International Journal of Basic & Clinical Pharmacology
Ngetich DK et al. Int J Basic Clin Pharmacol. 2025 Mar;14(2):140-145
http://www.ijbcp.com
pISSN 2319-2003 | eISSN 2279-0780

DOI: https://dx.doi.org/10.18203/2319-2003.ijbcp20250474

Original Research Article

Liver biomarkers of silymarin milk thistle on paracetamol induced liver toxicity in adult albino rats (*Rattus norvegicus*)

Davis Kiprono Ngetich[1]*, Scolastica Korir[2]

[1]Department of Human Anatomy, School of Medicine, Maseno University, Maseno-Kisumu, Kenya
[2]Department of Medical Microbiology, School of Medicine, Maseno University, Maseno-Kisumu, Kenya

Received: 19 September 2024
Revised: 25 October 2024
Accepted: 11 November 2024

***Correspondence:**
Dr. Davis Kiprono Ngetich,
Email: davis.ngetich@yahoo.com

ABSTRACT

Background: Paracetamol is the most common analgesic that is readily available with or without prescription over the counter and its toxicity is due to misuse or overdose associated with liver damage. Silymarin milk thistle is an edible herb believed to have antioxidant, scavenging and regulation of glutathione contents within the cell. Aim of the study was to determine the effects of different doses of silymarin milk thistle on liver biomarkers of paracetamol induced liver toxicity among albino rats.
Methods: Twenty-four adult albino rats were randomly grouped into four groups each group consisting of six animals. Paracetamol-only group given high dose paracetamol-750 mg/kbwt and control group received water and food only. The experimental group, were divided into three subgroups with all receiving high dose paracetamol for five days and thereafter given varying doses of silymarin milk thistle. SIL-G1 given 200 mg/kbwt of silymarin milk thistle, SIL-G2 given 400 mg/kbwt of silymarin milk thistle and SIL-G3 was given 600 mg/kbwt of silymarin milk thistle. All the animals were fed with standard rodent foods and water ad libitum. After completion of 24 hours of the last drug administration, they were humanely sacrificed, serum biochemical analysis of liver biomarkers (ALP, ALT and AST) was determined.
Results: High dose of paracetamol induced liver toxicity. Upon administration of high dose of silymarin milk thistle, there was restoration of ALP, AST and ALP of all these biomarkers in relation to the control group.
Conclusions: These findings showed that high dose (600 mg/kbwt) of silymarin milk thistle was found to have restorative effects and normalized liver biomarkers of ALP, AST and ALT.

Keywords: Hepato-restorative, Liver biomarkers, Paracetamol hepato-toxicity, Silymarin milk thistle

INTRODUCTION

Paracetamol (pcm) is the commonly used analgesic and antipyretic around the world readily available for use amongst the population. It is the first line drug of choice for mild-to-moderate acute pain that is the most utilized over the counter drug worldwide. It is regarded drug of choice for people who have sensitivities to non-steroidal anti-inflammatory drugs, the underage and also pregnant and lactating women, geriatrics and patients with peptic ulcers.[1] It has been found that paracetamol is the most common drug that causes liver toxicity since it is metabolized in the liver and its overdose may cause injury to the liver. Paracetamol has been readily found across the world with or without prescription over-the-counter thus steadily increasing the number of paracetamols induced liver intoxication.[2]

Prolonged use of paracetamol or at high doses causes oxidative metabolites N-acetyl-para-benzoquinone mine (NAPQI) to cause the liver cells to be under oxidative stress. This therefore will cause the hepatocellular mitochondria to burst leading to free oxygen radicals and

nitrogen ions to cause necrosis of hepatocellular cells leading to liver damage. PCM has been long time considered nontoxic when it is given in therapeutic doses though it might result in hepatotoxicity when taken singly or repeatedly in high dose or after it's been chronically ingested.[3] Toxicity may also arise when there is poor dietary intake and nutritional status of an individual or in alcohol intake even when administered at a therapeutic dose.[4]

Studies also suggested that there could unintentional acute liver injury due to acetaminophen toxicity for adults with small physique even though administered at lower or recommended doses. This could be due to acetaminophen dose per unit of body weight being higher for individuals with malnutrition, underweight or with a small frame.[5]

Silymarin (SIL), an edible herb has been found to have hepato-protective effects on the liver. It has antioxidant, scavenging and regulation of glutathione contents within the cell thus causing cell membrane stabilization and regulation of permeability into the cell thereby preventing hepatotoxic agents from getting into the hepatocytes.[6,7] These anti-oxidant properties of silymarin have been found to greatly reduce free radicals that are produced secondary to metabolism of toxic substances such as paracetamol and alcohol thereby improving the integrity of the mitochondria and maintaining redox balance thus maintaining liver function. Silymarin also increases hepatic glutathione which will promote the antioxidant defense of the liver.[8,9]

According to Jacobs et al and Vargas-Mendoza et al, silymarin milk thistle was found to have significant improvement in liver histology and liver biochemical markers for patients that had chronic liver disease with it also playing a critical role in liver defense by it increasing liver glutathione, hepatocyte protein synthesis by stimulating RNA polymerase I activity.[8,10]

Silymarin has been postulated to reduce the elevated levels of ALT and AST biochemical serum levels amongst patients who had non-alcoholic fatty liver though there is still inadequate data on it.[11] Similar studies conducted on Non-alcoholic fatty liver disease showed that there was significant reduction in liver enzymes post administration of silymarin milk thistle extracts.[12]

With the increasing level of liver failures arising from toxicities from various agents such as drugs or alcohol non-intervention group restorative remedy should be sought to avoid progression of the disease to help in improvement of quality of life and health in the population.[13] Scientific data on the dosage and administration is of great essence in application of various types of medicinal herbs to counter liver toxicities.

SIL is a medicinal herb that is widely growing in most of the climatic conditions worldwide and has been used in managing liver conditions. The findings that was obtained from the study will be helpful to health workers to administer silymarin milk thistle tablets to patients with drug induced hepatotoxicity hence breaching the gap in countering liver complications and restoration of the liver biomarkers.

METHODS

Study location

The study was conducted between May 2023 to July 2023 at the school of Biomedical Sciences of Maseno University. It's located along the equator in Kisumu County Western part of Kenya. All experimental studies appertaining feeding, weighing and administration of drugs were done at the animal house within the school

Study subjects

This study was conducted on pure bred albino rats of the species of Rattus norvegicus from a pure colony of both sexes sourced from the School of Biomedical Sciences of Maseno University, (Kisumu, Kenya). The albino rats weighing between 240g and 280g. The animals were fed with standard rodent pellets obtained from Unga Feeds Limited Kisumu city and water ad libitum. They were then put in cages and left to acclimatize for 5 days before the commencement of the study. All protocols for humane handling of the animals were strictly adhered to.

Experimental design

A post-test only true experimental study design was used. Animals were randomly selected into two groups of intervention group which did receive any silymarin milk thistle and non-intervention group did not receive any silymarin milk thistle. Sampling technique that was utilized was a simple random sampling method to obtain the 24 animals and assigned to the two major groups of experimental and control group.

Grouping was done into; A: Control group, this group did not receive any drug intervention. B- Paracetamol only group- high dose paracetamol (750 mg/kbwt) for five days. C- Low dose Silymarin group (SIL-G1)- received high dose paracetamol (750 mg/kbwt) for five days thereafter low dose of silymarin milk thistle (200 mg/kbwt) for the remaining days. D- Medium dose Silymarin group (SIL-G2)- received high dose paracetamol (750 mg/kbwt) for five days thereafter low dose of silymarin milk thistle (400 mg/kbwt) for the remaining days. E- High dose Silymarin group (SIL-G3)- received high dose paracetamol (750 mg/kbwt) for five days thereafter low dose of silymarin milk thistle (600 mg/kbwt) for the remaining days.

Sample size determination and sampling technique

A total number of 24 rats were sampled for this experiment. The sampled size was arrived at using Modified Resource equation method" of which there was

no previous research done to determine the standard deviation.[14]

Acquisition of study drugs

The paracetamol tablets (500 mg tablets) were obtained from Litein Central Chemist, Kericho-Kenya while the silymarin milk thistle tablets were obtained from Dynapharm Kenya Limited-Nakuru, Kenya. They were prepared by dissolving in water for injection before use.

Determination of doses for paracetamol and silymarin milk thistle

To induce liver toxicity, paracetamol a high dose paracetamol (750 mg/kbwt) was administered for five days.

Procedure for drug administration

The drugs were administered orally where they were dissolved in water for injection. Paracetamol was used to induce hepatotoxicity and silymarin milk thistle was used as a curative/ restorative drug. For the paracetamol-only, control group and the experimental group, they were given high dose paracetamol once daily for five days. Thereafter, those in the experimental group were given varying doses of silymarin according to the different groups.

Each albino rat was held and wrapped using a table towel to avoid the animal from soiling the primary researcher's garments. The animal was held behind the neck region with one hand. After that, the rat was put to resting position to lie on the researcher with its mouth put to face forward.

The gastric gavage needle was gently inserted into the albino rat's mouth while gently to maneuvering it through the esophageal constrictors and finally through the cardiac sphincter. Eventually the Paracetamol dose was eventually deposited into the rat's stomach. The gastric gavage needle was removed slowly and gently to avoid injuring the animal.

Procedure for anaesthetizing of albino rats

Concentrated chloroform was opened into a heavy tight fitting bell jar. The albino rats will be euthanized by being put into the bell jar for approximately 3-5 minutes. After being euthanized they were removed from the tight fitted lid jar and put on the dissecting board and well mounted using mounting pins to lie of the rear side facing supine.

Biochemical analysis

The rats in the control group and paracetamol-only group were anaesthetized on day 5 by using concentrated chloroform and the blood was collected via cardiac puncture.

The rats in the experimental group were anaesthetized on day 21 by using concentrated chloroform and the blood was collected via cardiac puncture The isolated blood was then centrifuged at 3000 rpm for 10 min and the serum was pipetted off carefully and stored at 20 degrees centigrade until used to assess liver enzymes.

Serum alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (ALP) was conducted at the UON Vet Teaching and Referral Hospital at the University of Nairobi, Kenya.

Data collection

The data obtained was entered into excel sheet, analyzed using SPSS for windows version 26 Chicago Illinois and statistically tested and analyzed using a One-way analysis of variance (ANOVA) for the group means with a $p \leq 0.05$ was considered statistically significant at a 95% confidence interval and comparison made between experimental and control group.

RESULTS

Animal mortality

There was no mortality observed during the period of experiment.

Biochemical parameters following administration of paracetamol followed by silymarin milk thistle

The normal adopted ranges of the biochemical parameters were as follow; Alanine transaminase (14-30 Units/l), Alkaline phosphatase (56.8–128 Units/l) and Aspartate transaminase (45.7- 80.0 Units/l).

There was statistical ($p=0.0001$) increase in in the levels of the Alanine transaminase, Alkaline phosphatase and Aspartate transaminase in the paracetamol-only control group as compared to the control group. One way ANOVA was used to test the difference between the means and post hoc test to find significance and the ($p \leq 0.05$) at 95% confidence interval was found significant. All the biomarkers tested were all above the normal ranges for the control group.

There was statistical significance ($p=0.0001$) increase in the levels of the Alanine transaminase, Alkaline phosphatase and Aspartate transaminase in the paracetamol-only group, Medium and Low dose group as compared to the control group. All biochemical parameter levels in the paracetamol-only group.

Medium and Low dose group were above the normal adopted reference ranges. One way ANOVA was used to test the difference between the means and post hoc test to find significance and the ($p \leq 0.05$) at 95% confidence interval was found significant. The paracetamol-only group, the low dose SIL and the medium dose SIL had deranged values in the liver biomarkers tested.

Table 1: Mean of the biochemical parameters between the control and paracetamol-only groups.

Groups	ALT	ALP	AST
Control group	22.00±0.95	77.00±0.70	94.62±0.71
Paracetamol-only group	35.84±0.51	108.30±1.39	113.27± 0.21
	P=0.0001	P=0.0001	P=0.0001

ALT-Alanine transaminase, ALP-Alkaline phosphatase, AST-Aspartate transaminase

Table 2: Mean of the biochemical parameters between the paracetamol-only group and the experimental groups.

Groups	ALT	ALP	AST
Paracetamol-only group	35.84±0.51 P=0.0001	108.30±1.39 P=0.0001	113.27± 0.21 P=0.0001
High dose SIL group	25.72±1.33 P=0.111	78.33±0.89 P=1.000	95.66±0.25 P=1.000
Medium dose SIL group	34.28±0.10 P=0.0001	106.81±1.86 P=0.0001	112.35±0.76 P=0.0001
Lose dose SIL group	35.22±0.70 P=0.0001	106.58±1.71 P=0.0001	113.60±0. 22 P=0.0001

Sig.-significance. ALT-Alanine transaminase, ALP-Alkaline phosphatase, AST-Aspartate transaminase, SIL-Silymarin milk thistle

DISCUSSION

Liver biochemical parameters (AST, ALT, ALP) following administration of silymarin milk thistle in paracetamol induced hepatotoxicity among adult albino rats

Paracetamol is listed as one of the essential drugs by the World Health Organization and it has a narrow therapeutic index. Toxicity can be due to accidental ingestion, self-harm overdose or even dose error prescription.[15] Paracetamol is the most commonly used analgesic worldwide, readily available over the counter with or without prescription. It has been attributed that its overdose leads to liver injury with several incidences of liver toxicity leading to high cost of treatment and hospitalization worldwide with almost 2 million deaths annually.[16] Paracetamol poisoning has become a leading medication poisoning in most countries posing a serious health concern worldwide.[17] Over the last decade, incidences of paracetamol toxicity have sharply risen in the America and the European countries hence becoming the leading cause of liver transplants.[18,19] This current study is designed to assess the restorative effects of silymarin milk thistle on paracetamol induced hepatotoxicity.

Several authors have used liver biomarkers to assess the level of liver toxicity and restoration on using different drugs. Paracetamol toxicity has been found to cause significant changes by increasing the total bilirubin, total protein, Alanine transaminase (ALT), Alkaline phosphatase (ALP) and Aspartate transaminase (AST) levels in the body.[20] The current study found a significant increase (P=0.0001) in the levels of ALP, AST, ALT in medium dose SIL group and low dose SIL group and paracetamol-only group (Table 1 and Table 2) as compared to the control group and high SIL group. It showed that high dose of silymarin milk thistle ameliorates these liver biomarkers. The persistent elevation of these markers even after giving silymarin milk thistle at low and medium doses is an indication that the restorative effects on the liver tissue did not occur using these doses. However; at higher doses of 600 mg/kbwt there was normalization of all liver biochemical markers (Table 2).

In agreement with the current results, a study on the effects of silymarin on the resolution of carbon tetrachloride induced liver fibrosis, observed that low doses of silymarin were ineffective and could not cause any restoration and normalization of the elevated liver biochemical markers.[21] In addition, other authors also reported that lower doses of less than 100 mg/kgbwt of silymarin milk thistle had no restorative effects on the liver histoarchitecture.[22] He further observed no normalization of liver biomarkers that had increased due to toxicity of acetaminophen on liver tissues. It was also found that silymarin milk thistle restores the elevated liver biomarkers of ALT, ALP and AST which suggest that it has hepatocyte membrane stabilizers hence preventing toxins from getting into the cells and ameliorating their effects.[23,24]

The levels of AST, ALP, ALT in the high dose SIL group and the control group were within the normal ranges indicating that restoration of the liver tissue was achieved through high dose silymarin. this confirms that phytochemical silymarin in the milk thistle may facilitate reconstruction of hepatic histo-architecture of the liver tissue and counteraction of the hepatotoxin effects of paracetamol, thereby improving the levels of glutathione thus improving the liver functional biochemistry. Authors from literature reviewed recorded that administration of silymarin milk thistle was found to normalize the elevated AST, ALP, ALT since the silymarin at high dose of 500 mg/kbwt was found to potentiate normalization of liver tissues causing improvements in liver biochemical parameters.[6,21,25] Moreover, Rahul et al, also reported restoration of liver histoarchitecture after administering Rhodiola Imbricata and silymarin milk thistle to injured

liver among rats therefore, these studies concluded that silymarin milk thistle may have a restorative effect on damaged liver tissues.[26]

The results of this study indicates that the restorative effects of silymarin milk thistle is dose dependent thus only higher doses may give the desired results. These findings mirror observations from other authors who found that the normalization of liver biomarkers was directly dependent on the dose of silymarin and Nigella sativa where a high dose of silymarin portrayed normalization of liver functions and improvement of its histoarchitecture. However, more studies may be needed to assess effects high doses of silymarin milk thistle and its pharmacodynamics.[27,28]

This current study found out that high dose of silymarin milk thistle caused normalization of the liver biomarkers of ALP, AST and ALT in paracetamol induced hepatotoxicity and therefore reinforced the evidence that injurious effect of paracetamol can be reversed by silymarin milk thistle.

CONCLUSION

Silymarin milk thistle was found to have restorative effects on paracetamol induced hepatotoxicity at a high dose (600 mg/kbwt). This led to normalization of liver biomarkers and this could be a possible therapeutic solution to paracetamol hepatotoxicity. Further studies should also be done to the human dosages and the pharmacokinetics and pharmacodynamics of silymarin milk thistle on human beings.

Funding: No funding sources
Conflict of interest: None declared
Ethical approval: The study was approved by the Institutional Ethics Committee

REFERENCES

1. Mallet C, Desmeules J, Pegahi R, Eschalier A. An updated review on the metabolite (AM404)-mediated central mechanism of action of paracetamol (acetaminophen): experimental evidence and potential clinical impact. J Pain Res. 2023;2:1081-94.
2. Jozwiak-Bebenista M, Nowak JZ. Paracetamol: mechanism of action, application and safety concern. Acta Poloniae Pharmaceutica. 2014;71(1):11-23
3. Tittarelli R, Pellegrini M, Scarpellini MG, Marinelli E, Bruti V, Di Luca NM, et al. Hepatotoxicity of paracetamol and related fatalities. Eur Rev Med Pharmacol Sci. 2017;21(1): 95-101.
4. Kurtovic J and Riordan SM. Paracetamol-induced hepatotoxicity at recommended dosage. J Int Med. 2003;253(2):240-3.
5. Fukuda M, Hirayu N, Nabeta M, Takasu O. Acute Liver Failure After Administration of Acetaminophen at the Recommended Daily Dose in an Adult: A Case Report. Cureus. 2023 Sep;15(9):567.
6. Khazaei R, Seidavi A, Bouyeh M. A review on the mechanisms of the effect of silymarin in milk thistle (Silybum marianum) on some laboratory animals. Veterinary Medicine and Science. 2022;8(1):289-301.
7. Saller R, Melzer J, Reichling J, Brignoli R, Meier R. An updated systematic review of the pharmacology of silymarin. Forschende Komplementärmedizin/Res in Complemen Med. 2007;14(2):70-80.
8. Nancy Vargas-Mendoza, Eduardo Madrigal-Santillan, Angel Morales-Gonzalez and Jaime. Hepatoprotective effect of silymarin. World J Hepatol. 2014;6(3):144.
9. Peter F Surai. Silymarin as a natural antioxidant: an overview of the current evidence and perspectives. Antioxidants. 2015;4(1):204-47.
10. Bradly P Jacobs, Cathy Dennehy, Gilbert Ramirez, Jodi Sapp and Valerie A Lawrence. Milk thistle for the treatment of liver disease: a systematic review and meta-analysis. The Am J Med. 2002;113(6):506-15.
11. Avelar CR, Pereira EM, Costa PRF. Effect of silymarin on biochemical indicators in patients with liver disease: Systematic review with meta-analysis. World J Gastroenterol. 2017;23(27):5004.
12. Hajagha MA, Ziaei A, Rafiei R. The efficacy of silymarin in decreasing transaminase activities in non-alcoholic fatty liver disease: A randomized controlled clinical trial. Hepatitis Month. 2008;3(8):191-5.
13. David S, Hamilton JP. Drug-induced liver injury. US Gastroenterol & Hepatol Review. 2010;6:73.
14. Arifin MZ. Sample size calculation in animal studies using resource equation approach Malaysian. J Med Sci. 2017;24(5):101-5.
15. Chidiac AS, Buckley NA, Noghrehchi F. Paracetamol (acetaminophen) overdose and hepatotoxicity: mechanism, treatment, prevention measures and estimates of burden of disease. Expert Opinion on Drug Metabol & Toxicol. 2023;19(5):297-317.
16. Asrani SK, Devarbhavi H, Eaton J. S. Burden of liver diseases in the world. J Hepatol. 2019;70(1):151-71.
17. Fontana RJ. Acute liver failure including acetaminophen overdose. Medical Clinics of North America. 2008;92(4):761-94.
18. Agrawal S, Khazaeni B. Acetaminophen toxicity. 2017.
19. Puri P, Lee WM, Fontana RJ, Kim NK, Durkalski V, McGuire BM, Liou I, Pezzia C, Stravitz RT, Acute Liver Failure Study Group. Alcohol consumption is associated with the severity and outcome of acute liver injury/failure. Liver International. 2020;40(2):360-7.
20. Chilvery S, Yelne A, Khurana A, Saifi MA, Bansod S, Anchi P, et al. Acetaminophen induced hepatotoxicity: An overview of the promising protective effects of natural products and herbal formulations. Phytomedicine. 2023;108:154510.
21. Tsai JH, Liu JY, Wu TT, Ho PC, Huang CY, Shyu JC, et al. Effects of silymarin on the resolution of liver fibrosis induced by carbon tetrachloride in rats. J Viral Hepat. 2008;15(7):508-14.
22. Freitag AF, Cardia GF, da Rocha BA. Hepatoprotective effect of silymarin (silybum marianum) on hepatotoxicity induced acetaminophen

in spontaneously hypertensive rats. Evid Based Complement Alternat Med. 2015;2015:538317.
23. Ahmed AF, Mahmoud MF, Ouf MA, Abd El-Fathaah E. Aminoguanidine potentiates the hepatoprotective effect of silymarin in CCL4 treated rats. Annals of hepatology. 2016;10(2):207-15.
24. Palanivel MG, Rajkapoor B, Kumar RS, Einstein JW. Hepatoprotective and antioxidant effect of Pisonia aculeata L. against CCl4-induced hepatic damage in rats. Scientia pharmaceutica. 2008;76(2):203-16.
25. Saeed M, Babazadeh D, Arif M, Arain MA, Bhutto ZA, Shar AH, et al. Silymarin: a potent hepatoprotective agent in poultry industry. World's Poultry Sci J. 2017;73(3):483-92.
26. Senthilkumar R, Chandran R, Parimelazhagan T. Hepatoprotective effect of Rhodiola imbricata rhizome against paracetamol-induced liver toxicity in rats. Saudi J Biol Sci. 2014;21(5):409-16.
27. Kidd P, Head K. A review of the bioavailability and clinical efficacy of milk thistle phytosome: a silybin-phosphatidylcholine complex (Siliphos). Alternative Medicine Rev. 2005;10(3):89.
28. Hamza RZ, Al-Harbi MS. Amelioration of paracetamol hepatotoxicity and oxidative stress on mice liver with silymarin and Nigella sativa extract supplements. Asian Pacific J Trop Biomed. 2015;5(7):521-31.

Cite this article as: Ngetich DK, Korir S. Liver biomarkers of silymarin milk thistle on paracetamol induced liver toxicity in adult albino rats (*Rattus norvegicus*). Int J Basic Clin Pharmacol 2025;14:140-5.