African Journal of Biology and Medical Research
ISSN: 2689-534X
Volume 4, Issue 1, 2021 (pp. 36-45)
www.abjournals.org

ACETAMINOPHEN INTAKE AND TAXIFOLIN SUPPLEMENTATION ALTERS IL-6, TNF-α AND TROPONIN LEVELS IN WISTAR RATS

Etah E. Nkanu[1], Kayode Dasofunjo[2] and Kebe Obeten[3]

[1]Department of Physiology, Cross River University of Technology, Calabar, Nigeria
[2]Department of Medical Biochemistry, Cross River University of Technology, Calabar, Nigeria
3Department of Human Anatomy, Cross River University of Technology, Calabar, Nigeria
Email: nkanuee@yahoo.com; nkanuee@gmail.com; ettahNkanu@crutech.edu.ng
Tel: +2347030299991; 08086127404

ABSTRACT: *Acetaminophen is an organic compound that is commonly used as an antipyretic and analgesic drug. Acetamenophen may cause liver injury and cardiac dysfunction because of complex cardiohepatic interactions.The aim of this study is to assess the possible effect of acetamenophen intake and taxifolin supplementation on some cardiovascular risk factors like tumour necrosis factor-alpha(TNF-α), interleukin-6(IL-6), troponin, and lipid profile in Wistar rats. Four groups of Wistar rats containing five rats each were used. Group 1(control) received normal feed and water. Group 2 received 500mg/kg body weight of acetaminophen(ACTMP), group 3 received 500mg/kg of ACTMP and 1mg/kg body weight of taxifolin (TXF) orally. Group 4 received 1mg/kg of TXF. Feeding and treatment lasted for 5 weeks. Blood samples were collected through cardiac puncture into blood sample bottles and centrifuged to obtain serum. Interleukin-6, troponin and TNF-α concentrations were determined using ELISA kits and following manufacturers procedures. Results showed that tumor necrosis factor-alpha (TNF-α), IL-6 and troponin in ACTMP were significantly($p<0.05$) higher than control while lactate dehydrogenase(LDH) was reduced in ACTMP. Lipid profile was significantly ($p<0.05$) reduced in ACTMP. Treatment with taxifolin significantly ($p<0.01$) lowered IL-6, troponin, and LDH. We conclude that Acetamenophen intake may alter serum level of IL-6, TNF-α and troponin which are risk factors of cardiotoxicity while taxifolin has little or no effect on TNF-α and troponin but may suppress IL-6 activity.*

KEYWORDS: Acetaminophen, Taxifolin, Interleukins, Troponin, Tumor Necrosis Factor, Lipids

INTRODUCTION

The use of acetaminophen as an analgesic and as an antipyretic drug has been a common practice as it is taken for pain relief. It is used majorly because it serves as an alternative to nonsteroidal anti-inflammatory drugs (NSAIDs) which are assumed to cause hepatic injury or increase risk of stroke and myocardial infarction.

The benefit of intake of acetaminophen has been very controversial. Some reports have claimed that it is cardioprotective because of its antioxidant property (Merrill and Goldberg, 2001, 2004.) while others have implicated it in progressive liver injury and having direct toxic effect

on the myocardium (Jones and Prescott, 1997). Evidences abound that suggest that activated macrophages from injured hepatocytes following ACTMP intake contribute to the release of cytotoxic mediators like TNF and IL – 6 that are possible candidates in the progressive phase of liver injury (Golden *et al.,* 1995;Blazka *et al.,* 1995, Blazka *et al.,* 1998). IL – 6 is a pro-inflammatory cytokine involved in immune activation, vascular function and modulation of TNF-α production (Akira *et al.,* 1993), while TNF-α modulates immune and inflammatory responses that are produced by macrophages and lymphocytes.

In an unquenchable quest to look for palliatives or alternative means to ameliorate the deleterious effect of acetaminophen, taxifolin was used. Taxifolin is found in the fruit of *Silybum marianum* also referred to as "milk thistle". Its extract is called sylimarin and contains a mixture of taxifolin and sylibin (Morazzoni and Bombardelli,1995). This plant product in cardiovascular disease is believed to reduce LDL oxidation and inhibit the activity of HMG-CoA reductase, thus exhibiting antioxidant and hepatoprotective property (Kandaswami and Middleton, 1994). The aim of this research work therefore, was to examine the possible effect of acetaminophen and taxifolin supplementation on some cardiovascular risk factors.

MATERIALS AND METHOD

Experimental Design

Twenty male Wistar rats weighing 180-200g and aged ten weeks were obtained from the animal house of Physiology Department, Cross River University of Technology, Calabar, Nigeria and randomly divided into **f**our groups containing five rats each. The animals were housed in plastic cages and kept in room temperature of 30^0c ± 3°C with 12 h light/dark cycle. Group 1(control) received normal feed and water. Group 2 received 500mg/kg body weight of acetaminophen(ACTMP), group 3 received 500mg/kg of ACTMP and 1mg/kg body weight of taxifolin (TXF) orally. Group 4 received 1mg/kg of TXF. Feeding and treatment lasted for 5 weeks after which Blood samples were collected through cardiac puncture and centrifuged at 1000rpm for 10minutes to obtain serum. Interleukin-6(IL-6), troponin and TNF-α concentrations were determined using standard methods. Ethical approval for the study was obtained from the Faculty of Basic Medical Science Animal Research Ethical Committee of Cross River University of Technology, Calabar, Nigeria (approval number FBMS/ CRUTECH/ 12/020).

Determination of Cholesterol

The methods of Siedel *et al*.,(1983) and Sullivan *et al*.,(1985) were used to determine total cholesterol (TC) and triglyceride, respectively. High-density lipoprotein cholesterol (HDL) was measured using the method of Warnick *et al.,* (1982); low density lipoprotein cholesterol was calculated using the equation of Friedewald, and Frederickson (1972).

Determination of TNF-α, Il-6, and Troponin

Tumor necrosis factor-alpha (TNF-α), Interleukin-6(IL-6), and troponin concentrations were determined by commercially available Elisa kits according to manufacturer's instructions.

African Journal of Biology and Medical Research
ISSN: 2689-534X
Volume 4, Issue 1, 2021 (pp. 36-45)
www.abjournals.org

Statistical analysis

All data obtained in this study were expressed as mean ± standard error of mean. Collected data was analyzed using ANOVA (analysis of variance) using GraphPad Prism version 5.0 for Windows (GraphPad Software, San Diego, CA, USA). Bonferroni multiple comparison post hoc tests were used to compare the level of significance between control and experimental groups. A value of $p<0.05$ was considered significant.

Table 1: Showing effect of acetaminophen intake and taxifolin supplementation on lipid profile in Wistar rats.

Parameters	Control	ACTMP	ACTMP+TXF	TXF
TC (mmol/L)	1.756±0.009	1.624±0.009	1.570±0.012	1.524±0.012
TG (mmol/L)	**0.7220**±0.009	**0.6180**±0.007**^{,cd}	**0.5980**±0.007**	**0.4880**±0.004**
HDL (mmol/L)	**0.4420**±0.003	**0.4060**±0.0022**	**0.3960**±0.002**	**0.3860**±0.002**^{b}
LDL (mmol/L)	**0.9760**±0.009	**0.9360**±0.002**	**0.9020**±0.004**	**0.9140**±0.009**^{,b}

*n=5, values are expressed in Mean±SEM, **=p<0.005 vs Control; b=p<0.05 vs ACTMP, d=p<0.05 vs. TXF.*

Effect of acetaminophen and Taxifolin on TNF-α. Interleukin-6 and Troponin.

Results obtained showed a significant($p<0.05$) increase in tumor necrosis factor, interleukin-6 and troponin in acetaminophen intake compared to control. Treatment with taxifolin did not show any significant difference in TNF and troponin but showed a significant ($p<0.05$) reduction in IL-6. This is represented in figures 1, 2, & 3 respectively.

Effect of acetaminophen and Taxifolin on lactate dehydrogenase:

LDH was significantly ($p<0.05$) reduced in all treatment groups. This is shown in figure 4.

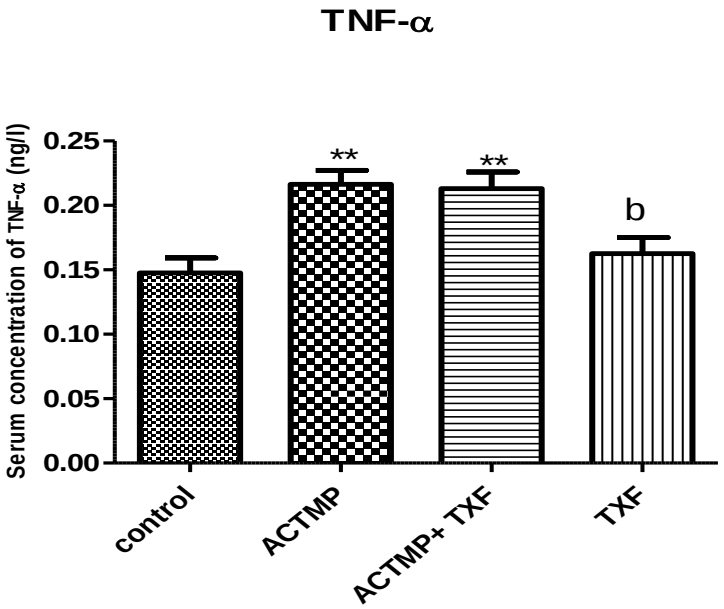

FIGURE 1 : Showing effect of administration of acetamenophen and supplementation of taxifolin in tumour necrosis factor in Wistar rats Values are expressed in Mean ± SEM **=p<0.05 vs Control; c=p<0.05 vs ACTMP

African Journal of Biology and Medical Research
ISSN: 2689-534X
Volume 4, Issue 1, 2021 (pp. 36-45)
www.abjournals.org

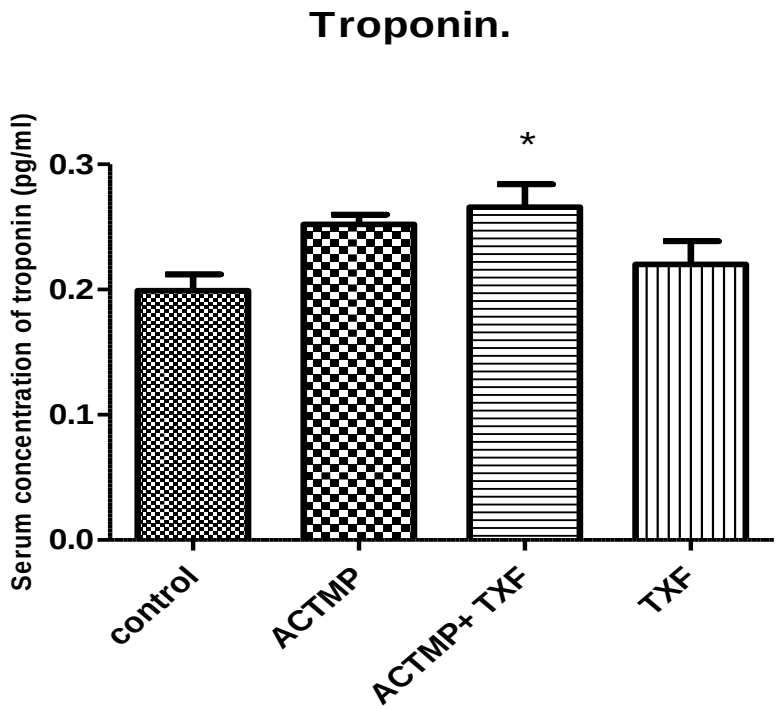

FIGURE 2 : Showing effect of administration of acetamenophen and supplementation of taxifolin on troponin I in Wistar rats
Values are expressed in Mean ± SEM; *=p<0.05 vs Control

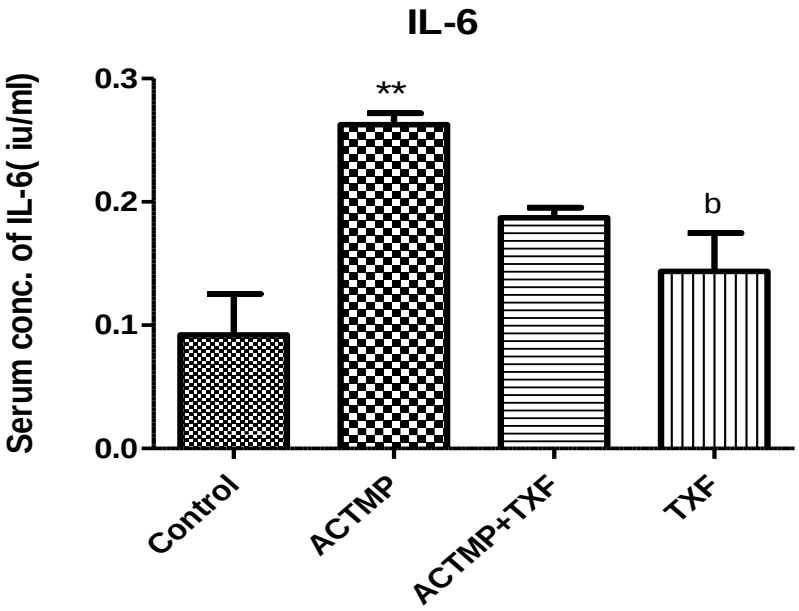

FIGURE 3 : Showing effect of administration of acetamenophen and supplementation of taxifolin on interleukin-6 in Wistar rats
Values are expressed in Mean ± SEM
; **=p<0.01 vs Control; b=p<0.05 vs ACTMP

African Journal of Biology and Medical Research
ISSN: 2689-534X
Volume 4, Issue 1, 2021 (pp. 36-45)
www.abjournals.org

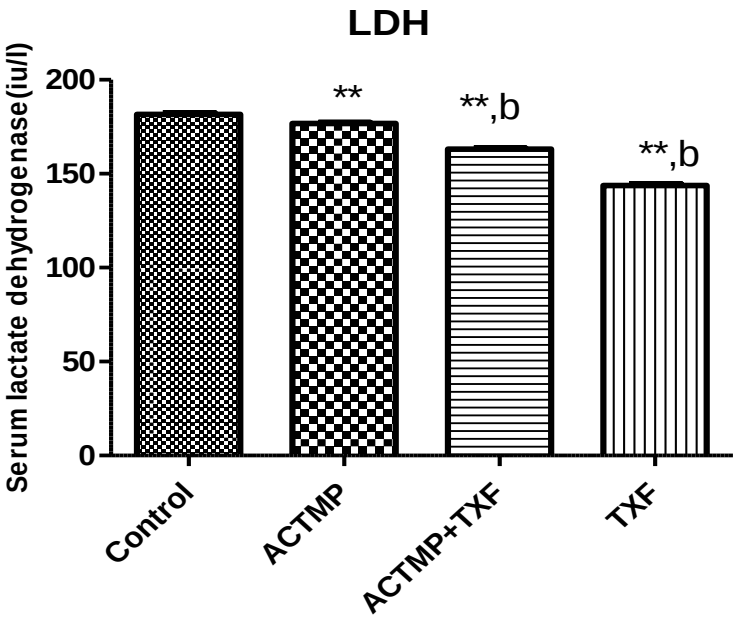

FIGURE 4 : Showing effect of administration of acetamenophen and supplementation of taxifolin on lactate dehydrogenase in Wistar rats
Values are expressed in Mean ± SEM
; **=p<0.01 vs Control; b=p<0.05 vs ACTMP

DISCUSSION

One of the most common drugs often taken to relieve body pain and headache is acetaminophen, an active ingredient in Tylenol. Even though it is reported not to have side effects within therapeutic doses, some research work has implicated its consumption in induced hepatocellular and cardiohepatic damage **(**Jones and Prescott,1997**).** In this work, we examined the effect of acetaminophen intake on some cardiovascular risk factors and the possible effect of taxifolin supplementation in Wistar rats.

Our results in this study showed that acetaminophen (ACTMP) intake up to a dosage of 500mg/kg body weight per day, elevates serum level of tumor necrosis factor-alpha (TNF-α), interleukin-6 (IL – 6) and troponin. Such variations in concentration of these circulating biomarkers are often used as diagnostic and prognostic assessment (Ridker *et al.*, 2010), rather than just clinical evaluations even though it has been described to be a pleiotropic cytokine displaying both pro-inflammatory and anti-inflammatory properties depending on the type of organ or cell in which it is acting (kamimura *et al.,* 2003).

Previous studies have considered the alteration in IL–6 and TNF-α levels as a marker of inflammation related to cardiovascular risk (Mendell *et al.,* 1997, Zakai *et al.,* 2007; Ridker *et al.,* 2000), and assumed that ACTMP may precipitate direct toxic effect on the myocardium and also deplete sulfhydryl groups causing interference with nitric oxide production leading to coronary ischemia (Jones and Prescott 1997; Lip and Vale 1996).

Increased IL–6 and TNF-α concentration in circulation has been reported in patients with cardiovascular heart failure and has been shown to present ventricular dysfunction, cardiac hypertrophy through the IL-6 signal transducing receptor component known as glycoprotein 130

(Tsugiyasu and Takahashi 2004.**)** and myocardial damage(Levine *et al.,* 1990;Tsutamoto *et al.,* 1998; Kopf *et al.,* 1995). Such surge in IL–6 and TNF-α may be resulting from its notable function of serving as an inducer of acute phase reactions as well as being a key player in potentiating cellular immune responses to affected cells (Kopf et al, 1994; Xing *et al.,* 1994; Ruzek *et al.,* 1997). The results obtained in our study may not have been unconnected with this systemic change in concentration and circulation of IL-6 and TNF-α following acetaminophen intake and suggestive of being causal to cardiovascular challenge.

Similarly, reports have also demonstrated that acetaminophen moiety may cause the hepatocytes to release substances that activate macrophages leading to the release of cytotoxic mediators such as IL-6 and TNF that are thought to contribute to hepatocellular damage (Golden *et al.,* 1995; . Blazka *et al.,* 1995; Blazka *et al.,*1998). Recent evidence has suggested that tumor necrosis factor (TNF) may be a possible candidate in the gradual development of liver injury **(**Golden et al, 1995;Blazka *et al.,*1995; Blazka *et al.,*1998 **).** Our limitation in this study was the inability to examine liver and heart histology following acetaminophen intake and taxifolin. However, studies have shown that most circulatory abnormalities are predominantly due to liver derived toxic factors and are associated with the development of cardiovascular insults (Wiese *et al.,* 2017) because of complex cardiohepatic interaction (Kubo *et al.,* 1987). In our previous work, we have demonstrated that acetaminophen intake increases liver enzymes production and threatens liver integrity (Dasofunjo *et al.,* 2018). In cirrhotic cardiomyopathy involving systolic dysfunction and diastolic relaxation, there is a reported defect in cardiac beta–adrenergic receptor system, alteration in plasma membrane fluidity, distortion in calcium channel activity and depletion of nitric oxide (Moller and Bernardi, 2013). Therefore, intake of acetaminophen above the therapeutic dosage may expose one to hepatocellular damage and a stand point on the possible alteration of cytokine expression and cardiovascular insults.

Furthermore, we investigated troponin and LDH activity as well as lipid profile in acetaminophen intake and our results showed elevated troponin level in acetaminophen intake. Numerous reports have shown that troponin I retains prognostic value (Freda *et al.,* 2005; Dezoya, *et al.,* 2004) and is a pathological evidence of myocardial damage (Mann *et al.,* 1989; Ooi, *et al.,* 2000 and Larson *et al.,* 2005). Elevated troponin levels of troponin have been associated with raised levels of IL – 6 and TNF-α, and C-reactive proteins in critical conditions and reflects increased left ventricular mass, depressed left ventricular ejection, diastolic dysfunction and atrial fibrillation (Wu *et al.,* 2004; Ammann *et al.,* 2003). This is evidenced by our results seen in this work.

Treatment with taxifolin, a potent flavonoid with antioxidant characteristics, did not suppress troponin and TNF-α release. This suggests that there may have been a non–interaction with the acetaminophen membrane receptors.

Lactate dehydrogenase is usually expressed in blood cells and in heart muscle. During tissue breakdown, LDH becomes released in good amounts into the blood and serum and serves as a

surrogate marker for heart or cellular injury. In this research work, acetaminophen did not elevate lactate dehydrogenase concentration.

Total cholesterol, triglyceride and low density lipoprotein were reduced in ACTMP intake and was further significantly reduced in taxifolin supplementation. The reduction in cholesterol level is attributed to the inhibition of HMG-CoA reductase (3-hydroxy-3-methyl-glutaryl-coenzyme A reductase) and suppression of cellular cholesterol esterification (De Whalley, *et al.,* 1990). In mammals, inhibitors of HMG-CoA reductase induce the expression of LDL receptors in the liver, and then increase the breakdown process of LDL and reduce cholesterol level (Humphries *et al.,* 1986). Taxifolin may be acting through this pathway.In cardiovascular disease, the bioavailability of flavonoid reduces LDL- oxidation known to be a vital step in atherogenesis (De Whalley, *et al.,* 1990; Hayal *et al.,* 1997).

Conclusion: Acetaminophen-induced cardiotoxicity may in part be the result of increased release of cytokines and troponin. Taxifolin has little or no effect on TNF-α and troponin but may suppress IL-6 activity.

Conflict of Interests

The authors have not declared any conflict of interests.

Acknowledgement

The authors of this research study wish to appreciate the technical assistance of Dr. Mrs Iya Eze of the Department of Medical Laboratory Science, University of Calabar, Calabar-Nigeria.

Authors Contribution

Nkanu, E.E designed, wrote the manuscript and revised the article for intellectual content; Dasofunjo K.; did data analysis, Kebe Obeten edited and finalized the manuscript.

Consent for publication

All authors agreed and gave consent to make public the current manuscript. The work described has not been published and is not under consideration for publication elsewhere

Ethics approval and consent to participate

All authors were in agreement to participate in the study and to have the data published. All participants signed a written informed consent, which was revised by the local Ethics Committee, which then approved the study protocol.

REFERENCES

Akira S, Taga T, Kishimoto T: Interleukin-6 in biology and medicine. Adv Immunol. 1993, 54: 1-78.

Ammann P, Maggiorini M, Bertel O, Haenseler E, Joller-Jemelka HI, Oechslin E, Troponin as a risk factor for mortality in critically ill patients without acute coronary syndromes. *J Amer Coll Cardiol*. 2003; 41: 2004– 2009.

Blazka ME, Elwell MR, Holladay SD,Wilson RE, Luster MI. Histopathology of acetaminophen-induced liver changes: role of interleukin 1 alpha and tumor necrosis factor alpha. Toxicol Pathol ; 1996; 24:181-189.

Blazka ME, Wilmer JL, Holladay SD, Wilson RE, Luster MI. Role of proinflammatory cytokines in acetaminophen hepatotoxicity. Toxicol Appl Pharmacol;1995; 133:43-52.

Borradaile N. M., Carroll K. K., Kurowska E. M. Regulation of HepG2 cell apolipoprotein B metabolism by the citrus flavanones hesperetin and naringenin. Lipids.1999; 34**:** 591–598.

Dasofunjo K., Berinyuy E.B., Nkanu, E.E., Mba CE1 ., Ujong P.U., Ati B.U1 and Monjok E. **N**..Maternal changes on histological and liver function indices exposed to *piliostigma thonningii* extract following acetaminophen toxicity in pregnant rats. *International Journal of Innovative Research and Review ISSN: 2018; Vol. 6 (4); 2347 – 4424 (Online) http://www.cibtech.org/jirr.htm*

DeZoysa JR. Cardiac troponins and renal disease. *Nephrology* 2004; 9: 83– 88.

Freda BJ, Tang WH, Van Lente F, Peacock WF, Francis GS. Cardiac troponins in renal insufficiency: review and clinical implications. *J Am Coll Cardiol* 2002; 40: 2065– 2071.

Friedwald WT, Levy RI, Fredrickson DS. Estimation of the concentration of low-density lipoprotein cholesterol in the plasma, without the use of preparative ultracentrifuge. Clin Chem. 1972; 18:449–502.

Goldin RD, Ratnayaka ID, Breach CS, Wickramasinghe SN.. Acetaminophen and macrophage activation. Int Hepatol Commun.1995; 4:16-18.

Humphries SE, Tata F, Henry I, Barichard F, Holm M, Junien C, Williamson R. "The isolation, characterisation, and chromosomal assignment of the gene for human 3-hydroxy-3-methylglutaryl coenzyme A reductase, (HMG-CoA reductase)". Human Genetics. 1986; **71** (3): 254–258. doi:10.1007/BF00284585.

Jones AL, Prescott LF. Unusual complications of paracetamol poisoning. Q J Med. *90*: 1997; 161–168.

Kandaswami C., Middleton E. Free radical scavenging and antioxidant activity of plant flavonoids. In Free Radicals in Diagnostic Medicine. D. Armstrong, editor. Plenum Press, New York.1994; 351–376.

Kopf M, Baumann H, Freer G, Freudenberg M, Lamers M, Kishimoto T, Zinkernagel R, Bleuthmann H, Kohler G. Impaired immune and acute-phase responses in interleukin-6-deficient mice. Nature.1994; 368:339–342. doi: 10.1038/368339a0.

Kubo, SH, Walter,BA, John, DH, Clark,M, Cody,RJ.(1987) Liver function abnormalities in chronic heart failure. Influence of systemic hemodynamics, Arch Intern Med. 1987. vol. 147(pg. 1227-1230)10.1001/archinte.1987.00370070041006

Larson AM, Fontana RJ, Davern TJ, Polson J, Lalani EK, Hynan LS. Acetaminophen-induced acute liver failure: Results of a United States multicenter, prospective study. *HEPATOLOGY*;2005; 42: 1367– 1372.

Lip GYH, Vale JA.(1996). Does acetaminophen damage the heart? Clin Toxicol. *1996. 34*: 145–147.

Mann JM, Peirre-Louis M, Kragel PJ, Kragel AH, Roberts WC. Cardiac consequences of massive acetaminophen overdose. *Am J Cardiol*; 1989; 63: 1018– 1021.

Mendall MA, Patel P, Asante M, Relation of serum cytokine concentrations to cardiovascular risk factors and coronary heart disease. Heart. 1997; 78:273–277.10.1136/hrt.78.3.273

Merrill GF, Rork TH, Spiler NM, and Golfetti R. Acetaminophen and myocardial infarction in dogs. Am J Physiol Heart Circ Physiol *287*:2004; H1913– H1920.

Møller Soren, Bernardi Mauro. Interactions of the heart and the liver .*European Heart Journal*, 2013;Volume 34, Issue 36, 2804–2811, https://doi.org/10.1093/eurheartj/eht246

Morazzoni P., Bombardelli E. Silybum marianum (Cardus marianus). Fitoterapia. 1995. 66**:** 6–42.

Ooi DS, Isotalo PA, Veinot JP. Correlation of antemortem serum creatine kinase, creatine kinase-MB, troponin I, and troponin T with cardiac pathology. *Clin Chem*; 2000; 46: 338– 344

Ridker PM, Rifai N, Stampfer MJ. Plasma concentration of Interleukin-6 and the risk of future myocardial infarction among apparently healthy men. Circulation.2000; 101:1767–1772. 10.1161/01.CIR.101.15.1767

Ruzek MC, Miller AH, Opal SM, Pearce BD, Biron CA. . Characterization of early cytokine responses and an interleukin-6-dependent pathway of endogenous glucocorticoid induction during murine cytomegalovirus infection. J Exp Med.; 1997; 185:1185–1192. doi: 10.1084/jem.185.7.1185.

Siedel J, Hagele EO, Ziegenhorn J,Wahlefield AW. Reagent for the enzymatic determination of serum cholesterol with improved lipolytic efficiency. Clin Chem. 1983; 20:1075–1080.

Sullivan DR, Kruijswijk Z,West CE, KohlmeierM, Katan MB. Determination of serum triglycerides by an accurate enzymatic method not affected by free glycerol. Clin Chem.1985; 31:1227–1228.

Tavakkol Afshari J, Ghomian,N Shameli A, Shakeri MT, Fahmidehkar, MA, Mahajer E, R Khoshnavaz, R & Emadzadeh M .Determination of Interleukin-6 and Tumor Necrosis Factor-alpha concentrations in Iranian-Khorasanian patients with preeclampsia. *BMC Pregnancy and Childbirth* volume 5, Article number: 14 (2005)

Tsugiyasu Kanda and Takashi Takahashi..Interleukin-6 and Cardiovascular Diseases.2004 45(2):183-93. doi: 10.1536/jhj.45.183.

Tsutamoto T, Hisanaga T, Wada A. Interleukin-6 spillover in the peripheral circulation increases with the severity of heart failure, and the high plasma level of interleukin-6 is an important prognostic predictor in patients with congestive heart failure. J Am Coll Cardiol. 1998; *31*:391–398.

Wiese S., Hove J.D., Moller S.*(2017)* Cardiac imaging in patients with chronic liver disease. *Clin Physiol Funct Imaging 37:347–356.*

Wilcox L. J., Borradaile N. M., Kurowska E. M., Telford D. E., Huff M. W. Naringenin, a citrus flavonoid, markedly decreases apoB secretion in HepG2 cells and inhibits acyl CoA: cholesterol acyltransferase. Circulation.1998; 98**:** I-537 (Abstract).

Wu T, Yuan A, Chen CY, Chen WJ, Luh KT, Kuo SC. Cardiac troponin I levels are a risk factor for mortality and multiple organ failure in noncardiac critically ill patients and have an additive effect to the APACHE II score in outcome prediction. *Shock*. 2004; 22: 95–101.

Xing Z, Braciak T, Jordana M, Croitoru K, Graham FL, Gauldie J. Adenovirus-mediated cytokine gene transfer at tissue sites: overexpression of IL-6 induces lymphocytic hyperplasia in the lung. J Immunol.;1994; 153:4059–4069.

Zakai NA, Katz R, Jenny NS. Inflammation and hemostasis biomarkers and cardiovascular risk in the elderly: the Cardiovascular Health Study. J Thromb Haemost.;5:1128–1135.10.1111/jth.2007.5.issue 6