Available online at http://www.journalcra.com

International Journal of Current Research
Vol. 3, Issue, 11, pp.317-321, October, 2011

INTERNATIONAL JOURNAL OF CURRENT RESEARCH

ISSN: 0975-833X

RESEARCH ARTICLE

MORPHOLOGICAL ALTERATIONS IN THE PODOCYTES FOLLOWING CHRONIC SIMULTANEOUS ADMINISTRATION OF ETHANOL AND ACETAMINOPHEN IN KIDNEYS OF ADULT WISTAR RATS (*RATTUS NORVEGICUS*)

[1]Fakunle P.B., [1]Ajibade A.J., [2]Ehighie L.O., [1]Oyewo O.O. and [1]Adeleke F.O

[1]Department of Anatomy, Ladoke Akintola University of Technology, Ogbomoso, Nigeria
[2]Department of Biochemistry, Ladoke Akintola University of Technology, Ogbomoso, Nigeria

ARTICLE INFO

Article History:
Received 12th May, 2011
Received in revised form
28th July, 2011
Accepted 7th September, 2011
Published online 30th October, 2011

Key words:
Ethanol,
Acetaminophen,
Podocytes,
Kidney,
Sucrose,
Wistar Rats.

ABSTRACT

Aims: Ethanol and acetaminophen have been separately reported with various effects on the functionality of the kidney but information on the level of impact when simultaneously administered chronically is scanty hence effects of chronic simultaneous administration of ethanol and acetaminophen were investigated.

Place and duration of study: This research work was carried out in the Anatomy department of Ladoke Akintola University of Technology,Ogbomoso,Nigeria between 2010 and July 2011.

Methodology: Forty adult wistar rats of average weight 200±3.21g, randomly distributed into 4 groups A,B, C and D (n=10).The animals were fed with standard mouse chow with water provided ad libitum. Animals in group A were given100mg/Kg.bwt. acetaminophen and 25% ethanol in 2% sucrose solution while group B animals were given 100mg/Kg.bwt. The group C animals received 25% ethanol in 2% sucrose solution and lastly the animals in group D were given only distilled water all for a period of 6 weeks. At the end of administration the animals were sacrificed by cervical dislocation and the wet weight of each kidney specimen was documented. The kidney specimens were fixed in 10% formol saline and then processed for routine histological techniques, sectioned at 6µ and stained with H&E.

Results: Significant body weight loss $P<0.05$ was observed in the treatment groups A and B compared to the control. Distortion of the podocytes as well as pyramidal cells in the proximal and distal convoluted tubules were observed in all the treatment groups although more marked in group A than in group B compared to the normal cells in the controls.

Conclusion: This may underline impaired filteration and tubular fluid reabsorbtion properties of the kidney.

INTRODUCTION

Acetaminophen is a widely used over-the-counter analgesic (pain reliever) and antipyretic (fever reducer). It is commonly used for the relief of fever, headaches, and other minor aches and pains, and is a major ingredient in numerous cold and flu remedies. Acetaminophen is one of the most commonly used medicines with a very high safety profile when used properly. If misused, either intentionally or accidentally, acetaminophen can cause significant injury (Amar and Schiff, 2007)). In combination with non-steroidal anti-inflammatory drugs (NSAIDs) and opioid analgesics, paracetamol is used also in the management of more severe pain (such as postoperative pain Alcohol plays an important role in the daily life of many healthy as well as unhealthy individuals, being a nutrient (energy source), psychoactive drug and a toxin (Suter, 2004). The characteristic feature of alcohol use disorders is the consumption of dangerous amounts of alcohol despite the knowledge that problems occur during drinking (Crews and Nixon, 2009).

***Corresponding author:** fakunsbobo@gmail.com*

However overindulgence in alcohol intake, which has always culminated into chronic alcohol consumption, has been a problem of public health concern (Jyrki, 2002). The kidneys have important physiological functions including maintenance of water and electrolyte balance, synthesis, metabolism and secretion of hormones, and excretion of the waste products from metabolism. In addition, the kidneys play a major role in the excretion of drugs, hormones, and xenobiotics (James *et al.,* 1998). The podocytes are the most differentiated cell type in the glomerulum, which forms a crucial component of the glomerular filtration barrier (Hermann, 2000). Podocytes are important for maintaining the selective filtration barrier of the renal glomerulus. They are complex cells that develop primary and secondary foot processes that form the slit diaphragm, an unique cell-cell contact that serves as a final filtration barrier (Kowoh *et al.,* 2006 and Pavenstadt *et al.,* 2003). The slit diaphragm is formed by specialized adhesion proteins that connect the foot processes, forming an open space between them. Injury of the slit diaphragm or the actin cytoskeleton as well as interference with the podocyte-glomerular basement membrane (GBM) interaction have been shown to cause foot

process effacement and proteinuria (Kowoh *et al.,* 2006). An additional factor that can also affect the mechanisms of progression/regression of fibrosis is the plasticity of podocytes controlling glomerular filtration (Dussaule *et al.,* 2011).It is well established that toxic nephropathies are not restricted to a single type of renal injury. Some chemicals target one discrete anatomical region of the kidney and may affect only one cell type. Yet chemical insults to the kidney may result in a spectrum of nephropathies that are indistinguishable from those that do not have a chemical etiology. Since cell's function depends not only on receiving a continuous supply of nutrients and eliminating metabolic waste products but also on the existence of stable physical and chemical conditions in the extracellular fluid bathing it, in this study we attempt to investigate the integrity of podocyte when chronically and simultaneously assaulted by acetaminophen and alcohol.

MATERIALS AND METHODS

Forty adult healthy wistar rats of both sexes of average weight 200±3.23g were maintained under standard laboratory conditions for an acclimatization period of 2 weeks in the animal holdings of Anatomy Department, Ladoke Akintola University of Technology Ogbomoso. During this course, the rats were fed with standard laboratory mouse chow (Ladokun feeds, Ibadan) and were given water *ad libitum*. Daily weights were taken and documented. After acclimatization, the rats were randomly assigned into four groups (N=10) such that A, B and C served as treatment groups, while D served as the control group. Group A animals received 100mg/kg body weight acetaminophen and 2% sucrose in 25% ethanol solution as their drinking water, group B animals as well only took 2% sucrose in 25% ethanol solution as their drinking water while group C animals received 100mg/kg body weight of acetaminophen and distilled water. The 2% sucrose in 25% ethanol solutions were replaced afresh daily at 18.00 hours G.M.T. The rats in group D received distilled water *ad libitum*. All the animals were exposed for a period of 6 weeks. Changes in body weights and volume of ethanol consumed were documented. Bottles containing absolute ethanol were obtained from Sigma Laboratory Ltd, San Francisco, U. S. A. while acetaminophen was obtained from Emzor Phamaceutical industrial limited, Nigeria.At the end of administration, all the rats were sacrificed by cervical dislocation. The kidney specimens were processed for routine histological techniques sectioned at 6µ and stained using Heamtoxylin and Eosin stains.

Statistical analysis*:* The data were analyzed using the computerized statistical package 'SPSS Version 11'. Mean and standard error of mean (SEM) values for each experiment group was determined. The means were compared by analysis of variance at a level of significance of 95% and 99% as previously described by Fakunle *et al.,* 2011.

RESULTS AND DISCUSSIONS

Body weights: At the end of drug administration, significantly decreased body weight ($P < 0.05$) of mean±sem (138.7 ± 6.63 and 144.8 ± 14.53g) respectively compared to the control group of mean±sem (213.3 ± 5.59g) as seen in Table I was observed in the Treatment groups A and B. However Treatment group C showed an insignificant ($P > 0.05$) weight gain of Mean±SEM (209.6 ± 10.20)g as compared to the control group of mean±sem (213.3 ± 5.59g) as seen in Table I. At the end of the first week of acetaminophen administration, rats in this group presented an insignificant ($P > 0.05$) decrease in body weight of Mean±SEM (168.4 ± 3.24g). Insignificantly decreased ($P<0.01$) wet weight loss of Mean±SEM (0.46±0.13 and 0.47±0.14)g was obtained in the of the kidneys of the treatment groups A and B compared to the control group of (0.49±0.26) as seen in Table II while the treatment group C also revealed an insignificantly decreased ($P>0.01$) value of (0.48±0.31)g compared to the control from Table II.

Feed consumption pattern: Ethanol was observed to severely depressed feeding as the two treatment groups T_1 and T_2 show a significantly ($P<0.001$) of Mean±SEM (200.0±9.3 and 206.0±8.4)g compared to the control group of Mean±SEM (272.0± 9.4)g as shown in Table III. There was no significant difference in the feeding pattern of C compared to the controls.

Ethanol consumption pattern: The two treatment groups A and B showed an initial decrease in volume of ethanol consumption in the first three weeks as shown in Figure I. However, gradual increase in the volume of ethanol consumed was seen from the end of the third week and throughout of the experiment.

Histological findings: The histoarchitecture revealed by the control group D showed normal sized capsular space with intact podocytes in the glomerulus while the cells of the proximal and distal convoluted tubules also appeared normal in Figure I. The treatment group TI showed marked microanatomical changes from wider capsulr space when compared to those of the control with distorted podocytes as well as pyknotic cellular appearance of the cells of proximal and distal convoluted tubules as seen in Figure II. The treatment group T2 in Fig. III also revealed microanatomical alterations in the podocytes and cells of proximal and distal convoluted tubules when compared to the control group but not as marked as obtained in the treatment group T1.The treatment group T3 presented a cytoarchtecture similar to that of control with very less pronounced microhistological changes as seen in Figure IV. As evident from the results of obtained from this study that long term simultaneous consumption of acetaminophen and ethanol clearly affects body weight, the feeding pattern as well as histomorphology of the kidney most especially the podocytes with degeneration and necrosis of renal tubular epithelia.

The significant body weight loss seen in the treatment groups A and B as shown in Table I appeared depicted by the pattern of ethanol consumption as shown in Chart I with an initial decrease in volume of ethanol consumption within the first three weeks followed by gradual increase in the volume of ethanol consumed from the end of the third week and throughout of the experiment. Correlating the pattern of ethanol consumption to the quantity of feed consumed Table II, significant feeding depression is clearly evident to be a sole function ethanol as this was noticed only in groups A and B. The depressed feeding noticed here can be as result of a gradual process of ethanol dependence which is the need for continued alcohol consumption to avoid a withdrawal

syndrome that generally occurs from 6 to 48 hours after the last drink. (Linnoila,1987 and Morrow *et al.,* 1988). Hence

Table I. Mean±SEM weight distribution at the end of experiment

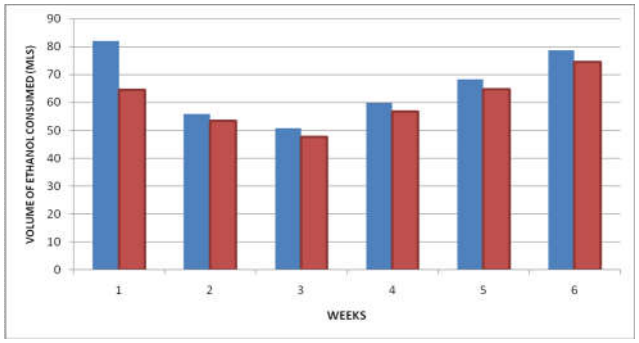
WEEKS	NO OF RATS	A	B	C	D
0	10	169.3± 7.00	190.8± 7.98	170.5± 1.86	154.8± 3.53
1	10	167.8± 8.06	185.6± 14.37	168.4± 3.24	163.8± 2.18
2	10	159.3± 6.97	173.0± 20.24	171.2± 7.01	178.3± 5.19
3	10	150.5± 4.94	166.6± 6.46	178.8± 5.82	191.3± 2.06
4	10	146.7± 4.98	156.4± 10.19	184.4± 10.79	197.8± 2.81
5	10	140.6± 6.80	150.7± 13.92	195.1± 10.53	207.8± 5.51
6	10	138.7± 6.63*	144.8±14.53*	209.6± 10.20	213.3± 5.59

* (P < 0.05) Significance difference when compared with control using t-test

Table II. Mean + SEM of Feed consumption at the end of administration

Group	N	Mean±SEM (g)	D.O.F	2-Prob
A	10	200.0±9.3*	10.2	0.004
B	10	206.0±8.4*		
C	10	240.0±10.6		
D	10	272.0± 9.4		

* (P<0.01) Significance difference when compared with control using t-test



Group A is represented in Blue bars; Group B is represented in Red bars

Fig. 1. Histogram of volume of ethanol consumption pattern

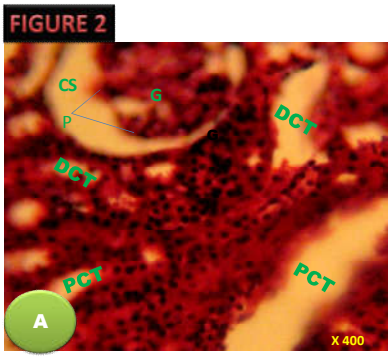


Fig. 2: Photomicrograph of kidney (Treatment section A) showing enlarged capsular space CP, distorted podocytes P within glomerullus G and distal and proximal convoluted tubules(DCT&PCT) with pyknotic cells. *H&E x400.*

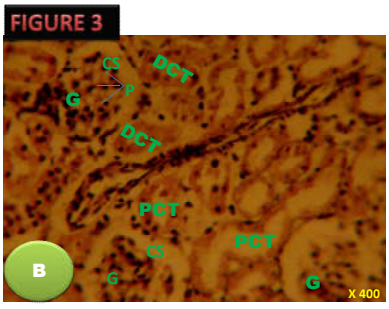


Fig. 3 : Photomicrograph of kidney (Treatment section B) showing slightly enlarged capsular space with few distorted podocytes in the glomerulus as well as distal and proximal convoluted tubules(DCT&PCT) with some pyknotic cells.

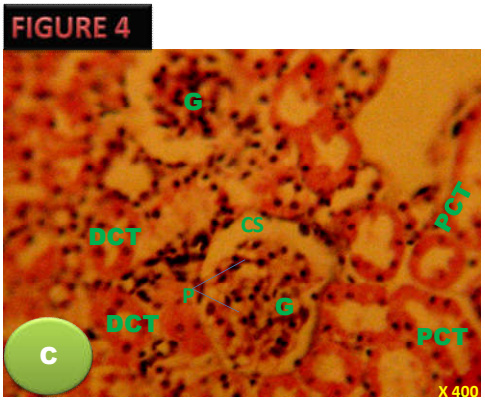


Fig. 4: Photomicrograph of kidney (Treatment section C) showing normal capsular space (CP) with averagely normal podocytes and normal distal and proximal convoluted tubules(DCT&PCT) with intact cells having normal parietal pleura. *H&E x400*

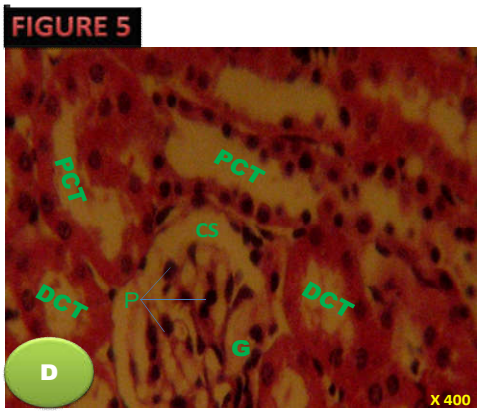


Fig. 5: Photomicrograph of kidney (control section C) showing normal capsular space(CP) with normal podocytes (P) in the glomerulus (G).Distal and proximal convoluted tubules(DCT&PCT) also appear normal with intact cells having normal parietal pleura. *H&Ex400*

gradually there appears to be more urge to drink ethanol than for the feed consumption. In other words the weight loss observed here is in conformity with the earlier reports of effects of alcohol on digestion, absorption, storage, utilization and excretion of essential nutrients such as vitamins, minerals and proteins (Lieber, 2003). Over the long term, excessive ethanol intake is metabolized predominantly by the microsomal ethanol-oxidizing system, which requires high ethanol concentrations for half-maximal activity and which leads to an increased loss of energy from ethanol as heat (Suter *et al.,* 1992). The impairment of nutrient absorption by ethanol via damaging cells lining the stomach and intestines, and disabling transport of some nutrients into the blood as previously by Feinman and Lieber (1998) can also play active role in the recorded weight loss. Many reports also have it that hepatotoxicity of paracetamol (acetaminophen) is increased in chronic alcoholics, and that such individuals not only carry an increased risk of severe and fatal liver damage after acute overdosage (Artnak and Wilkinson 1998, Bridger *et al.,* 1998 and Schiødt *et al.,* 1997) although that similar serious liver damage may also occur with 'therapeutic' use (Andreu *et al.,* 1999). As postulated by Corcoran *et al.* (1980), paracetamol has been implicated in liver damage through

conversion by hepatic cytochrome P450 enzymes to a minor but toxic intermediate metabolite subsequently identified as **N**-acetyl-p-benzoquinoneimine .In accordance with this mechanism, the susceptibility to paracetamol-induced liver damage in animals was increased when these enzymes were stimulated by prior administration of inducing agents such as phenobarbitone and 3-methylcholanthrene, and decreased by inhibitors such as piperonyl butoxide and 4-methylpyrazole (Thummel *et al.,* 1989).

Histological observations noted in the treatment groups A and B in Figures 2 and 3 revealed marked microanatomical changes from wider capsular space with distorted degenerating podocytes as well as pyknotic cellular appearance of the cells of proximal and distal convoluted tubules as a result of ethanol and acetaminophen intake, although more pronounced in groups A and B, it was very mild in group C as seen in Figure control group D as seen in Figure 5. Alcohol may have both detrimental and salutatory effects on renal function (Chung *et al.,* 2005). Cell degeneration has been classified mainly into two types as necrosis and apoptosis. Apoptosis is a genetically determined, biologically meaningful, active process playing a role opposite to mitosis in tissue size regulation, shaping organs and removing cells that are immunologically reactive against self, infected or genetically damaged whose continuous existence pose a danger to the host. It is actively involved in physiological and developmental processes (Kerr, *et al.,*1995). Lipid peroxidation has been suggested as one of the possible mechanisms whereby chemicals may produce membrane damage and cell death. Free radicals, generated either directly by the metabolism of a chemical or from the reduction of oxygen (forming O_2^-, H_2O_2 and OH*), can initiate lipid peroxidation via hydrogen abstraction from polyunsaturated fatty acids. This interaction will form lipid peroxyradicals and lipid hydroxyperoxides, propagating the chain reaction. Such a chain reaction may destroy cellular membranes and thereby result in increased plasma membrane permeability or altered fluidity and cell death. Lipid peroxidation may also cause cell death through the formation of potent toxic lipid metabolites (such as hydroxyalkenals). However, several lines of evidence indicate that lipid peroxidation is most often independent (or is a consequence rather than the cause) of cell death (Witschi *et al.,* 1987). One or more of these mechanisms of cellular injury could closely interact. Analgesic nephropathy may be a consequence of the excessive consumption. Originally, phenacetin was common to all of these mixtures, which led to the conclusion that this drug was the only cause of "phenacetin kidney". Subsequently, a variety of analgesics, NSAIDs, and a number of industrial and environmental chemicals including ethanol have been shown to have the potential to cause RPN and interstitial nephritis (Schwarz *et al.,* 1988). The degeneration of the podocytes noticed in this work appears more of an apoptotic reaction.With increasing recognition of an important role for podocytes in the progression of renal disease (Mundel and Shankland, 2002). It has recently become clear that initial glomerular injury affects the podocyte as an important target cell for progression (Kriz and Lemley, 1999). Proximal renal tubular cells are particularly vulnerable to the toxic action of chemicals, owing to their high energy demand (such as reabsorptive and secretory functions). Redox-active agents may cause extensive oxidation of GSH to oxidized glutathione (GSSG). Under such conditions, often referred to as "oxidative stress", reduction of GSSG back to GSH by the NADPH-dependent GSSG reductase is lower than the rate of GSH oxidation. This may lead to gluthathione depletion and cause oxidation of cellular enzymes, depletion of cellular ATP, and loss of mitochondrial function (Trump et al., 1989).It is clearly evident from the results obtained from this work that nephrotoxicity is more pronounced when both ethanol and acetaminophen are simultaneously administered than when either dugs are used particularly acetaminophen and this can be related to the earlier reports that acetaminophen induced a mild degree of tubular cell apoptosis, even at concentrations found during therapeutic dosing (Corina *et al.,* 2004).

Conclusions

This study thus conclusively underlines the impairment of podocyte functions in stabilizing glomerular architecture by counteracting distensions of the glomerular basement membrane (Kriz *et al.,*1999) and maintaining a large filtration surface through the slit diaphragms and also strong possibilities of impairment of tubular fluid reabsorbtion from tubular necrosis as observed strongly due to chronic simultaneous administration of ethanol and acetaminophen.

REFERENCES

Amar, P.J., and Schiff, E. R. 2007. Acetaminophen safety and hepatotoxicity—Where do we go from here? Exp Opin Drug Safety 6:341–355.

Andreu, V., Gomez-angelats E, and Bruguera, M.1999. Severe hepatitis from therapeutic doses of paracetamol in an alcoholic patient. *Gastroenterol Hepatol.* **22**:235–237

Artnak, K.E., Wilkinson, S.S., and Fulminant, 1998. Hepatic failure in acute acetaminophen overdose. *Dimensions Crit. Care Nursing.*17:135–144

Bridger, S, Henderson K, Glucksman E, 1998. Deaths from low dose paracetamol poisoning. *Br Med J.* 316**:**1724 – 1725

Chung, Fu-Mei, Yi-Hsin Yang, Tien-Yu Shieh, Shyi-Jang Shin, Jack C.-R. Tsai and Yau-Jiunn Lee, 2005. Effect of alcohol consumption on estimated glomerular filtration rate and creatinine clearance rate. Nephrol Dial Transplant. 20: 1610–1616

Corcoran, G.B., Mitchell, J.R., Vaishnav, Y.N, 1980. Evidence that acetaminophen and N-hydroxy acetaminophen form a common arylating intermediate, N-acetyl-p-benzoquinoneimine. *Mol. Pharmacol.,*18**:**536–542

Corina Lorz, Pilar Justo., Ana Sanz., Dolores Subirá., Jesús Egido., and Alberto Ortiz, 2004. Paracetamol- Induced Renal Tubular Injury: A Role for ER Stress. *J Am Soc Nephrol.,* 15:380- 389

Crews, F.T., and Nixon, K. 2009. Mechanisms of neurodegeneration and regeneration in alcoholism. *Alcohol Alcohol.,* 44: 115–127

Dussaule Jean-Claude, Dominique Guerrot, Anne-Ce´cile Huby, Christos Chadjichristos, Nasim Shweke, Jean-Jacques Boffa and Christos Chatziantoniou, 2011. The role of cell plasticity in progression and reversal of renal Fibrosis. *Int. J. Exp. Path.,* 92:151–157

Fakunle, P.B., Fakoya, F.A., and Shallie, P.D. 2011. Impairment activities of succinic dehydrogenase in the lateral geniculate body and superior colliculus of adult

wistar rats (*Rattus norvegicus)* as a result of long term intake of ethanol consumption. *Journal of Neuroscience and Behavioural Health*, Vol. 3(5), pp. 57-65.

Feinman, L. and Lieber, C. S.1998. Nutrition and diet in alcoholism. In: Shills, M.E., Oslon, J.A., Shike, M.and Ross,A.C; eds. *Modern Nutrition in Health and disease*. 9th ed. Baltimore: Williams and Wilkins. Pp1523-1542

James, W., Lorh, F.N., Gail, R.W., and Margaret A.A. 1998. *Pharmacological Reviews* . vol. 50 no. 1 107-142.

Jyrki, R. 2002. Neuropathology of aging and long-term ethanol consumption. Ph. D.Thesis. University of Tampere.

Kerr J.F.R., Gobe, D.C., Winterford, C.M., and Harmon, B.V 1995. Anatomical methods in cell death. Methods cell biology.46:1-27.

Kriz, W., and Lemley K.V. 1999. The role of the podocyte in glomerulosclerosis. *Curr Opin Nephrol Hypertens* 8: 489-497

Kwoh, C.**,** Shannon, M.B., Miner, J.H., and Shaw, A. 2006. *Pathogenesis of nonimmune glomerulopathies. Annu Rev Pathol.,* 1*:* 349*–374.*

Lieber, C.S. 2003. Relationships between Nutrition, Alcohol Use, and Liver disease. *Alcohol* Health & Research World. 27: 220-231.

Linnoila, M**.** 1987. Alcohol withdrawal and noradrenergic function. *Annals of Internal Medicine* 107(6):875-889.

Morrow, A. L., Suzdak, P.D., Karanian, J.W., and Paul, S.M.1988. Chronic ethanol administration alters *-aminobutyric acid, pentobarbitol and ethanol-induced 36Cl* uptake in cerebral cortical synaptoneurosomes. *Journal of Pharmacology and Experimental Therapeutics* 246(1):158-164.

Mundel, P and Shankland, S.J. 2002. Podocyte biology and response to injury. *J Am Soc Nephrol.,* 13: 3005-3015.

Pavenstadt, H**.** Kriz, W. and Kretzler M. 2003. Cell biology of the glomerular podocyte. *Physiol Rev.,* 83*:* 253–307.

Schwarz A., Krause, P.H., Keller, F., Offermann, G., Mihatsch, M.J. 1988. Granulomatous interstitial nephritis after nonsteroidal anti-inflammatory drugs. *Am J Nephrol.,*8**:**410–416.

Suter, P. M, Yves Schutz, M.S and Eric Jequier, 1992. The Effect of Ethanol on Fat Storage in Healthy Subjects .the new england journal of medicine. *N Engl J Med.,* 326:983-98.

Suter, P.M. 2004. Alcohol,nutrition and health maintenance: selected aspects. *Proc.Nutr.Soc.,* 63:81-88

Thummel, K.E., Slattery, J.T., and Nelson, S. D.1989. Effect of ethanol on hepatotoxicity of acetaminophen in mice and on reactive metabolite formation by mouse and human liver microsomes. *Toxicol Appl Pharmacol.* 100**:**391–397

Trump, B.F., Berezesky, I.K., Smith, M.W., Phelps, P.C. and Elliget, K.A. 1989. The relationship between cellular ion deregulation and acute and chronic toxicity. *Toxicol. Appl. Pharmacol.,* 97: 6-22.

Witschi, H.P., Aldridge, W.N., Aust, S.D., Autor, A.P., De Matteis, F., Druet, P., Elbers, R., Fowler, B.A., Groniowski, J.A., Hagman, W., Orrenius, S., Petering, D.H., Seinen, W., Summer, K.H., and Trump, B.F. 1987. Mechanisms and target cell injury. In: Fowler, B.A., ed. Mehanisms of cell injury: Implications for human health, New York, Chichester, Brisbane, Toronto, John Wiley and Sons. 385-403.

****