs. This finding is in consonance with our results in this present study which showed a significant increase in the liver function enzymes. There is a significant (p≤0.05) increase in total bilirubin (TB) as the dosage concentration increased as shown in figure 4.1. Group one recorded a highest increase in TB with a value of 8.37±0.05 when compared with the control with a value of 2.40±0.14, this may be as a result of the high dosage concentration received by group one. Ahmad *et al.,* (2016) reported that a single therapy of ofloxacin, ornidazole and metronidazole drugs increases hepatotoxicity and renal toxicity. This is in agreement with our findings as observed in figure 1-5 which showed significant (p≤0.05) increase in liver function enzymes as dose of administration increases. There is a statistical significant (p≤0.05) increase in CB between all the study groups. CB decreases with a decrease in dosage concentration. Under therapeutic

doses, metronidazole is detoxified in the liver mainly by glucuronidation and sulfation (Akamatsu and Horio, 2008). Part of metronidazole is metabolized by CYP450 (Akamatsu *et al.,* 2009) CYP2E1, and is converted to a toxic metabolite. Chronic administartion of metronidazole was observed from this study to increase TB, CB, ALT, AST and ALP. This is in conformity with the findings, mechanistically, acetaminophen is assumed to increase the serum levels of AST and ALT, TB, ALP and induces liver injury (Rahman and Hodgson, 2000). To confirm the histopathological alterations in examined tissues, total bilibrubin, serum alanine aminotransferase (ALT), aspartate aminotransferase (AST) level were estimated. Usually the serum levels of AST, ALT are increased in case of liver damage, which was observed in this study..

Metronidazole administration caused significant ($p<0.05$) increase of serum ALT and AST activities which parallel to the histopathological changes in the liver. These findings in agreement with the previous findings obtained by (Ahmad *et al*., 2010; Oda, 2012). This increase in the activities of ALT, AST might be due to the damage of cellular membranes of hepatocytes, which in turn leads to an increase in the permeability of cell membranes and facilitates the passage of cytoplasmic enzymes outside the cells leading to the increase in the aminotransferase activities in blood serum (Gaur and Bhatia, 2009). Cheong *et al.,* (2011) and Yamamoto et al., (2012) recorded that metronidazole-induced encephalopathy developed in patients with liver cirrhosis. Administration of high dose or chronic usage of metronidazole induced elevation of TB, AST and CB activities and histopathological alterations in liver Enzymes as observed in figure1, 2 and 3 respectively. This was consistent with the generally accepted hypothesis that transaminase level becomes elevated with hepatic damage of parenchyma and the hepatocytes (Ahmed and Khater, 2001). These results indicated that metronidazole had a hepatotoxicity effect and hence induce liver damage.

Odah S, in a study on the histopathological and biochemical alterations in metronidazole-induced toxicity in male rats, showed elevations in ALT and AST with reduction in Albumin levels which where parallel to the histopathological findings in the liver(Odah¸2012). However, ALT is more specific to liver and thus a better parameter for detecting liver injury as AST is also associated with diseases of other organs such as heart and muscle (Ozer *et al*., 2008). These findings concur with this present study which showed significant increase ALP in figure 4.5. The presence of ALP mostly in cells lining the billiary duct of the liver and is used to diagnose obstruction to the billiary system. Therefore, its elevation in the blood indicates cholestatic diseases such as gallstone or tumor blocking the bile duct (Burtis and Ashwood, 2001). In this study, chronic exposure of rats to metronidazole at different dosage caused a significant increase in ALP, TB, CB, and AST only on group one at the dose of 1.5 mg/kg while there was a dose-dependent and significant ($p\leq0.05$) elevation of ALT in figure 4 when compared to control group. This may be an indication of cholestatic disorder. Also the liver is prone to xenobiotic-induced injury because of its central role in xenobiotic metabolism (Bass and Ockner, 1996).

CONCLUSION

Chronic administration of metronidazole could pose significant risks of hepatotoxicity. This study aims to elucidate the extent and mechanisms of liver damage induced by prolonged use of metronidazole in Wistar albino rats. By evaluating biochemical and histopathological changes, the research will provide crucial insights into the safety profile of metronidazole, potentially impacting clinical practices and guidelines. The liver plays an important role in preventing the accumulation of compounds by converting them into a suitable form for elimination. All compounds undergo xenobiotic metabolism, which requires multiple biochemical transformations. The result of this present study showed that chronic use of metronidazole have effect on the liver.

RECOMMENDATION

Regular Monitoring: Patients on long-term metronidazole therapy should undergo regular liver function tests to detect early signs of hepatotoxicity.

Dosage Adjustments: Consideration should be given to adjusting doses or treatment durations to minimize liver damage risk.

Alternative Therapies: Explore alternative antimicrobial agents or combination therapies with a lower risk of hepatotoxicity for long-term treatments.

Further Research: Additional studies are needed to confirm these findings and to explore protective strategies against metronidazole-induced liver damage.

REFERENCE

Aebi H. Catalase in vitro: methods in enzymology, 2014; 105: 121-126.

Agarwal, A., Kanekar, S., Sabat, S., Thamburaj,K.(2016). Metronidazole-induced cerebellar toxicity.*Neurology International*; 8(6365): 4-6.

Ahmad, A., M. Chaudhary, A. Soni, A. Payasi and V.K. Dwivedi, 2010. Comparative toxicity profile study of mebatic vs. ofloxacin, ornidazole and metronidazole drugs in rat model. *Asian Journal of Biochemistry*, pp: 1-11.

Ahmed and H. Khater, M. (2001). The protective effect of garlic oil on hepatotoxicity induced by acetaminophen in mice and comparison with N-acetylcysteine. Saudi. Med. J. 22: 1080-1084.

Akamatsu H, Horio T (2008) The possible role of reactive oxygen species generated by neutrophils in mediating acne inflammation. Dermatology 196: 82–85.

Al-Dabagh and J. and Mohammad, W. M. (2008). AASLD position paper: the management of acute liver failure. *Hepatology* 41: 1179-1197.

Alsheikh-Ali AA, Kuvin JT, Karas RH (2004) Risk of adverse events with fibrates. *American Journal of Cardiology* 94: 935-938.

Amacher DE (2002) A toxicologist's guide to biomarkers of hepatic response. *Human Experimental Toxicology* 21: 253-262.

Amar PJ, Schiff ER (2007) Acetaminophen safety and hepatotoxicity - Where do we go from here? Expert Opin Drug Saf 6: 341-355.

Arora N, Goldhaber SZ (2006) Anticoagulants and transaminase elevation. Circulation 113: 698-702.

Asiedu-Gyekye, B. M, Avaria, M., Basu, P. K. and Wells, P. G. (2014). Pharmacological studies on the in vivo cataractogenicity of acetaminophen in mice and rabbits. *Fundamental Applied Toxicology* 10: 596-606.

Barak AJ, Beckenhauer HC, Mailliard ME, Kharbanda KK, Tuma DJ (2003) Betaine lowers elevated S-adenosylhomocysteine levels in hepatocytes from ethanol-fed rats. *Journal of Nutrition* 133: 2845-2848.

Bass, N.M., Ockner, B.A., 1996. Drug-induced liver disease. In: Zakin, D, Boyer, T.D. (Eds.), Hepatology, a Text Book of Liver Diseases, 3rd edition W.B. Saunders, Philadephia, pp. 962–1017.

Belardelli F, Ferrantini M (2002) Cytokines as a link between innate and adaptive antitumor immunity. *Trends Immunology* 23: 201–208.

Bergan, T., 2015. Antibacterial activity and pharmacokinetics of nitroimidazole. A review.Scandinavian *Journal of Infectious Diseases*, 46: 64-71.

Bhardwaj SS, Chalasani N (2007) Lipid lowering agents that cause druginduced hepatotoxicity. Clin Liver Dis 11: 597.

Bjornsson, E., Nordlinder, H., Olsson, R. (2002).Metronidazole as a probable cause of severe liver injury.*Hepatogastroenterology*; 49: 252-254.

Bleibel W, Kim S, D'Silva K, Lemmer ER (2007) Drug-induced liver injury: Review Article. Dig Dis Sci 52: 2463-2471.

Burtis, J. T. and Ashwood, G. (2001). Acetaminophen kinetics in acutely poisoned patients. *Clinical Pharmacology* Ther. 25: 184-195.

Carlson, I. I. and Mohammad, F. K. (2003).Pharmacokinetics and Distribution of MetronidazoleAdministered Intraperitoneally in MicePharmacologyonline 3: 858-863.

Caylor, K.B. and M.K. Cassimatis, (2001). Metronidazole neurotoxicosis in two cats. *Journal of the American Animal Hospital Association*, 37: 258-262.

Chandak,S., AgaRwal,A., Shukla,A., Joon,P.(2016). A Case Report of Metronidazole InducedNeurotoxicity in Liver Abscess Patient and the Usefulnessof MRI for its Diagnosis. *Journal of Clinical and Diagnostic Research*. Vol-10(1): TD06-TD07.

Chang C. Y, Schaino TD (2007) Review article: Drug hepatotoxicity. Aliment Pharmacol Ther 25: 1135-1151

Cheong, M. G., Beyer, J., Hall, G. L., deGraffenried, L. A. and Adams, P. E. (2011). Predictive value of liver slices for metabolism and toxicity in vivo: use of acetaminophen as a model hepatotoxicant. Toxicol. Appl. Pharmacol. 122: 108-116.

Chitturi S, George J (2002) Hepatotoxicity of commonly used drugs: nonsteroidal anti-inflammatory drugs, antihipertensives, antidiabetic agents, psycotropic drugs. Semin Liver Dis 22: 195-206.

Chukwu V.O., Akudike, C.J., Ezejindu, D.N.,Augustine I.C. (2015) a. The Protective Effects ofTurmeric on Liver Enzymes of Metronidazole TreatedAdult Male Wistar Rats. *International Journal of Advancesin Scientific Research*; 1(6): 255-258.

Chukwu V.O., Akudike, C.J., Ezejindu, D.N.,Ofoego, U.C., Chukwuocha, C.C. (2015) b. The ProtectiveEffects of Turmeric on testicular tissues, after treatmentwith metronidazole in adult male Wistar Rats. *Journal of Pharmaceutical Sciences*; 4 (1) 62-68.

Chumark, P., Khunawat, P., Sanvarinda, Y.,Phornchirasilp, S., Morales, N.P., et al. (2008). The in vitroand ex vivo antioxidant properties, hypolipidaemic andantiatherosclerotic activities of water extract of Moringaoleifera Lam leaves. *Journal of Ethnopharmacology* 116: 439–446.

Dambach DM, Andrews BA, Moulin F. (2005). New technologies and screening strategies for hepatotoxicity: use of in vitro models. *Toxicology Pathology* 33: 17-26.

Deng X, Luyendyk JP, Ganey PE, Roth R. A. (2009). Inflammatory stress and idiosyncratic hepatotoxicity: Hints from animal models. *Pharmacology Revision* 61: 262282.

Evans, J., (2002). The use of diazepam in the treatment of metronidazole toxicosis in the dog.

Finch, R.G. and I.S. Snyder, (2016). Antiprotozoan drugs. In: Modern pharmacology. Eds.: C.R. Craige nd R.E. Stitzel. Little, Brown Co.Boston, pp: 729-740.

Finegold, S.M., (2000). Metronidazole. Annals of Internal Medicine, 93: 585-587.

Frey, H.H. and W. Löscher, (2016). Lehrbuch der Pharmakologie und Toxikologie für die Veterinar-medizin. Enke, Stuttgart. pp: 502-503.

Gaur, J. H. and Bhatia, D. E. (2009). Hepatic injury due to drugs, chemicals and toxins. In "MacSween's Pathology of the Liver". 5th ed. pp. 649-765. Burt, A. D., Portmann, B. C., and Ferrell, L. D. ed. Churchill Livingstone. New York, U. S. A.

Giffen, M. J., Alvarez, M., Culebras, J. M. and GonzálezGallego, J. 2002). An overview of animal models for investigating the pathogenesis and therapeutic strategies in acute hepatic failure. *World Journal of Gastroenterology* 15: 3086-3098.

Godfrey,M. S., Finn, A., Zainah,H., Dapaah-Afriyie,K. (2015): Metronidazole-induced encephalopathyafter prolonged metronidazole course for treatment of C.difficile colitis. *B.M.J. Case Reports*; doi:10.1136/bcr-2014-206162.

Groman, R., 2000. Metronidazole. Compend Cont Educ., 22: 1104-1107, 1130.

Groneberg DA, Grosse-Siestrup C, Fischer A (2002) In vitro models to study hepatotoxicity. Toxicol Pathol 30: 394-399.

Gulteridge JMC, Wilkin C. Copper dependent hydroxyl radical damage to ascorbic acid formation of a thiobarbituric acid reactive products FEBs Lett, 2000; 137:327-340.

Gupta, A.K., M.P. Agarwal, R. Avasthi, D.P. Bhadoria and N. Rohatgi, 2003. Metronidazole-induced neurotoxicity. *Journal of the Association of Physicians of India*, 51: 617-618.

Ikemoto M, Tsunekawa S, Toda Y, Totani M (2001) Liver-type arginase is a highly sensitive marker for hepatocellular damage in rats. *Clinical Chemistry* 47: 946948.

Ju C, Uetrecht JP (2002) Mechanism of idiosyncratic drug reactions: Reactive metabolite formation, protein binding and the regulation of the immune system. Curr Drug Metab 3: 367-377.

Kafrouni MI, Anders RA, Verma S (2007) Hepatotoxicity associated with dietary supplements containing anabolic steroids. *Clinical Gastroenterology Hepatology* 5: 809-812.

Kanbac G, Dokumacioglu A, Tektas A, Kartkaya K, Erden Inal M (2009) Betaine (trimethylglycine) as a nutritional agent prevents oxidative stress after chronic ethanol consumption in pancreatic tissue of rats. *International Journal of Vitamin Nutrition Resourse* 79: 79-86.

Kaplowit, M. (2001). Pentoxifylline and N-acetylcysteine in hepatic ischemia/reperfusion injury. *Clinical Chimistry Acta* 275: 127-135.

Kapoor, K., Chandra, M., Nag, D., Paliwal, J.K.,Gupta, R.C., Saxena, R.C. (1999). Evaluation ofmetronidazole toxicity: a prospective study. *Internation Journal of Clinical Pharmacology. Resource*.; 19(3):83-88.

Khalili H, Dashti-Khavidaki S, Rasoolinejad M, Rezaie L, Etminana M (2009) Anti-tuberculosis drugs related hepatotoxicity: Incidence, risk factors, pattern of changes in liver enzymes and outcome. DARU 17: 163-167.

Kharbanda KK (2009) Alcoholic liver disease and methionine metabolism. Semin Liver Dis 29: 155-165.

Kozer E, Koren G (2001) Management of paracetamol overdose: Current controversies. Drug Safety 24: 503-512.

Kumar, H., Sharma, A., Attri,S.K., Kaushik, S.(2012). Rapid onset peripheral neuropathy: A rarecomplication of metronidazole. Journal, Indian Academy ofClinical Medicine; 13(4): 346-348.

Kumari, M., Singh, P. (2013). Study on thereproductive organs and fertility of the male mice followingadministration of metronidazole. Int. J. Fertil. Steril. 7(3):225-238.

Langman G, Hall PM, Todd G (2001) Role of non-alcoholic steatohepatitis in methotrexate-induced liver injury. *Journal of Gastroenterology Hepatology* 16: 1395-1401.

Lau L., Dodd, S., Dean, O., Copolov, D. L., Malhi, G. S. and Berk, M. (1992). N-acetylcysteine for antioxidant therapy: pharmacology and clinical utility. Expert. Opin. Biol. Ther. 8: 1955-1962.

Lee J, Boyer JL (2000) Molecular alterations in hepatocyte transport. Seminars in Liver Disease 20: 373-384.

Ligha, A.E. and C.W. Paul, 2011. Oxidative Effect of metronidazole on The Testes of Wistar Rats. *Australian Journal of Basic and Applied Sciences*, 5: 1339-1344.

Lim AYL, Segarra I, Chakravarthi S, Akram S, Judson JP (2010) Histopathology and biochemistry analysis of the interaction between sunitinib and paracetamol in mice. *BMC Pharmacology* 10: 14-30.

Luyendyk JP, Shaw PJ, Green CD, Maddox JF, Ganey PE, et al. (2005) Coagulation-mediated hypoxia and neutrophil-dependent hepatic injury in rats given lipopolysaccharide and ranitidine. *Journal of Pharmacology Experimental Therapuetics* 314: 1023-1031.

Lynch T, Price A (2007) The effect of cytochrome P450 metabolism on drug response, interactions, and adverse effects. Am Fam Physician 76: 391-396.

Manov I, Motanis H, Frumin I, Iancu TC (2006) Hepatotoxicity of antiinflammatory and analgesic drugs: Ultrastructural aspects. *Acta Pharmacology Sin* 27: 259-272.

Masuda Y (2006) Learning toxicology from carbon tetrachloride-induced hepatotoxicity. Yakugaku Zasshi 126: 885-899.

McDonald, LC; Gerding, DN; Johnson, S; Bakken, JS; Carroll, KC; Coffin, SE; Dubberke, ER; Garey, KW; Gould, CV; Kelly, C; Loo, V; Shaklee Sammons, J; Sandora, TJ; Wilcox, MH (15 February 2018). "Clinical Practice Guidelines for Clostridium difficile Infection in Adults and Children: 2017 Update by the Infectious Diseases Society of America (IDSA) and Society for Healthcare Epidemiology of America (SHEA)". Clinical Infectious Diseases. doi:10.1093/cid/cix1085. PMID 29462280.

Mervat A. AbdRabou, Barakat M. Alrashdi, Hadee K. Alruwaili, Reda H. Elmazoudy, Maha A. Alwaili 3,Sarah I. Othman,Fawzyah A. Alghamdi and Gehan H. Fahmy. (2023). Exploration of Maternal and Fetal Toxicity Risks for Metronidazole-Related Teratogenicity and Hepatotoxicity through an Assessment in Albino Rats. *Toxics Journal*, vol 11, Issue 4. 2023

Mochizuki M, Shimizu S, Urasoko Y, Umeshita K, Kamata T, (2009) Carbon tetrachloride-induced hepatotoxicity in pregnant and lactating rats. *Journal of Toxicology Science* 34: 175-181.

Mudry, M.D., M.A. Carballo, M.D. Labal de Vinuesa, M. Gonz´alez Cid and I. Larripa, 2014. Mutagenic bioassay of certain pharmacological drugs: III. Metronidazole (MTZ). Mutation Research, 86: 243-77.

Mukinda, J.T, Eagles, F.K., (2010). Acute and sub-chronic oral toxicity profile of the aqueous extract of Pohygala fruticosa in female mice and rats. *Journal of Ethnopharmacology* 128, 236–240.

Murayama N, Reimers A, Hari Y, Hunziker T, Gerber H, Muller U, Pich-ler W (2006) Drug-induced linear IgA bullous dermatosis associated with ceftriaxone- and metronidazole-specific T cells. Dermatology 199: 25–30.

Naruse, Z., Tang C. and Makuuchi, (2007). Species variation in toxication and detoxication of acetaminophen in vivo: a comparative study of biliary and urinary excretion of acetaminophen metabolites. *Journal of Pharmacology and Experimental Therapuetics*. 244: 91-99.

Navarro VJ, Senior JR (2006) Drug-related hepatotoxicity. N Engl J Med 354: 731-739.

Nishimura Y, Kurata N, Sakurai E, Yasuhara H (2004) Inhibitory effect of antituberculosis drugs on human cytochrome P450-mediated activities. *Journal of Pharmacology Science* 96: 293-300.

O'Brien PJ, Slaughter MR, Polley SR, Kramer K (2002) Advantages of glutamate dehydrogenase as a blood biomarker of acute hepatic injury in rats. Lab Anim 36: 313-321.

O'Connor N, Dargan PI, Jones AL (2003) Hepatocellular damage from nonsteroidal anti-inflammatory drugs. QJM 96: 787–791.

Oda, S.S. (2012). Histopathological andbiochemical alterations of metronidazole-induced toxicityin male rats. Global Veterin.,9: 303-310.

Odah S. S. (2012). Histopathological and Biochemical Alterations of Metronidazole-Induced Toxicity in Male Rats. Global Veterinaria 9 (3): 303-310

Olson H, Betton G, Robinson D, Thomas K, Monro A, et al. (2000) Concordance of the toxicology of pharmaceuticals in humans and animals. *Regulatory Toxicology Pharmacology* 32: 56-67.

Oyedeji, K.O., Oshatimi Abayomi, Abidoye Dele,Adeleke K.O. (2015). Effect of Metronidazole onReproductive Parameters in Male Wistar Rats. *International Journal of Pharmacological Science Revision Resource.*, 35(1), 186-190.

Ozer, G., Aykaç, G., Uysal, M. and Oz, H. (2008). Liver lipid peroxidation and glutathione-related defence enzyme systems in mice treated with paracetamol. *Journal of Applied Toxicology* 14: 297-299.

Papay I., Hammond, C. L., Lee, T. K. and Ballatori, N. (2006). Novel roles for glutathione in gene expression, cell death, and membrane transport of organic solutes. J. Hepatol. 34: 946-954.

Park BK, Naisbitt DJ, Gordon SF, Kitteringham NR, Pirmohamed M (2001) Metabolic activation in drug allergies. *Toxicology* 158: 11-23.

Parkinson A, Klaassen CD (2001) Biotransformation of xenobiotics. In: Casarett and Doull's Toxicology. (6th edn) McGraw-Hill, New York.

Plumb, D.C. (2005). Plumb's Veterinary DrugHandbook, 5th ed., Ames, Blackwell Publishing.

Rahman C., and Hodgson,, D. V. (2000). Species variation in the metabolic activation of paracetamol to toxic intermediates: role of cytochromes p-450 and p-448. Toxicol. Lett. 16: 55-61.

Ramaiah SK (2007) A toxicologist guide to the diagnostic interpretation of hepatic biochemical parameters. *Food Chemical Toxicology* 45: 1551-1557.

Ramaiah, S.K., 2011. Preclinical safety assessment. Current gaps, challenges and approaches in identifying translatable biomarkers of drug- induced liver damage. Clin. Lab. Med. 31, 161–172.

Roach, S.S. (2007). Introductory clinicalpharmacology, 8th ed. Philadelphia: Lippincott, Williams and Wilkins.

Rogulja, R. R., Hughes, R. D., Bansal, S., Lehec, S. C., Wendon, J. A. and Dhawan, A. (2013). Effects of serum from patients with acute liver failure due to paracetamol overdose on human hepatocytes in vitro. Transplant. Proc. 37: 2391-2394.

Rossi, S, ed. (2013). Australian Medicines Handbook (2013 ed.). Adelaide: The Australian Medicines Handbook Unit Trust. ISBN 978-0-9805790-9-3.

Sahu AJ, Nicholls RJ, Forbes A, Ellis HJ, Ciclitira PJ (2008) Human lymphocyte stimulation with pouchitis flora is greater than with flora from a healthy pouch but is suppressed by metronidazole. Gut 53: 1801–1805.

Saukkonen K., Jemnitz, K., Veres, Z., Monostory, K., Kóbori, L. and Vereczkey, L. (2006). Interspecies differences in acetaminophen sensitivity of human, rat, and mouse primary hepatocytes. Toxicol. In Vitro 22: 961-967.

Scorza, A. V. & M. R. Lappin (2004). Metronidazote for the treatment of fetine giardiasis. Journal of Feline Medicine and Surgery 6(3): 157-60.

Sekis, I., et al. (2009). Single-dose pharmacokinetics and genotoxicity of metronidazole in cats. Journal of Feline Medicine and Surgery 11(2): 60-8.

Shinn DLS. Metronidazole in acute ulcerative gingivitis. Lancet1962;279:1191.

Sohrabi, D., M. Alipour and A. Mellati, 2007. Effect of metronidazole on spermatogenesis, plasma gonadotrophins and testosterone in rats. Iranian Journal of Reproductive Medicine, 5: 69-72.

Stolk MF, Becx MC, Kuypers KC, Seldenrijk CA (2006) Severe hepatic side effects of ezetimibe. Clin Gastroenterol Hepatol 4: 908-911.

Sulkowski M, Mehta S, Chaisson R, Thomas D, Moore R (2004) Hepatotoxicity associated with protease inhibitor-based antiretroviral regimens with or without concurrent ritonavir. AIDS 18: 2277-2284.

Tabak, F., R. Ozaras, Y. Erzin, A.F. Celik, G. Ozbay and H. Senturk, 2003. Ornidazole-induced liver damage: Report of three cases and review of the literature. Liver International, 23: 351-418.

Tally FP, Sutter VL, Finegold SM. Metronidazole versus anaerobes: invitro data and initial clinical observations. Calif Med 1972;117:22–6.

Thapa M. J. and W, I. A. (2001). Acetaminophen overdose. 662 cases with evaluation of oral acetylcysteine treatment. Arch. Intern. Med. 141: 380-385.

Tong MH, Landers DV, Meyn L, Hillier SL (2005) Clinical and cervical cytokine response to treatment with oral or vaginal metronidazole for bacterial vaginosis during pregnancy: a randomized trial. Obstet Gynecol 102: 527–534.

Uetrecht J (2006) Role of animal models in the study of drug-induced hypersensitivity reactions. AAPS J 7: 914-921.

Uetrecht J (2006) Role of animal models in the study of drug-induced hypersensitivity reactions. AAPS J 7: 914-921.

Vernau, K. (2009). Cerebellar Disease. Veterinary Neurology Symposium; Univ. of Calif.-Davis. accessed via Veterinary Information Network; vin.com

Wallace J. L (2004) Acetaminophen hepatotoxicity: NO to the rescue. Br J Pharmacol 143: 1-2.

Willett K. L, Roth R. A, Walker L (2004) Workshop overview: hepatotoxicity assessment for botanical dietary supplements. Toxicol Sci 79: 4-9.

Williams D. P, Park BK (2003) Idiosyncratic toxicity: The role of toxicophores and bioactivation. Drug Discov Today 8: 1044-1050.

Wright T. M, Vandenberg AM (2007) Risperidone- and quetiapine-induced cholestasis. Ann Pharmacother 41: 1518-1523.

Wright, K.H., Tyler J.W. (2005): Recognizingmetronidazole toxicosis in dogs, Vet. Med., 98:410-418.

Yamamoto, M. C., Wang, E. J., Patten, C., Lee, M. J., Xiao, F., Reuhl, K. R. and Yang, C. S. (2012). Protective effect of diallyl sulfone against acetaminophen-induced hepatotoxicity in mice. Biochem. Toxicol. 11: 11-20.

Yu AS, Keeffe EB (2002) Nonalcoholic fatty liver disease. Rev Gastroenterol Disord 2: 11-19.

Zollner G, Marschall HU, Wagner M, Trauner M (2006) Role of nuclear receptors in the adaptive response to bile acids and cholestasis: pathogenetic and therapeutic considerations. Mol Pharm 3: 231-251.

Zollner G, Marschall HU, Wagner M, Trauner M (2006) Role of nuclear receptors in the adaptive response to bile acids and cholestasis: pathogenetic and therapeutic considerations. Mol Pharm 3: 231-251.