Wild Strawberry Fragaria vesca L. extracts Hepatoprotective Activities Against Paracetamol-Induced Hepatotoxicity in Male Wistar Rats

by Sihning E. J. T., Diah Purwaningsari, Nita Pranitasari, Liliawanti

Submission date: 16-Feb-2023 07:41AM (UTC+0700)
Submission ID: 2015224193
File name: Wild_Strawberry_Fragaria_vesca_L._extracts_Hepatoprotective.pdf (693.81K)
Word count: 3417
Character count: 19562

Wild Strawberry Fragaria vesca L. extracts Hepatoprotective Activities Against Paracetamol-Induced Hepatotoxicity in Male Wistar Rats

Sihning E. J. T.[1], Diah Purwaningsari[1], Nita Pranitasari[1], Liliawanti[1]

[1]*Lecturer at Department of Anatomy and Histology, Faculty of Medicine, Hang Tuah University, Surabaya, East Java, Indonesia*

Abstract

Background: Administration of high-dose paracetamol had been indicated to induce several molecular and biochemical cascades of hepatotoxicity. The abundant amount of ROS formation following the uptake of the medication exceeds the physiologic antioxidant capacity of the body, leading to the occurrence of oxidative stress. Wild strawberries (*Fragaria vesca L*)extractsare thought to have hepatoprotective activities regarding the high activity of antioxidants contained.

Method: Aspartate Transaminase (AST) activity was used as a marker of hepatotoxicity. In the 10 days trial, randomized samples of 24 male Wistar rats (*Rattus Norvegicus*) were used and divided into three groups: 1 group without treatment, 1 group receiving oral paracetamol 1750 mg/kg in CMC-Na suspension, and 1 group receiving oral paracetamol 1750 mg/kg following administration of strawberry extract 400 mg/kg. The strawberry extract was administered from day 1 to 10, while high-dose paracetamol was administered on day 9. AST activity analysis was done on day 10 of the trial. Significant results were found showing an increased AST activity in the paracetamol-only group (p=0.001) and a decreased AST activity in the group receiving strawberry extract (p=0.001).

Conclusion: *Fragaria vesca L* extracts were proven *in vivo* to have hepatoprotective activities against paracetamol-induced hepatotoxicity.

Keywords: *Fragaria vesca L, paracetamol, liver, AST, SGOT.*

Introduction

Increasing trends in self-medication had reached a quite worth-considering activity. A recent study showed a prevalence of 89.6% of self-medication among the locals[1]. Over-the-counter (OTC) medication purchases were reported to be the most source to obtain drugs for self-medication, with NSAIDs being the most purchased medication. Fever was reported to take a major part as symptoms triggering this medication habits[2]. This unrestricted access to medications leading to bizarre doses, frequencies, and durations in drug usage; a habitual use that commonly taken place when symptoms persisted but there was a lack of capability to afford some medical helps[3].

Corresponding Author:
Diah Purwaningsari
Faculty of Medicine, Hang Tuah University
1, Gadung St. Surabaya, East Java, 60111, Indonesia
+6287728843166
diah.purwaningsari@hangtuah.ac.id

Paracetamol is one of the most frequently prescribed and purchased antipyretic agents. Paracetamol (N-acetyl-p-aminophenol, Acetaminophen) is a para-aminophenol derivative that served as a non-steroidal analgesic and antipyretic agent. Although it appeared that Paracetamol seems like a non-harmful medication with a broad range of therapeutic doses and a minimum number of adverse effects reported, Paracetamol is often associated with hepatotoxicity and acute liver failure, particularly in high-doses and frequent unintentional administrations, or in simultaneous ingestion with precipitating substances such as metoclopramide, anticholinergics, and several CNS (central nervous system) medications, which increased the absorption or decreased the rate of metabolism of Paracetamol[4].

The adverse effect of hepatotoxicity of Paracetamol is thought to occur mainly through two mechanisms of molecular interactions, covalent and non-covalent. Covalent bonds between NAPQI and hepatocytes' proteins were established due to lack of the supposed binder cytosol GSH following administration of high-dose Paracetamol, leading to destructions of the hepatocytes. Non-covalent interaction, on the other hand, involving formations of free radicals such as N-Acetyl-p-semiquinones (NAPSQI), triggers of reactive oxygen substances (ROS) and superoxide anions, and disruption in Ca^{2+} homeostasis. All those cascades ended up with an occurrence of oxidative stress and hepatocytes destructions[5].

Many chemical substances had been suggested to have hepatoprotective activities. Extracts of wild strawberries, *Fragaria vesca L*, were found to contain substantial amounts of antioxidants that are hoped to have the ability to neutralize the over-accumulation of free radicals[6]. It is a family of *Rosacea* plants and is a vastly cultivated agricultural plant. It has a high ability to adapt to various geographic and climate settings that it could be accessed without further difficulties[7].

The extract of these wild strawberries was reported to have a high activity of ascorbic acid, ellagic acid, and several forms of flavonoid, including anthocyanin, catechin, quercetin, and kaempferol[7]. Qualitative analysis of phytochemical substances in *Fragaria vesca L* showed accumulation of phenols, flavonoids, anthocyanin, and terpenoids. These substances were suggested to have antioxidant activities particularly through *in silico* dockings. Maximum potencies were reported to be achieved via oral or rectal administration with antioxidant properties 2 to 11 times more potent than those in apples, peaches, pears, grapes, tomatoes, or even oranges[8].

With the basic pathophysiology of hepatotoxicity being a depletion of physiologic antioxidant capacity and an over-exceeding amount of free radicals, *Fragaria vesca L* with its antioxidant properties could be an organic reversal of the cascades. Thus, this study aims to analyze the significance of *Fragaria vesca L* hepatoprotective activities against Paracetamol-induced hepatotoxicity.

Materials and Methods

Study design

Measurable and controllable results in this study could be obtained by conducting true experimental research in a laboratory setting with controlled samples and treatments. The design used was the Randomized, post-only control group design.

Sampling method

Simple random sampling was used to randomized the result probabilities in each study animal both in the control group and the treatment group. The sample size was determined using statistical consideration.

Paracetamol-induced hepatotoxicity

Paracetamol was administered in adjusted human doses. The daily therapeutic dose of 15 to 20 g per

day of Paracetamol for humans weighed about 70 kg was adjusted for rats weighed about 200 g[9]. The adjustment dose of 1750 mg/kg was administered to the second (II) and third (III) sample groups.

***Fragaria vesca L* extract**

Fresh fruits of wild strawberries *Fragaria vesca L* were obtained from the Argo Wisata plantation in Malang, Indonesia. Initial preparation was done. The fruits were rinsed with clean tap water, dabbed with a soft cloth, leaves-removed, then cut into small fragments. The cut was then dried in a 45º Celsius degree oven for 2 to 3 days and ground into powder. 10 g of *Fragaria vesca L* powder was dissolved in 100 ml 70% Ethanol (1:10 ratio) then continuously stirred for 6 hours and left in for a night. The solvent was filtrated then evaporated using the vacuum rotatory evaporator to remove the ethanol. Extracts of *Fragaria vesca L* were obtained through an extraction process using a suspension of 1:50 70% Ethanol in 0.5% CMC-Na. The extract was administered to the third (III) sample group for 10 days in 200 mg/kg daily doses.

Animals

24 male Wistar rats (*Rattus norvegicus*) aged 10 to 12 weeks and weighted 150 to 200 g were included. All ethical considerations had been cleared and approved by the Ethics Committee. The selection of healthy rats was done. Rats with shiny-coated hair, active movements, and no scar, was cared for and adapted in the Biochemical Laboratory, Faculty of Medicine, Hang Tuah University, Surabaya, Indonesia. Standard laboratory condition was maintained at 23±20Celsius degree temperature, 45 to 55±10% humidity, and 12 by 12 light and dark cycles. The samples were then divided into three groups as followed.

(I) Negative control group: the samples were given filtrated water via NGT for 10 days and CMC-Na 0.5% on day 8. CMC-Na 0.5% was given 2 hours after administration of filtrated water on the same day.

(II) Positive control group: the samples were given filtrated water for 10 days and oral Paracetamol with 1750 mg/kg doses on day 9. Paracetamol was given 2 hours after administration of filtrated water on the same day.

(III) Treatment group: the samples were given *Fragaria vesca L* extract in 200 mg/kg doses for 10 days and oral Paracetamol with 1750 mg/kg doses on day 9. Paracetamol was given 2 hours after administration of *Fragaria vesca L* extract on the same day.

All three groups were given euthanasic anesthesia on day 11 of the trial using ketamine hydrochloride 40-60 mg/kg through intramuscular injection on femoral quadriceps or triceps.

Hepatotoxicity biochemical marker

Aspartate transaminase (AST) activity is extensively used as an indicator of liver injuries. It is a glycolysis and electron transport facilitator, NAD+/NADH balancing enzyme, in cellular metabolism, particularly in malate-aspartate shuttle where cytosol-NADH are oxidized and mitochondrial NAD+ are reduced. The early release of AST in liver injuries and the later release of the mitochondrial isoform made this enzyme suitable as a hepatotoxicity marker, respectively to determine the onset of the injury and the extent of liver necrosis[10]. The early appearance of AST following a liver injury, even before any presence of clinical signs or symptoms, added the superiority of AST as a marker[11].

Liver cell destruction in hepatotoxicity raised a specific pattern concordant to the vasculature flows. The injury would mainly occur in the 3 central zones of the hepatic acinus which receive the least oxygen supply from the triad portal. In the benefits of using AST as a marker, these areas are happened

to have the highest AST activity. It makes any hepatocytes destruction due to toxicities would be able to be sensitively screened[12].Serum AST activity was observed using the Cobra Integra method then analyzed through photometric measurements using 340 nm wavelength light with results in U/L unit.

Statistical Analysis

A descriptive analysis on the activity of serum AST activity was done. Mean and standard deviation was calculated. The significance of the results was analyzed using one-way Analysis of Variance (one-way ANOVA) followed by post-hoc Least Significance Difference (LSD) for normally distributed data. Kruskal Wallis analysis was done for non-normal distributed data. The value of $p<0.05$ is considered significant.

Results

Descriptive analysis of serum AST showed a mean level of 187.23 ± 33.69 U/L**(Table 1)** in the group given only 0.5% CMC-Na, served as a negative control group (I). In accordance with the hypothesis, the activity of the enzyme was increasedin the group given only Paracetamol (II). There was a significant reduction of serum AST activity from 269.48 ± 45.52 in the group given only high-dose Paracetamol (II) to 185.69 ± 21.27 in the groupgiven Fragaria vesca L extract (III). The activity in the intervention group (III) was found even lesser compared to the group without Paracetamol induction (I).

Table 1.Mean and Standard Deviation of Serum AST Between Groups.

Group	Mean ± SD (U/L)
Ia	187.23 ± 33.69
IIb	269.48 ± 45.52
IIIc	185.69 ± 21.27

[a]study group without treatment; [b]study group receiving high-dose Paracetamol; [c]study group receiving high-dose Paracetamol and *Fragaria vesca L* extract; SD = standard deviation

A normality test was done to ensure that all data in each group were normally distributed before determining the significance of the results. Shapiro-Wilk test was chosen as the normality test method due to the limited size of the sample in each group**(Table 2)**. All data in each group were found normally distributed, except for the positive control group (II).

Table 2: Normality Test of Serum AST Distribution in Each Group.

Group	p-value	Interpretation
Ia	0.453	Normal distribution
IIb	0.039	Non-normal distribution
IIIc	0.939	Normal distribution

[a]study group without treatment; [b]study group receiving high-dose Paracetamol; [c]study group receiving high-dose Paracetamol and *Fragaria vesca L* extract; *p*-value >0.05 considered normally distributed

Kruskal-Wallis test was then chosen as the significance test due to the presence of non-normal distribution data. A significant result was obtained (x^2 = 15.440, df = 2, p = 0.001) showing that there were significant differences in the serum AST activity between the study groups. A further significance test was done using a post-hoc analysis, the Mann-Whitney test, to locate specifically the differences between the study groups **(Table 3)**. It appeared that a significant differencewas found between the group without Paracetamol induction(I) and the group given Paracetamol (II) [p = 0.001]. A significant difference was also found between the group given only high-dose Paracetamol(II) and the group given *F. vesca L* extract following Paracetamol induction (III)[p = 0.001]. Serum AST activity in subjects receiving *Fragaria vesca L* extract (III) interestingly showed

no significant difference compared to the negative control group (I) [$p = 0.674$].

Table 3: Mann-Whitney Test of Serum AST Between Groups.

Group	I[a]	II[b]	III[c]
I[a]		0.001	0.674
II[b]			0.001
III[c]			

[a]study group without treatment; [b]study group receiving high-dose Paracetamol; [c]study group receiving high-dose Paracetamol and *Fragariavesca L* extract

Discussion

The significant increase in the serum AST activity between the group without intervention (I) and the group given only Paracetamol (II) showed that the Paracetamol-induced hepatotoxicity had been successfully recreated in the Wistar rats in this study. The presence of additional reaction in the liver metabolism, particularly due to Acetaminophen, would positively correlate to an increase of the serum AST activity[14]. The massive elevation, nearly 1.5 times higher, had also proven that there was a presence of liver damage or necrosis to the extent of liver injuries. It has been demonstrated in several studies that administration of Paracetamol particularly in high doses would cause drug-induced hepatotoxicity in a form of liver damage or necrosis[11].

High-dose Paracetamol would saturate the glucuronidation and sulfation pathway of liver metabolism, thus increasing the utilization of the Cytochrome P-450 oxidation pathway. The oxidation pathway would produce destructing end-products including free radicals and toxic metabolites as NAPQI (N-acetyl-p-benzoquinone imine). Supposedly,these NAPQIs would have been bound by the GSH enzyme (glutathione sulfhydryl) and turned into hydrophilic cysteine and mercapturic metabolites. Yet in a high dose administration of Paracetamol, the slow nature of GSH production along with increased NAPQIs formation would both exhaust the endogenous enzyme. These toxic metabolites would accumulate in hepatocytes, underwent nucleophilic reactions with the cells' macromolecules such as protein, leading to necrosis of the cells[14,15].

On the other hand, the significant reduction of serum AST activity from the group given only high-dose Paracetamol to the group given*Fragaria vesca L* extract following Paracetamol-induction supported our hypothesis that administration of the extract could neutralize the hepatotoxicity that occurred due to administration of a high-dose Paracetamol. Escalation of serum AST activity indicated a presence of cellular leakage and functional disintegration of the hepatocyte membranes[9]. Thus, this reduction of serum AST activity might occur due to the ability of *Fragaria vesca L* extract to stabilize the cell membrane of hepatocytes following the injury which prevented any leakages of cytosol enzymes[16].

Anthocyanin, particularly the pelargonidin-3-glycoside contained in *Fragaria vesca L*, is thought to be the main substance that provides the antioxidant capacity of the extract. The work might center on preventing the free radicals from oxidizing the macromolecules of the hepatocytes. These findings are in accordance with the suggested pathophysiology of Paracetamol-induced hepatotoxicity. These exogenous antioxidants would assist the GSH enzyme in binding NAPQIs preventing the exhaustion of the endogenous antioxidants[7].

Furthermore, there was no significant difference in serum AST activity between subjects receiving *Fragaria vesca L* extract (III) and subjects without intervention (I). Through this finding, we can roughly

infer that there was a nearly complete reversal of the hepatic conditionfrom Paracetamol-induced hepatotoxicity, including hepatocytes restoration and hepatic regeneration[16], following the administration of *Fragaria vesca L* extract.

Fragaria vesca L extracts hepatoprotective and antioxidant capacities in reversing the Paracetamol-induced hepatotoxicity had been proven. Nonetheless, it still should be noted that hepatotoxicity is a complex process and may occur through different pathophysiology. Thus, we suggest conduction of further studiesin discovering other hepatoprotective pathways and agents.

Certain boundaries had given several limitations in this study to observe the hepatoprotective activities of *Fragaria vesca L* extract. Administration of various doses of the extract would give us a better view of the optimum amount and frequency that should be given to evoke the antioxidant properties. Multiple administration of Paracetamol with various doses, rather than fixed-dose single administration, would give us the ability to explore the maximum antioxidant capacity of the extract. Analysis of more hepatic markers should also provide a more holistic evaluation. Regarding several limits that we'd foreseen, we hope that this study could be a help in acknowledging the significance of *Fragaria vesca L* as a hepatoprotective agent.

Conclusion

Fragaria vesca L fruit extract is a promising alternative as a hepatoprotective agent particularly in reversing the hepatotoxicity following an administration of high-dose Paracetamol.

Ethical Clearance: All ethical consideration had been cleared and approved by the Ethics Committee of Hang Tuah University.

Source of Funding: Financial funding was provided by Hang Tuah University and private sources.

Conflict of Interest: None should be declared.

References

1. Abdi A, Faraji A, Dehghan F, Khatony A. Prevalence of self-medication practice among health sciences students in Kermanshah, Iran. BMC Pharmacol Toxicol [Internet]. 2018;**19**(36). Available from: https://www.ncbi.nlm.nih.gov/pmc/articles/PMC6029137/
2. Khan H, Maheen S, Alamgeer, Abbas G, Mahmood A, Sarfraz RM, et al. Determinants of Increasing Trend of Self-Medication in a Pakistani Community. Trop J Pharm Res [Internet]. 2014;**13**(3). Available from: https://www.tjpr.org/home/abstract.php?id=1587&aTitle=Determinants of Increasing Trend of Self-Medication in a Pakistani Community
3. Jayawardhani V, Kamtane RA, Kulkarni AK. Pharmaco-epidemiological study of self-medication pattern in Hyderabad. Res J Pharm Technol [Internet]. 2012;**5**(8):1114–7. Available from: https://rjptonline.org/HTMLPaper.aspx?Journal=Research Journal of Pharmacy and Technology;PID=2012-5-8-15
4. Bunchorntavakul C, Reddy KR. Acetaminophen (APAP or N-Acetyl-p-Aminophenol) and Acute Liver Failure. Clin Liver Dis [Internet]. 2018;**22**(2):325–46. Available from: https://pubmed.ncbi.nlm.nih.gov/29605069/
5. Larson AM, Polson J, Fontana RJ, Davern TJ, Lalani E, Hynan LS, et al. Acetaminophen-Induced Acute Liver Failure: Results of a United States Multicenter, Prospective Study. Hepatology [Internet]. 2005;**42**(6):1364–72. Available from: https://pubmed.ncbi.nlm.nih.gov/16317692/
6. Pham-Huy LA, He H, Pham-Huy C. Free radicals, antioxidants in disease and health. Int

J Biomed Sci [Internet]. 2008 Jun;**4**(2):89–96. Available from: https://www.ncbi.nlm.nih.gov/pmc/articles/PMC3614697/

7. Seeram NP. Strawberry Phytochemicals and Human Health: A Review [Internet]. University of California; 2007. Available from: https://www.researchgate.net/publication/228983423_Strawberry_phytochemicals_and_human_health_a_review
8. Tanideh N, Baseri FA, Jamshidzadeh A, Ashraf MJ, Kuhi O, Mehrabani D. The Healing Effect of Strawberry Extract on Acetic Acid-Induced Ulcerative Colitis in Rat. World Appl Sci J [Internet]. 2014;**31**(3):281–8. Available from: https://www.idosi.org/wasj/wasj31(3)14/1.pdf
9. Trevor AJ, Katzung BG, Kruidering-Hall M, Masters SB. Katzung & Trevor's Pharmacology, Examination & Board Review. 10th ed. New York: Lange, McGraw Hill; 2013.
10. McGill MR. The past and present of serum aminotransferases and the future of liver injury biomarkers. EXCLI J [Internet]. 2016;**15**:817–28. Available from: https://www.ncbi.nlm.nih.gov/pmc/articles/PMC5318690/
11. Johnson DB, Rajesh CSKR, Venkatnarayanan R, VK MA. Hepatoprotective Activity of Borreria hispida on Paracetamol induced Liver Damage. Res J Pharm Technol [Internet]. 2013;**6**(1):61–5. Available from: https://rjptonline.org/HTMLPaper.aspx?Journal=Research Journal of Pharmacy and Technology;PID=2013-6-1-3
12. Hall P, Cash J. What is the Real Function of the Liver 'Function' Tests? Ulster Med J [Internet]. 2012;**81**(1):30–6. Available from: https://pubmed.ncbi.nlm.nih.gov/23536736/
13. Steel RD, Torrie JH, Dickey DA. Principles and Procedures of Statistics: A Biometrical Approach. Subsequent. New York: McGraw-Hill College; 1996. 672 p.
14. Hinson JA, Roberts DW, James LP. Mechanisms of Acetaminophen-Induced Liver Necrosis. In: Handb Exp Pharmacol [Internet]. 2010. p. 369–405. Available from: https://pubmed.ncbi.nlm.nih.gov/20020268/
15. Kalsi SS, Dargan PI, Waring WS, Wood DM. A review of the evidence concerning hepatic glutathione depletion and susceptibility to hepatotoxicity after paracetamol overdose. Open Access Emerg Med [Internet]. 2011;**3**:87–96. Available from: https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4753970/
16. Karwani G, Sisodia SS. Hepatoprotective activity of Chenopodium album Linn. in Ethanol induced Hepatotoxicity in Rats. Res J Pharm Technol [Internet]. 2015;**8**(6):669. Available from: https://rjptonline.org/HTMLPaper.aspx?Journal=Research Journal of Pharmacy and Technology;PID=2015-8-6-1

Wild Strawberry Fragaria vesca L. extracts Hepatoprotective Activities Against Paracetamol-Induced Hepatotoxicity in Male Wistar Rats

ORIGINALITY REPORT

4%	3%	1%	0%
SIMILARITY INDEX	INTERNET SOURCES	PUBLICATIONS	STUDENT PAPERS

PRIMARY SOURCES

1	Matthew G. Kirkpatrick, Margaret C. Wardle. "Experimental Methods for Understanding the Role of Social Context in Drug Addiction", Wiley, 2013 Publication	1%
2	jurnal.unej.ac.id Internet Source	1%
3	www.hindawi.com Internet Source	1%
4	moam.info Internet Source	<1%
5	www.tandfonline.com Internet Source	<1%
6	journals.plos.org Internet Source	<1%
7	www.ust.edu Internet Source	<1%

8	docplayer.net Internet Source	<1%
9	storage.googleapis.com Internet Source	<1%
10	www.bielcorp.com Internet Source	<1%

Exclude quotes	On	Exclude assignment template	On
Exclude bibliography	On	Exclude matches	Off