# The Effectiveness of Pentoxifylline in NAFLD: A Meta-Analysis

John Mark K. Torres, M.D.*; Carlos Rolando Cuaño, M.D.**; and Janus P. Ong, M.D.***

## Abstract

**Introduction:** Rising prevalence of non-alcoholic fatty liver disease (NAFLD) suggests its correlation with liver failure worldwide. To date, there is no proven pharmacologic therapy for NAFLD. Pentoxifylline (PTX) with its anti-tumor necrosis factor properties has shown improvement of histological parameters, reductions in transaminase levels and serum cytokines among patients with NAFLD. The main objective is to determine the effectiveness of PTX in the reduction of progression of NAFLD in terms of reducing levels of aspartate transaminase (AST) and alanine transaminase (ALT), improving liver histology parameters and in decreasing TNF-α, IL-6 and IL-8.

**Methods:** A comprehensive literature search showed seven randomized controlled trials (N=222) comparing PTX (1,200mg/day) with placebo. Two reviewers independently selected studies, assessed quality, and extracted and pooled outcomes including AST levels, ALT levels, serum cytokines and liver histology. All selected studies were found to be of low risk of bias based on Cochrane risk of bias assessment tool for randomized trials. Statistical analysis and forrest plot generation were done using the Review Manager Software 5.3.

**Results:** Pooled results showed that PTX significantly reduced the ALT (WMD= -20.08; 95% CI: -40.20, 0.05; *p*=0.05) and AST (WMD= -11.38; 95% CI: -20.47, -2.29; *p*=0.01) in NAFLD patients. PTX significantly improved lobular inflammation (WMD= -0.45; 95% CI: -0.89, -0.01; *p*=0.04), fibrosis (WMD= -0.39; 95% CI: 0.83, 0.05; *p*=0.08) and NAS score (WMD= -0.52; 95% CI: -1.06, 0.0; *p*=0.051). Among serum cytokines, greater reduction was demonstrated in TNF-α (WMD= -20.20; 95% CI: -50.46, 10.41; *p*=0.20).

**Conclusion:** Pentoxifylline (PTX) decreases the amino-transferase activities, improves the liver histology and TNF-α of NAFLD patients. Demonstrating effects on serum TNF-α which plays a key role in progression to hepatic steatosis, it may be used as an adjunct to diet and lifestyle modifications in the treatment of NAFLD.

**Keywords:** meta-analysis, nonalcoholic fatty liver disease, nonalcoholic steatohepatitis, pentoxifylline

## Introduction

The increasing prevalence of chronic liver disease parallels the burden of non-alcoholic fatty liver disease (NAFLD) worldwide estimated to be at 24%. NAFLD, and its histological phenotype non-alcoholic steatohepatitis (NASH), through fibrogenic cascade of mechanisms can potentially progress to advanced liver disease, cirrhosis and hepatocellular carcinoma (HCC).[1] Cirrhosis and HCC account for the 13th most common cause of mortality worldwide and has become a major public health problem. Global cirrhosis deaths have increased from 1.54% of all deaths in 1980 to 1.95% in 2010, causing more than one million deaths each year.[2] Chronic hepatitis B and C, alcoholic liver disease, and NAFLD are among the most common primary etiologies of cirrhosis. In recent years, with the rising incidence of obesity and insulin resistance, NAFLD has become one of the leading causes of cirrhosis in some countries. And by 2020, NAFLD cirrhosis will become the leading indication for liver transplantation exceeding those predicted from hepatitis B and C-related cirrhosis.[3]

The prevalence at least in the Asia-Pacific region has remarkably risen to about 30%. The worldwide prevalence of NAFLD is constantly increasing (15% in 2005 to 25% in 2010) and similarly the rate of NASH in the same timeframe has almost doubled (59.1% versus 33%).[4] In developed countries, it occurs in 30% of the population, 85-95% of obese and overweight adults, 40-50% of patients with diabetes mellitus and in 90% of patients who have dyslipidemia.[1]

*Department of Medicine, University of the Philippines – Philippine General Hospital, Manila, Philippines
** Section of Gastroenterology, Department of Medicine, University of the Philippines – Philippine General Hospital, Manila, Philippines

Corresponding author: John Mark K. Torres, M.D., University of the Philippines – Philippine General Hospital, Manila, Philippines
Email: torres_johnmark@yahoo.com

In a local study,[5] the prevalence of NAFLD in Philippine General Hospital, was 12.2%. NAFLD was diagnosed in 134 patients seen at the gastroenterology clinic. Diagnosis was based on clinical, ultrasonographic and histologic findings. Fatty liver was diagnosed by ultrasonography using an abdominal probe at 2.0-5.0 MHz. Record review was done to account for patient's history, demographics, comorbidities, physical examination findings, and laboratory findings. Results revealed that Filipino patients with NAFLD appear to be of younger age compared to previous studies. Female sex, obesity, overweight, elevated alanine transaminase (ALT) and aspartate transaminase (AST), and diabetes were clinical features of Filipino patients with NAFLD that are consistent with worldwide data.

In NAFLD, lipids accumulate in the liver, which alters the normal hepatic histologic architecture. These changes cause impairment in hepatic glucose and lipid metabolism. By this mechanism, NAFLD increases the risk of diabetes and cardiovascular disease respectively. The prevalence and severity of complications are proportional to the histologic changes seen. The rising incidence and prevalence of NAFLD suggest that this disease will soon be a primary cause of liver disease in the population. Measures to prevent progression must be sought to decrease the risk for cardiovascular and other NAFLD related complications.[6]

The American Gastroenterological Association, defined NAFLD by; (a) presence of hepatic fat accumulation, as evidenced by imaging or histology, (b) no associated comorbidities to account for steatosis such as excessive alcoholic consumption about >60-80 g/day x 10yrs, women 20-40g/day, certain steatotic medication, or hereditary disease.[7] A part of spectrum of liver histologic changes, from simple hepatic steatosis to cirrhosis to hepatocellular carcinoma or primary liver carcinoma, NAFLD studies suggest that the degree of histologic changes correlate with the natural history of the disease. Among individuals with NAFLD, particularly with NASH histology who are untreated, 15%-25% will progress to cirrhosis and its complication in 10-20 years.[8]

The diagnosis of NAFLD is based on clinical and histologic criteria. Most patients are evaluated because of elevated serum aminotransferase levels and/or hepatomegaly on ultrasonography. While liver biopsy is the standard means of diagnosis and the only test that can reliably differentiate simple steatosis from NASH, some noninvasive methods for assessing fibrosis are emerging. Serum aminotransferases, imaging evidence of fatty liver, and histopathologic findings of steatosis are essential in its diagnosis. The changes in these serve as markers of liver injury, and progression to advanced disease like cirrhosis. In the past years, investigations on underlying pathophysiologic mechanisms have led to discovery of cytokines' role in NAFLD as well.

Serum aminotransferases activity is commonly used as an indicator for liver disease or injury. In six to eight percent of an American population studied, elevation of ALT could not be explained by comorbidities, excessive alcoholic consumption (greater than 60 or 80 g/day for 10yrs, women 20-40g/day), steatotic medication, or hereditary disease. Such elevated ALT was associated with increased BMI. These biochemical changes are presumed to be NAFLD. This condition developed fibrosis and was more prevalent in older, overweight or obese patients, with type 2 DM and those with hepatitis C infection.[9]

Liver aminotransferases are markers of liver injury and are useful markers for NAFLD and progression. Patients with NAFLD have chronically elevated aminotransferases that usually are found incidentally and trigger further evaluation.[10] A population-based study showed that high normal ALT levels were associated with an increased 10-year risk of coronary heart disease independent of cardiovascular disease (CVD) risk factors indicating that ALT may be a useful marker in assessment of CVD risk in patients who may have NAFLD.[11] The ALT levels are higher than the AST levels in most instances. A reversal of the ALT/AST ratio to more than one has been reported to predict the presence of more advanced fibrosis. The World Gastroenterology Organization global guidelines suggest that morbid obesity, diabetes and ALT/AST more than 27 IU/L are independent predictors for progression to NASH and mortality in NAFLD.[12] Reduction in ALT levels correlate with improvement in hepatic lipid accumulation and inflammation and thus better outcome and delays progression to cirrhosis or fibrosis. Measures to control these conditions may reverse or delay progression of NAFLD.

The pathogenesis of NAFLD is a multifactorial interplay of chronic inflammatory mechanisms with increased oxidative stress, cytokine production, direct lipotoxicity and autoimmunity, all leading to development of NASH. Patients with NASH have significantly higher levels of serum TNF-α and IL-6 than seen in patients with simple steatosis. These cytokines including proinflammatory cytokines and adiponectin, an anti-inflammatory cytokine, are believed to play an important role in hepatocellular damage, inflammation and fibrogenesis in NASH.[13]

Insulin resistance, as one of the explored pathogenesis in NAFLD, is almost a universal finding in NASH.[14] from five studies have shown that TNF-α, plays a key role in its development by interacting with tyrosine kinase receptors of insulin.[15] Recent studies have demonstrated raised concentrations of serum TNF-α as well as increased TNF-α mRNA in hepatic tissue in patients with NASH.[16,17] In mice, fatty liver disease is improved by inhibition of hepatic TNF-α production[18] and by infusion of anti-TNF-α neutralizing antibody.[19]

With insulin resistance, there is a concomitant increase in free fatty acids. The concentration of the latter leads to lysosomal destabilization and stimulation of TNF-α, as well as sustained up-regulation of peroxisomal proliferator–activated receptor (PPAR)-α. These events further promote

fatty acid oxidation and disposal thereby increasing oxidative stress through the production of dicarboxylic acid derivatives and free radicals.[20]

Proven as alternative to steroids among patients with alcoholic hepatitis, pentoxifylline (PTX) has been explored for potential benefit in NAFLD with its ability to reduce free radical oxidative stress, possible anti-fibrotic properties and TNF-α levels. [21] PTX, a methylxanthine derivative, is known to increase red blood cell flexibility, reduce blood viscosity, and decrease platelet aggregation.[11,12] PTX inhibits a number of pro-inflammatory cytokines including TNF-α,[13-15] supporting the potential role in improving NAFLD. Hepatoprotective effects of PTX include upregulation of hepatic glutathione levels in mice with steatohepatitis induced by a methionine choline deficient diet[18] and reduction of the production of oxygen radicals induced by prolonged ischemia time in rat livers.[19] In vitro studies in hepatic stellate cells have shown the potential antifibrogenic effects of PTX as well. In some trials, PTX, has also demonstrated relative improvement in lobular inflammation and ballooning degeneration and steatosis in comparison to baseline.[22]

Currently there is no approved pharmacologic therapy for prevention of NAFLD in the market. Several agents like thiazolidinedione metformin, statins, fibric acid derivatives, ursodeoxycholic acid, and vitamin E have been used for NAFLD but failed to show effectivity. The only known effective management is lifestyle modification, which includes diet and exercise and the reversal of conditions associated with NAFLD like diabetes and obesity. Since lifestyle modification as first line therapy is hard to sustain and implement, a need for a single pharmacotherapy in conjunction with lifestyle modification is needed. An agent that is safe, acceptable, accessible and available is still an area of research for the successful treatment and prevention of progression of NAFLD. PTX is commercially available and can be an alternative treatment for Filipinos with NAFLD. It has been subject of research for its protective effect on the liver. A meta-analysis has been published regarding this topic in 2014, but since then three additional randomized controlled trials have been performed. Though previous meta-analysis of five randomized, double-blind controlled studies has shown reduction in the aminotransferase activities and improvement in the histological parameters in NAFLD patients,[23] the pooled analysis on effects of PTX not only on serum aminotransferases but more importantly on histologic improvement and on the serum cytokine was not yet explored.

The primary aim of this study was to compare the effectiveness of PTX over one year with placebo in patients with NASH. The primary outcome measures including improvement on aminotransferases, histologic changes and serum cytokine levels.

The general objective is to determine the effectiveness of PTX in the reduction of progression of NAFLD. More specifically;

1. To measure reduction of aminotransferase (ALT and AST) levels from baseline
2. To describe improvement of liver histology based on NAFLD activity score (NAS) and histology parameters like ballooning, fibrosis, lobular degeneration and steatosis
3. To measure reduction of serum cytokine levels of TNF-α, IL-6 and IL-8 from baseline

## Methods

The study was conducted in reference to the preferred reporting items for systematic reviews and meta-analyses (PRISMA). All studies were identified and aggregated from a pool of available data.

Studies were eligible for inclusion if they met the following criteria; 1) randomized controlled trial as study design, 2) population of patients with age of 18 years and older, diagnosed with NAFLD on the basis of liver biopsy and/or suggestive ultrasound findings, 3) population of patients were randomly divided into case (PTX 1,200 mg/day for about three to 12 months duration) and control groups, 4) with composite outcomes of changes in liver enzymes, liver histology from baseline and serum cytokine levels determined pre- and post-treatment. Studies were excluded if the intervention included concomitant use of other drugs in addition to PTX. There was no restriction on the date of publication. Two independent reviewers identified the trials for inclusion by applying the selection criteria.

A systematic literature search without language restriction was conducted by the researchers using the PubMed, EMBASE, NEJM, Cochrane, Chinese Biomedicine Database, Science Citation Index Expanded and Google scholar to identify randomized, double-blind, placebo-controlled clinical trials about the effects of PTX on the biochemical and/or histological parameters of NAFLD patients. Unpublished articles were also sought by searching for ongoing trials or recently finished trials that have not yet been submitted to journals through ClinicalTrials.gov. For studies without complete text published online, correspondence with the author was made in order to obtain a copy of the complete text to enable a comprehensive analysis of the study. The literature search was updated to December 1, 2018.

The researchers search strategy was as follows: (((“pentoxifylline”(MeSH Terms) OR “pentoxifylline”(All Fields)) OR “pentoxifylline”(MeSH Terms)) AND (((“non-alcoholic fatty liver disease”(MeSH Terms) OR (“non-alcoholic”(All Fields) AND “fatty”(All Fields) AND “liver”(All Fields) AND “disease”(All Fields)) OR “non-alcoholic fatty liver disease”(All Fields) OR (“nonalcoholic”(All Fields) AND “fatty”(All Fields) AND “liver”(All Fields) AND “disease”(All Fields)) OR “nonalcoholic fatty liver disease”(All Fields)) OR (“non-alcoholic fatty liver disease”(MeSH Terms) OR (“non-alcoholic”(All Fields) AND

"fatty"(All Fields) AND "liver"(All Fields) AND "disease"(All Fields)) OR "non-alcoholic fatty liver disease"(All Fields) OR "nafld"(All Fields))) OR (("non-alcoholic fatty liver disease"(MeSH Terms) OR ("non-alcoholic"(All Fields) AND "fatty"(All Fields) AND "liver"(All Fields) AND "disease"(All Fields)) OR "non-alcoholic fatty liver disease"(All Fields) OR ("nonalcoholic"(All Fields) AND "fatty"(All Fields) AND "liver"(All Fields) AND "disease"(All Fields)) OR "nonalcoholic fatty liver disease"(All Fields)) OR "non-alcoholic fatty liver disease"(MeSH Terms)))) AND ((((("non-alcoholic fatty liver disease"(MeSH Terms) OR ("non-alcoholic"(All Fields) AND "fatty"(All Fields) AND "liver"(All Fields) AND "disease"(All Fields)) OR "non-alcoholic fatty liver disease"(All Fields) OR ("nonalcoholic"(All Fields) AND "fatty"(All Fields) AND "liver"(All Fields) AND "disease"(All Fields)) OR "nonalcoholic fatty liver disease"(All Fields)) OR ("non-alcoholic fatty liver disease"(MeSH Terms) OR ("non-alcoholic"(All Fields) AND "fatty"(All Fields) AND "liver"(All Fields) AND "disease"(All Fields)) OR "non-alcoholic fatty liver disease"(All Fields) OR "nafld"(All Fields))) OR (("non-alcoholic fatty liver disease"(MeSH Terms) OR ("non-alcoholic"(All Fields) AND "fatty"(All Fields) AND "liver"(All Fields) AND "disease"(All Fields)) OR "non-alcoholic fatty liver disease"(All Fields) OR ("nonalcoholic"(All Fields) AND "steatohepatitis"(All Fields)) OR "nonalcoholic steatohepatitis"(All Fields)) OR NASH(All Fields))) AND ("pentoxifylline"(MeSH Terms) OR "pentoxifylline"(All Fields)))

Manually scanned bibliographies revealed two more articles found only through Google Scholar and was published in a medical subspecialty society publication. Of the records screened, only five were found to be eligible for the meta-analysis. The four rejected articles did not meet the criteria for inclusion of this meta-analysis.

Two independent reviewers extracted the data of interests using standardized data collection form (Cochrane data collection form). The internal validity of each trial was independently assessed using the validity assessment tool after completing the review of the articles. The reviewers were blinded to the authors and institution of the studies undergoing review. When the two independent assessors had a disagreement on assessing the validity of the study, a third independent will perform the analysis for the article of interest in order to settle the disagreement.

Treatment effects were based on reduction of AST and ALT levels, serum cytokine levels and histologic changes from baseline to end of the study after the intervention compared to placebo.

## Results

A total of 64 non-duplicate citations were screened through electronic searches: PubMed, Cochrane, and Google Scholar. No ongoing trials were identified in both HERDIN at the time of the writing of this meta-analysis. There was a total of 23 irrelevant or ineligible studies were excluded by reading the titles and abstracts. The full copy of the remaining 41 studies were then retrieved and assessed. Thirty-four of the studies were excluded since they were not controlled clinical trials. A total of seven articles met the inclusion criteria set by this meta-analysis as shown in Figure 1.

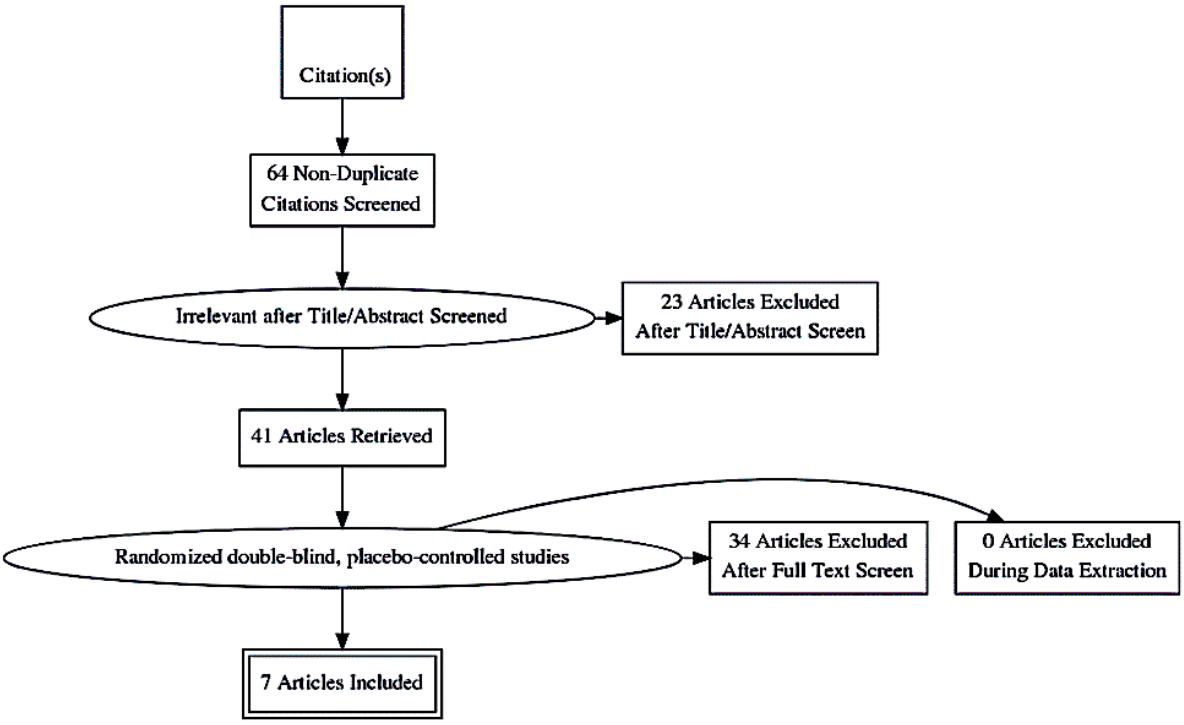


**Figure 1.** PRISMA diagram of literature search.

Details on the seven studies which fulfilled the inclusion criteria are summarized in Table I. All studies were randomized controlled studies and patients were recruited from the different medical centers. Van Wagner and Zein's studies were both done in USA. Rest of the studies were done in Asia (Thailand, Singapore, Iran, Bangladesh) and Egypt.

The total number of participants in all the studies was 222 (98 from the intervention and 124 from the control groups), with majority coming from the Zein's study. Sample sizes ranged from 10 (Lee 2008 and Amin 2009) to 26 (Zein 2011) from the intervention and nine (Lee 2008 ) to 29 (Zein 2011) participants from the control group. The population of all of the studies had a mean age of greater than 40. Male to female ratio was at 1.52:1 (122:80). BMI of the participants was around 25 to 32 with majority of the subjects were overweight by WHO classification. As high as 32% of study participants have concomitant diabetes (Alam 2017) Across all studies, there was no significant difference in the baseline characteristics in the intervention and control arms. (Table I)

All of the studies except Amin 2009 utilized 1,200mg per day dosing of PTX in the treatment arm. Three of the studies (Van Wagner, Zein and Alam) run for 12 months while three more studies had a total of six months duration. Only three studies (Buranaati, Alam and Lee) had defined additional dietary advice among their participants. Rest either did not offered dietary advice or did not specify in the treatment arm.(Table I)

The mean changes of the biochemical and metabolic parameters from the baseline, and the decrease in the histological scores, were treated as continuous variables. The pooled weighted mean difference (WMD) with 95% confidence interval (CI) was calculated to compare the effects of PTX and placebo.

All studies included aminotransferases measured at baseline and at sixth or 12th month depending on study duration as shown in Figures 3 and 4. For the serum cytokines, four studies (Van Wagner, Lee, Baniasadi and Zein) measured TNF-α as shown in Figure 6.1. Only two studies however measured interleukins-6 and interleukins-8 as shown in Figure 6.2 and 6.3.

Histological improvement with the treatment arm was also measured in terms of five parameters (NAS, steatosis,

**Table I. Characteristics of studies included in the meta-analysis**

| References and Year | Country | Duration (months) | Dose (mg/day) | Number (Dropout) | | Outcomes | Dietary Advice |
|---|---|---|---|---|---|---|---|
| | | | | PTX | Placebo | | |
| Buranawati 2007 | Thailand | 6 | 1200 | 16 (0) | 16 (0) | ALT, AST, glucose, BMI, insulin, HOMA, adiponectin, TNF-α | Yes |
| Lee 2008 | Singapore | 3 | 1200 | 11 (0) | 9 (0) | ALT, AST, TC, LDL, HDL, TG, TNF-α, IL-6, HA | Yes |
| Amin 2009 | Egypt | 6 | 400 | 10 (0) | 10 (0) | ALT, AST, glucose, BMI, TG, TC, HA | NA |
| Van Wagner 2011 | USA | 12 | 1200 | 21 (2) | 9 (2) | Biochemical, histological, and genetic endpoints | No |
| Zein 2011 | USA | 12 | 1200 | 26 (3) | 29 (3) | Histology, HOMA, adiponectin, TNF-α | NA |
| Baniasadi 2015 | Iran | 6 | 1200 | 15(0) | 15 (0) | ALT, AST, glucose, BMI, LDL, HDL, TG TC, Cytokines (IL-6, IL-8, and TNF-α) | No |
| Alam 2017 | Bangladesh | 12 | 1200 | 25 (5) | 10 (0) | ALT, AST, glucose, BMI, LDL, HDL, TG TC, HOMA, NAS | Yes |

*Abbreviations: ALT-alanine aminotransferase, AST- aspartate aminotransferase, BMI-body mass index, HOMA-homeostasis model assessment, TC-total cholesterol, TG-triglyceride, HDL-high density lipoprotein, LDL-low density lipoprotein, HA-hyaluronic acid, TNF-α-tumor necrosis factor alpha, IL-interleukin.*

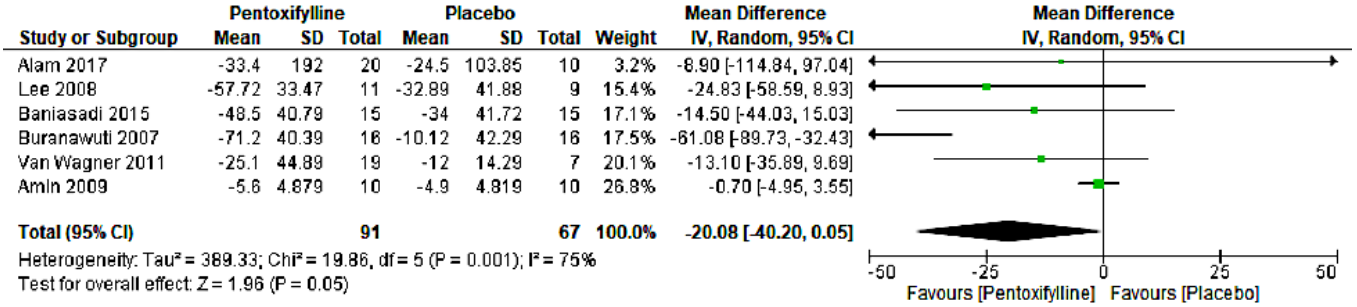


**Figure 3.** Forrest plot on the effect of PTX vs placebo in ALT reduction

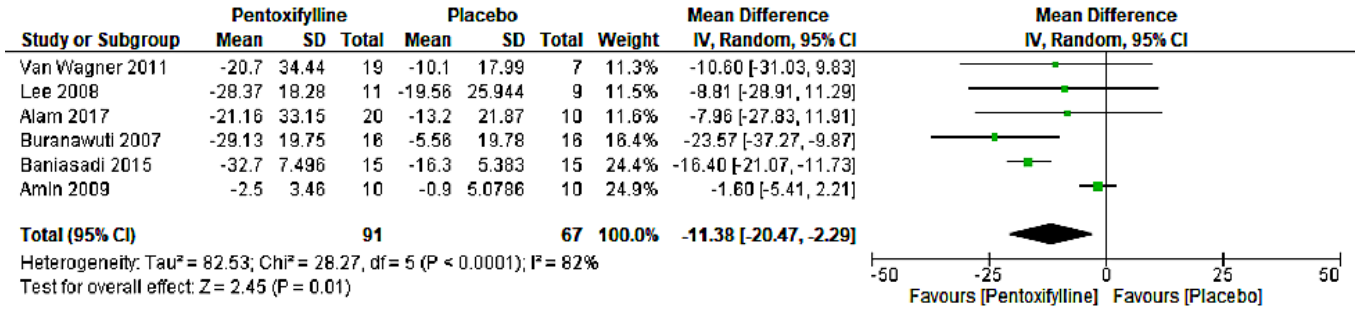


**Figure 4.** Forrest plot on the effect of PTX vs placebo in AST reduction

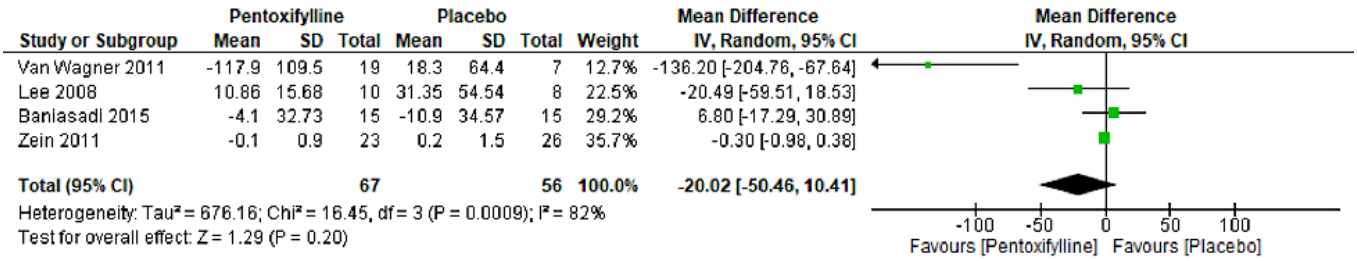


**Figure 6.1** TNF Alpha - Forrest plots on effects of PTX vs placebo in serum cytokine reduction

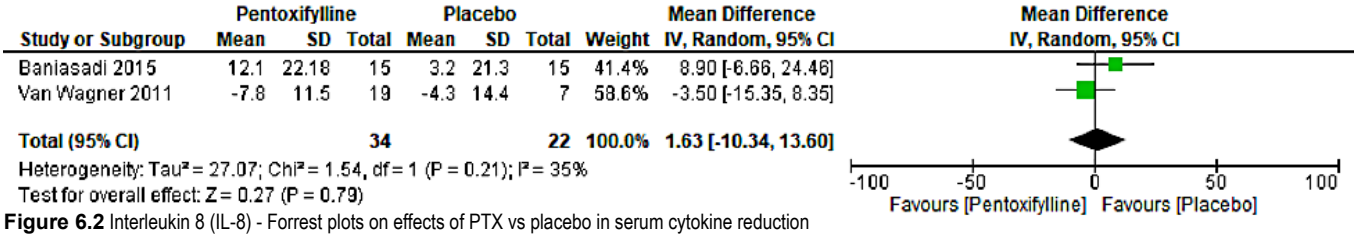


**Figure 6.2** Interleukin 8 (IL-8) - Forrest plots on effects of PTX vs placebo in serum cytokine reduction

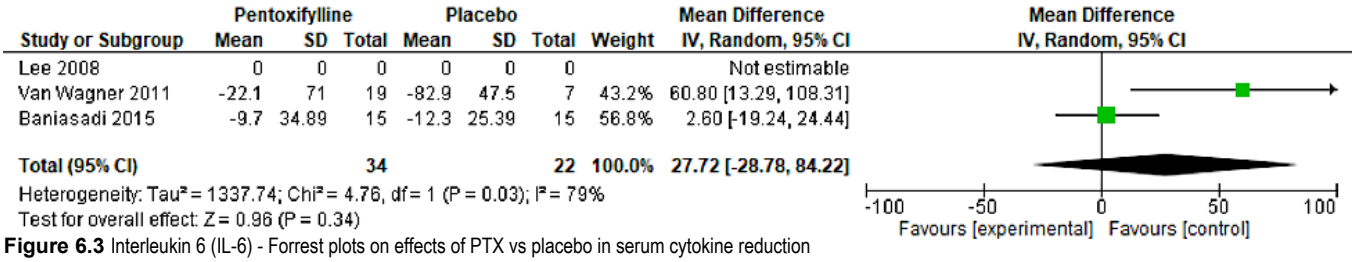


**Figure 6.3** Interleukin 6 (IL-6) - Forrest plots on effects of PTX vs placebo in serum cytokine reduction

lobular inflammation, ballooning, and fibrosis) as shown in Figure 4. This was compared to that of the control arm.

Other biochemical parameters including triglyceride (TG), total cholesterol (TC), high- and low-density lipoproteins, glucose etc were also measured but was not the focus of this meta-analysis.

The seven included trials were randomized placebo-controlled studies. The study by Alam et al. was an open label trial while the rest were double blind. All studies used PTX as an intervention, with six of the seven used 1,200mg/day dosing while one study used 400mg/day. The total number of patients was 222. The mean age ranged from 29 to 69 with males comprising 55-59%. The mean BMI in all studies was obese based on their ethnic specific BMI classifications. (Table II)

The studies included were deemed of low risk of bias. However, the study by Buranawuti (2007) was open label thus the participants, and outcome assessors were not blinded. (Figure 2)

PTX therapy caused a significant decrease in ALT and AST activities compared to placebo when the studies were combined using the random effects model. There was significant heterogeneity in the studies included. Seven well-designed studies retrieved with pooled results showed that PTX significantly reduced the serum ALT activity (WMD= –20.08; 95% CI: – 40.20, 0.05) and AST activity (WMD= -11.38; 95% CI: -20.47, -2.29) in NAFLD patients compared with placebo. (Figure 3 and Figure 4)

PTX caused significant improvement in steatosis, ballooning, lobular inflammation, and fibrosis by combined mean difference at 95% CI. PTX significantly improved steatosis (WMD= –0.33; 95% CI: -0.87,0.21), lobular inflammation (WMD= –0.45; 95% CI: –0.89, –0.01), and fibrosis (WMD= –0.39; 95% CI: –0.83, 0.05). The results showed heterogeneity only in the fibrosis parameter. (Figure 5.1-5.5)

PTX-treated patients showed decreased TNF-α, interleukin 8 and 6 but was not statistically significant. None of the above three cytokines was significantly affected by PTX when compared with placebo. The data for the effect of PTX on cytokines was heterogenous. (FIgures 6.1-6.3)

**Table II. Baseline characteristics of NAFLD patients in included studies.**

| | Age (years) | | Sex (male/female) | | BMI (kg/m²) | | Diabetics (%) | | Race | |
|---|---|---|---|---|---|---|---|---|---|---|
| References | PTX | Placebo | PTX | Placebo | PTX | Placebo | PTX | Placebo | PTX | Placebo |
| Alam et al. | 41.52±9.9 | 38.8±6.2 | 7/18 | 5/5 | 27.9±3.3 | 24.3±1.5 | 32 | 20 | NA | NA |
| Amin et al. | 50.1±13.4 | 50.1±7.7 | NA | NA | 32.0±2.7 | 31.0±1.4 | NA | NA | NA | NA |
| Baniasadi et al. | 35.7±6.6 | 37.4±12.5 | 12/3 | 12/3 | 26.9±3.1 | 26.6±7.8 | NA | NA | NA | NA |
| Buranawati et al. | 48.4±11.4 | 49.5±12.2 | 12/4 | 7/9 | 25.8±2.4 | 27.1±3.8 | 18.7 | 12.5 | NA | NA |
| Lee et al. | 47.0±8.4 | 47.8±14.1 | 7/4 | 6/3 | 26.7±3.4 | 29.9±5.4 | 18.2 | 0 | NA | NA |
| Van Wagner et al. | 48.0±2.0 | 53.0±2.0 | 13/8 | 3/6 | 34.0±0.9 | 35.1±2.6 | 10 | 13 | White: 17/21 | White: 7/9 |
| Zein et al. | 50.5±12.7 | 49.6±9.6 | 18/8 | 20/9 | 32.9±4.6 | 34.0±5.4 | 3.8 | 13.8 | White: 25/26 | White:26/29 |

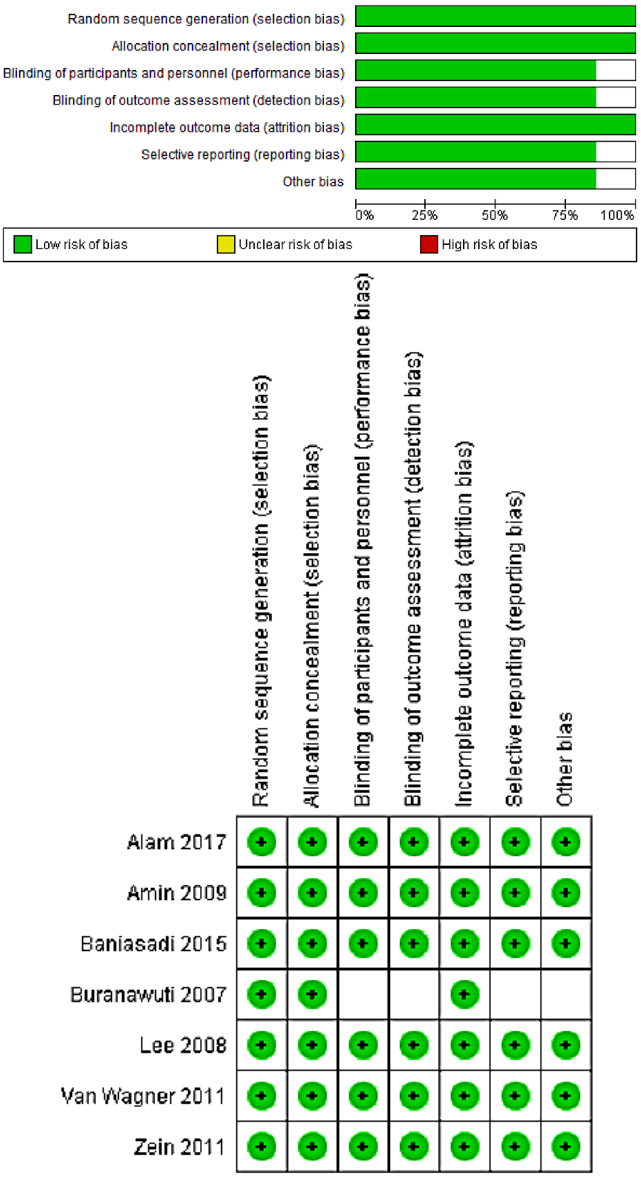


**Figure 2.** Risk of bias summary showing the authors' assessment on each domain and for each included study.

## Discussion

Non-alcoholic fatty liver disease (NAFLD) is a multifactorial disease with metabolic, genetic, environmental and stress-related elements. In the advent of lifestyle changes leaning towards obesity and development of insulin resistance, the prevalence of NAFLD was observed to be increasing.[3] With the identified risk of development of liver cirrhosis and liver failure, efforts to identify primary therapeutic approach has gained wide attention.

This is a meta-analysis on the effect of PTX on reducing transaminase levels and improvement of liver histopathology as measures of liver disease activity among NAFLD patients. ALT, GGT and AST are markers of liver injury and may be useful surrogate measures of NAFLD. ALT is in the hepatocellular cytosol, whereas AST is mostly within the mitochondria. AST can also be synthesized from other organs and is thus nonspecific for the liver.[18] Hence, based on the results, PTX versus placebo provided a more substantial reduction in ALT level compared to that of AST level.

Non-alcoholic fatty liver disease activity score (NAS) is a tool developed to measure histologic changes in NAFLD during trials and encompasses the spectrum of NAFLD. NAS measures hepatic lipid accumulation (steatosis), degree of lobular inflammation and ballooning scores to determine changes. NAS scores of five to eight is largely considered diagnostic of NASH. NAS correlates closely with the spectrum of disease from NAFLD to NASH and is a good system for comparing pre- and post-treatment histology for use in clinical and research settings. By virtue of its antiTNF-α properties, PTX may have a plausible role as an effective therapy for NASH and improving histologic changes, a process in which oxidant injury has been implicated.

With the rising obesity and diabetes epidemics, the prevalence of NAFLD continues to increase.[2] Despite the urgent need to halt the progression of NAFLD, there are no

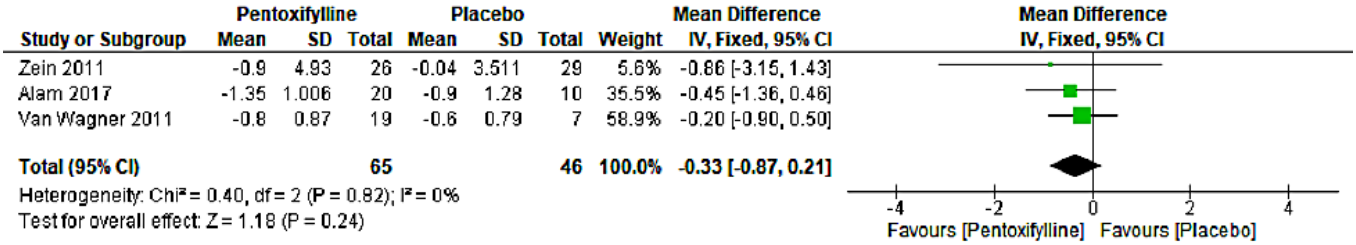


**Figure 5.1** Steatosis - Forrest plots on the effect of PTX vs placebo in improving liver histology

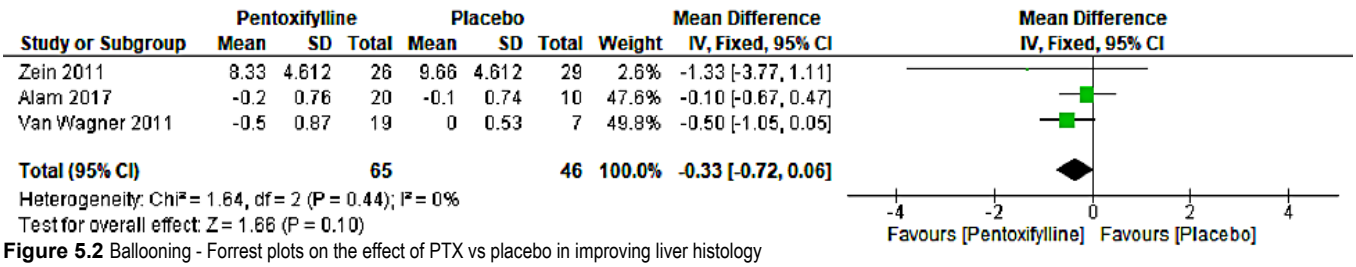


**Figure 5.2** Ballooning - Forrest plots on the effect of PTX vs placebo in improving liver histology

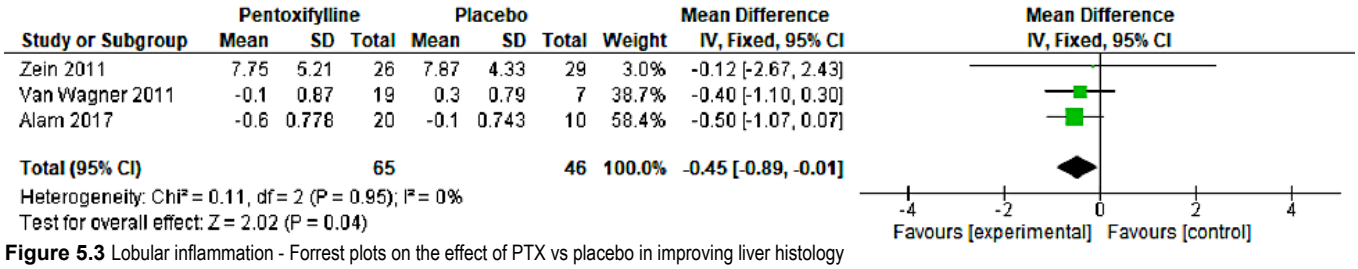


**Figure 5.3** Lobular inflammation - Forrest plots on the effect of PTX vs placebo in improving liver histology

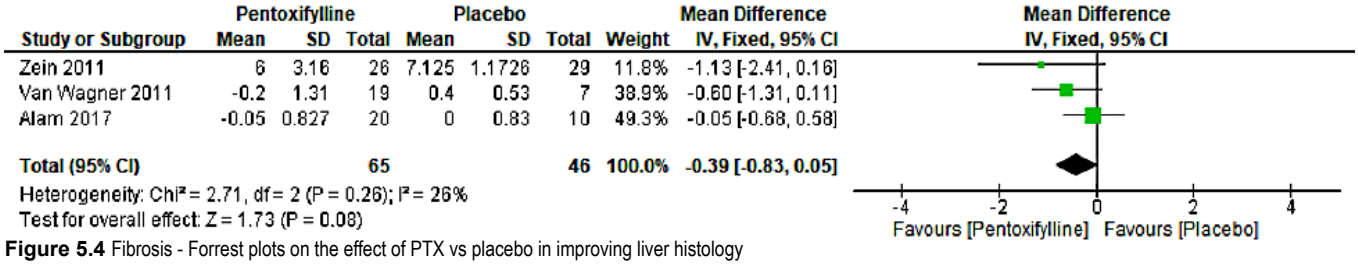


**Figure 5.4** Fibrosis - Forrest plots on the effect of PTX vs placebo in improving liver histology

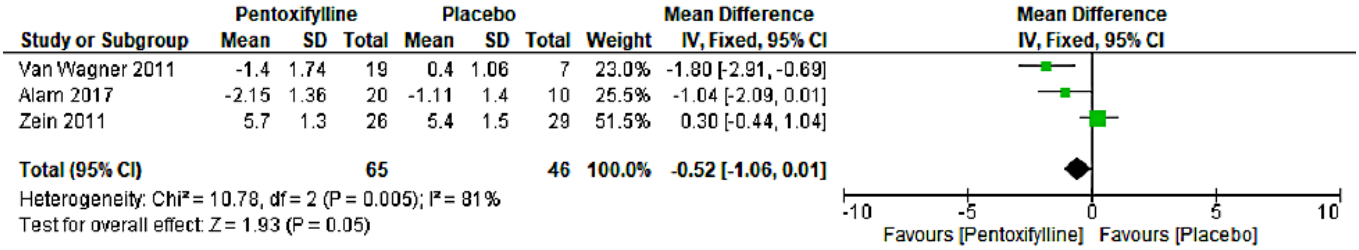


**Figure 5.5** NAS score - Forrest plots on the effect of PTX vs placebo in improving liver histology

pharmacologic interventions that have been proven to do this.

This meta-analysis shows that PTX decreases aminotransferase activities in patients with NAFLD. It also improves their histologic parameters such as steatosis, ballooning, lobular inflammation, and fibrosis. The current study supports the data of the previous meta-analysis and gives our claims more statistical power by adding two high quality trials and enlarging the previous sample size of 157 to 222 and the inclusion of the intervention's effects on one of the pathophysiologic basis in the development of NAFLD, the serum cytokines specifically the TNF-α.[10]

TNF-α has been implicated in causing progression of NAFLD to NASH.[5,6,7,8] PTX is a derivative of methylxanthine with anti TNF-α properties. A study shows that it achieves this by inhibiting nuclear factor-kappa B17.Our meta-analysis is the first to pool data on the effect of PTC on TNF-α and IL 6 and 8. The pooled effects of PTX showed trends towards benefits with the decreasing TNF-α levels from baseline although statistically insignificant. This reduction in TNF-α could explain the effect of PTX in improving biochemical markers of liver activity and injury in terms of aminotransferases and improving liver histology specifically steatosis, ballooning, lobular inflammation and fibrosis.

The data on AST, ALT, TNF-α, and IL-6 had significant heterogeneity. The wide age range, different durations and doses of PTX can explain this. Despite this, the study used high quality randomized controlled trials with low risk of bias, the best evidence available to support the use of PTX on NAFLD.

## Conclusion

This meta-analysis confirmed that PTX could be used to reduce disease progression in NAFLD. There is a significant reduction in both ALT and AST levels; enzyme highly specific to the liver treated with PTX. The study revealed that it can also reduce rates of fibrosis, lobular inflammation and total NAFLD activity score as well as reduction of TNF-α levels.

This meta-analysis generated the following outcomes: (1) there is a statistically significant effect of PTX on ALT and AST reductions, (2) there is statistically significant improvement on liver histology parameters post treatment, and (3) there is substantial reduction in serum cytokines particularly with TNF-α. Overall suggestive of beneficial effects in preventing disease progression in NAFLD.

### Implication to practice and research

At the moment, there is no strong supporting evidence for any effective therapeutic agents for reducing inflammation and fibrosis as core mechanism in the progression of NAFLD. PTX in this meta-analysis proved to significantly reduce ALT and AST levels (as associated with histological NAFLD and serum cytokines) indicating reduction in the progression of liver disease. It may also be used then at least as a

complementary therapy to other proposed treatment modalities. PTX can be used as an adjunct to lifestyle and dietary modifications in patients with NAFLD.

Future endeavors in the field of research include investigation of safety profile and possible adverse events. Addition of well-designed randomized, placebo-controlled studies in the future with a larger sample size might have added benefit in establishing effects of PTX to the defined outcomes.

### Declaration of conflict of interest

The authors certify that there are no existing relevant conflicts of interests to declare.

The authors certify that there were no funding sources supporting this work and output.

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