INNSPUB

International Journal of Biosciences | IJB |
ISSN: 2220-6655 (Print), 2222-5234 (Online)
http://www.innspub.net
Vol. 21, No. 3, p. 139-145, 2022

RESEARCH PAPER OPEN ACCESS

# Hepatoprotective Property of *Parameria laevigata* Leaf Extracts in CCl4- hepatotoxicity Induced Sprague Dawley Rats

**Jandy S. Danzalan[1], Julius T. Capili[2*]**

[1]*Philippine Normal University North Luzon, Alicia, Isabela*

[2]*Cagayan State University Andrews Campus, Tuguegarao City*

**Key words:** Hepatoprotective, *Parameria laevigata*, Sprague Dawley, SGOT, SGPT.

http://dx.doi.org/10.12692/ijb/21.3.139-145 Article published on September 12, 2022

## Abstract

This study investigated the hepatoprotective property of *Parameria laevigata* leaf extracts in CCl4-hepatotoxicity-induced Sprague Dawley rats. An experimental design consisting of four (4) treatments, positive control and negative control, was used in the study. It has two (2) major phases, namely: plant authentication and screening of secondary metabolites and enzymometric analysis (SGOT and SGPT) using a blood chemistry analyzer. Results of the study revealed that the leaf extract of *Parameria laevigata* exhibits hepatoprotective properties as evidenced by the SGPT and SGOT levels and that of the histopathological analysis when induced at 100% concentration. The results were comparable to that of the positive control. The hepatoprotective activity of the *Parameria laevigata* leaf extracts is attributed to the collective presence of secondary metabolites such as flavonoids, saponins, and tannins. Fractionation and isolation of the active secondary metabolite must therefore be carried out before advancing to the pre-clinical and clinical trials phases of experimentation.

* **Corresponding Author:** Julius T. Capili ✉ jchua@csu.edu.ph

## Introduction

The liver performs essential functions that sustain life. It creates new molecules, acts as a blood filter, and makes a protein that regulates blood clotting among all these functions, the liver has an extraordinary ability to regenerate its damaged tissue. However, liver regeneration primarily works in healthy livers. Thus, it is imperative to protect the liver from any diseases. By virtue of its position, structure, function, and biochemistry, the liver is especially vulnerable to damage from toxic compounds (Timbrell, 2009). Injury may result from direct toxicity via hepatic conversion of a xenobiotic to an active toxin or through immune mechanisms, usually by a drug or a metabolite acting as a hapten to convert a cellular protein into an immunogenic (Kumar *et al.,* 2006). Liver disease is a considerable health burden across the world. Additionally, 43 000 die of liver disease, of which about 50% are alcohol-related (Rayfield, 2013). Furthermore, hepatotoxicity occurs when liver regeneration capabilities are exhausted and cell damage follows.

Natural products from plants used traditionally have been investigated for the development of new drugs. Studies have shown that plants with hepatoprotective properties have phytoconstituents and classified them under phenyl compounds, coumarins, essential oils, monoterpenoids, diterpenoids, triterpenoids, steroids, alkaloids, and others (Adewusi and Afolayan, 2010). It is vital to realize that despite the great advances in modern medicine, there is a limited effective drug available to stimulate chronic diseases, particularly in liver function, liver protection, or in the regeneration of hepatic cells. This calls for the need to seek novel potential hepatoprotective substances and to search for alternative drugs to treat liver diseases.

Natural products, including herbal extracts, could significantly contribute to the recovery processes of the intoxicated liver. Among these plants with great potential for further scrutiny is the Tagulauai plant (*Parameria laevigata* or lupluppiit in the dialect). This plant offers great medical benefits, although not much research has been examined on its medicinal potential.

With this, the researcher aimed to investigate the hepatoprotective potential of *P. laevigata* leaf extracts in CCl4- hepatotoxicity induced Sprague Dawley rats considering biochemical effects.

## Materials and methods

*Collection of plant specimen*

The plant samples were collected from Barangay Malannit and Barangay Divinan of Jones Isabela. Matured leaves were randomly collected at Barangay Malannit and Divinan of Jones, Isabela. The leaves were washed with fresh water to remove dirt and were initially air-dried at room temperature.

*Authorization of experimental animals*

Before the conduct of the investigation for the hepatoprotective potential of *P. laevigata*, authorization or clearance was secured from the Department of Agriculture Central Office – Bureau of Animal Industry (BAI).

*Plant extraction and processing*

The plant material was allowed to dry and extracted by maceration for three days with ethanol. The ethanolic extract was subjected to rotavap. The extracted product was placed in a water bath for 24 hours and maintained at a temperature not exceeding 60 degrees to dryness. The ethanolic extract was subjected to phytochemical screening and toxicity test, and the anti-hepatoprotective assay was conducted next.

*Phytochemical analysis*

The extract was allowed to evaporate to incipient dryness and, after the procedure, was tested for the presence of saponins, tannins, and flavonoids using the protocol of Guevara *et al.* (2005).

*Animal bioactivity testing (in vivo)*

*Animal husbandry*

The selection, caring, and handling of the experimental animals were done as per the guidelines

of the authorized/certified IACUC (Institutional Animal Care and Use Committee). A total of 35 healthy Sprague Dawley rats (170-300 g), aged twelve weeks of either sex, bred by a licensed veterinarian was used. The animals were purchased from PITAHC-CVHPP and kept at their Pharmacology Laboratory under room temperature and 10 h:14 h of light and dark cycles, respectively. They were provided with water and rat feed ad libitum and kept in 60 cm × 30 cm × 25 cm plastic cages with a wire screen to allow air ventilation. Grounded corn cobs and rice husks were used as bedding to allow the test animals to maintain their body temperature and remain comfortable inside the cages.

*Preparation of different concentrations of extracts*

Different concentration of extracts was prepared to test their hepatoprotective potential. There were six treatments with five (5) replicates. Group 1 to Group 4 were treated with a mixture of $CCl_4$ and leaf extracts with various concentrations (25%, 50%, 75 %, and 100%, respectively). Group 5, the positive control, was induced with $CCl_4$ and silymarin mixture. Group 6, the negative control, was treated with $CCl_4$ and distilled water only. The different doses were given orally at 1ml/kg.

*Acute oral toxicity procedure*

Preliminary dosing of the crude extract was conducted to determine the expected dose that causes 50% death of the experimental animals following OECD 423 guidelines (OECD/OCDE. 2002). Four increasing doses of the extract were given orally to the test animals in four groups of three females, including a negative control. The following dose ranges includes 0, 1.0, 2.0, and 5.0 ml/kg bodyweight.

The concentration of test extract was 100mg/ml obtained by diluting 1 ml from 1/1g (100%) stock solution of test extract with 10ml sterile water.

*Hepatoprotective testing*

Thirty (30) rats were divided into six groups of five. Blood was collected from the tail before and after dosing with the plant extracts. A scalpel and/or sharp scissors were used to quickly remove up to 1 cm of the tail. The blood is placed in a red top tube as drops appear (Hoff, 2000). Pretest and post-test Serum analysis was conducted at the Cagayan United Doctors Medical Center laboratory. Serum was separated by centrifuging at 2 500 rpm for 15 min and was used for the analysis of various biochemical parameters, Serum glutamic oxaloacetic transaminase, and Serum glutamic pyruvic transaminase. SGOT and SGPT activities were measured by Reitman and Frankel (1957) and Schmidt and Schmidt (1963). The normal values of SGOT and SGPT for rats are 74-143 IU/L and 63- 175 IU/L (Giknis *et al.,* 2008), respectively.

*Data analysis*

Data obtained from this work were analyzed statistically using a t-test and One-way analysis of variance (ANOVA) using SPSS version 22. Histopathological processing was done at Cagayan Valley Medical Center, and a Veterinary pathologist was tapped to assess and read the results. Differences between means were considered significant at a 0.1% level of significance.

**Results and discussion**

The results during the pretest indicate that the SGOT level of all the treatment groups lies at the acceptable level of 74-143 IU/L (Giknis *et al.,* 2008). After the induction of specific dosages to the different concentrations of *P. laevigata* leaf extracts, the Sprague Dawley rats induced a marked increase in their serum glutamic oxaloacetic transaminase (SGOT). Notably, treatment 4 marked a decrease in SGOT level with a mean of 136.78, which indicates a normal level of SGOT even after the treatment. Also, the result is comparable to that of the positive control. This can be greatly attributed to the secondary metabolites which were found to be present in the *P. laevigata* crude extracts.

Table 1 shows the difference in the hepatoprotective potential on SGOT among the *P. laevigata* leaf extracts compared to the positive and negative control.

**Table 1.** Test of difference on the hepatoprotective potential on SGOT among the *Parameria laevigata* leaf extracts compared to the positive and negative control.

| Source of Variance | Sum of Squares | Df | Mean Square | F-ratio | *p*-value |
|---|---|---|---|---|---|
| Between Groups | 760296.37 | 5 | 152059.274 | 9.830[8] | 0.000 |
| Within Groups | 371241.37 | 24 | 15468.390 | | |
| Total | 1131537.74 | 29 | | | |

The single-factor analysis of variance revealed that there are highly significant differences in the SGOT level of the treatments used, as shown in the F-ratio of 9.830 (p= 0.000). The different concentrations of extracts differed in favor of the positive and negative control thus, the null hypothesis is rejected. The results reveal that the best treatment is Treatment 4 (100% concentration) of the *P. laevigata* ethanolic extracts. Aside from the fact that it contains active secondary metabolites like alkaloids, saponins, and tannins, the dilution theory holds true to this experiment, reiterating that the higher the concentration of the plant extract, the more effective it becomes. Further, this finding is also attributed to the phenol coefficient. To determine which concentration of the *P. laevigata* leaf extracts significantly differ, the least significant difference test was used. Results showed that the mean differences between 50% leaf extract with the positive control, negative control, and 100% leaf extract significantly differed in SGOT level. The SGOT level for 75% leaf extracts significantly differed from the positive control and 100% leaf extracts. On the other hand, the 25% leaf extract showed a significant difference from the 100% concentration of leaf extract in terms of SGOT level.

**Table 2.** Comparison among means of the hepatoprotective potential on SGOT among the *Parameria laevigata* leaf extracts compared to the positive and negative control.

| Treatment | Mean | T2 | T3 | T1 | Negative Control | Positive Control | T4 |
|---|---|---|---|---|---|---|---|
| T3 | 609.53 | - | | | | | |
| T2 | 473.88 | 135.55 | - | | | | |
| T1 | 391.02 | 218.50 | 82.95 | - | | | |
| Negative Control | 337.48 | 272.05* | 136.50 | 53.54 | - | | |
| Positive Control | 200.22 | 409.31* | 273.75* | 190.80 | 137.25 | - | |
| T4 | 136.78 | 472.74* | 332.19* | 254.24* | 200.69 | 63.44 | - |

*Mean differences are significant.

Based on the analyses, the 100% leaf extract concentration is best against the positive and negative controls (Table 2). This finding is attributed to the fact that *P. laevigata* leaf extracts contain tannins, flavonoids, and saponins. Studies suggested that tannins from different plant sources can inhibit various cellular signaling pathways and several oncogenic proteins that involve in the development of cancer to wit condensed tannins possess anti-cancer effects in the lungs, and gallotannins inhibit the malignant properties of lung cancer cells (Rajasekar *et al.,* 2021; Raju *et al.,* 2021). Moreover, the hepatoprotective activity of the *P. laevigata* leaf extracts may be due to its rich contents of flavonoids. The hepatoprotective activity of flavonoids is well documented in the study by Gupta *et al.* (2012) and Prada *et al.,* (2014).

The results during the pretest indicate that all the treatment groups are within the acceptable range (63-175 IU/L) of SGPT (Giknis *et al.*, 2008).

The Sprague Dawley rats induced a marked increase in their serum glutamic pyruvate transaminase (SGPT) after the induction of specific dosages to the different concentrations of *P. laevigata* leaf extracts.

**Table 3.** Test of difference on the hepatoprotective potential on SGPT among the *Parameria laevigata* leaf extracts compared to the positive and negative control.

| Source of Variance | Sum of Squares | df | Mean Square | F-ratio | *p*-value |
|---|---|---|---|---|---|
| Between Groups | 187697.61 | 5 | 37539.523 | 4.214* | 0.007 |
| Within Groups | 213781.35 | 24 | 8907.556 | | |
| Total | 401478.96 | 29 | | | |

Interestingly, treatment 4 (m= 80.74, SD= 51.71) and positive control (m= 99.52, SD= 22.36) reported the least increase of SGPT level. Consistent with the result of the SGOT enzymometric analysis, the result can be greatly attributed to the secondary metabolites which were found to be present in the *P. laevigata* crude extracts, namely: flavonoids, saponins, and tannins. To compare the mean values of the hepatoprotective potential on SGPT among the *P. laevigata* leaf extracts compared to the positive and negative control, an analysis of variance was conducted (Table 3). The computed F-ratio of 4.214 has an associated variability of 0.007; thus, the null hypothesis that there is no difference in the hepatoprotective potential of the dosages prepared from *P. laevigata* leaf extracts in terms of SGPT and the positive and negative controls was rejected. It means that there are significant differences in the hepatoprotective potential of the extract. The study further reveals that Treatment 4 remains to be the best concentration and is comparable to the effect of the positive control, which further can imply that it can be a good alternative for Silymarin. Again, the significant result is attributed to the secondary metabolites, which have an anti-hepatocellular damage effect. The concept of dilution reiterates that the more concentrated the extract is, the more effective it becomes.

**Table 4.** Comparison among means of the hepatoprotective potential on SGPT among the *Parameria laevigata* leaf extracts compared to the positive and negative control.

| Treatment | Mean | T2 | T3 | T1 | Negative Control | Positive Control | T4 |
|---|---|---|---|---|---|---|---|
| T2 | 301.00 | - | | | | | |
| T3 | 259.47 | 41.58 | - | | | | |
| T1 | 198.58 | 102.42 | 60.84 | - | | | |
| Negative Control | 171.44 | 129.56 | 87.98 | 27.14 | - | | |
| Positive Control | 99.52 | 201.48* | 159.90 | 99.06 | 71.92 | - | |
| T4 | 80.74 | 220.26* | 178.68 | 117.84 | 90.70 | 18.78 | - |

To determine whether the different treatments significantly differ when compared, the least significant difference comparison between means was made. Results reveal that the mean differences between 50% leaf extract and positive control and that of the 100% leaf extract are significantly higher. All other treatments displayed no significant results as to their mean differences.

The hepatoprotective potential of the leaf extract is attributed to saponins, flavonoids, and tannins. Saponins extracts from plants exert various pharmacological effects to control many diseases, including hepatoprotective activities (Huang *et al.,* 2012). This is supported by Qu (2012) in her study where liver histopathology indicated that total saponins from Actinidiavalvata Dunn root alleviated/ cured $CCl_4$-induced inflammatory infiltration and focal necrosis.

Furthermore, several studies demonstrated that flavonoids improved insulin resistance and blood glucose level and regulated liver function indexes and inflammatory cytokines levels, regulating oxidative stress in liver tissues (Wang *et al.,* 2021; Zhu *et al.*, 2019; Bangayan *et al.,* 2020). Also, tannins have been

found to possess protective properties, such as anti-inflammatory, anti-fibrotic, anti-microbial, anti-diabetic, and so on.

Table 4 presented below reveals that the 100% leaf extract of *P. laevigata* and the positive control (silymarin mixture) are equipotent and almost the same in terms of hepatoprotective potential as indicated by the SGPT results. It must be worth noting that SGPT is a very efficient marker to indicate the presence of hepatic damage. This result implies that both the Silymarin and 100% aqueous leaf extract of *P. laevigata* can protect the liver from possible causes of injury.

## Conclusion

Based on the study's findings, the 100% ethanolic extract from the matured leaves of *Parameria laevigata* exhibits hepatoprotective potential, and the effect was comparable to the positive control. This means that it can be an efficient and alternative source of herbal raw material to develop drugs that can protect the liver damage and cure liver diseases as well. The hepatoprotective potential is attributed to active secondary metabolites such as tannins, flavonoids, and saponins.

## Acknowledgment

Special thanks to the heads of various government agencies which the researchers have coordinated with, PITAHC-CVHPP, CUDMC, CVMC, DOST, and DA-BAI, for their support and technical advice.

## References

**Adewusi EA, Afolayan AJ.** 2010. A review of natural products with hepatoprotective activity. Journal of Medicinal Plants Research **4,** 1318–34.

**Bangayan JMB, Palattao JRM, Chua JMT, Capili JT, Ramirez Jr, RB.** 2020. Evaluation of *Equisetum hyemale* and *Euphorbia hirta* leaf extracts in increasing platelet count of sprague dawley rats. International Journal of Biosciences **17(2),** 58-65.

**Giknis ML, Clifford CB.** 2008. Clinical Laboratory Parameterfor Crl: WI (Han) Rats. Wilmington: Charles River Laboratories.

**OECD-423.** 2001. Guidance document on acute oral toxicity testing classification schemes to cover the transition period until full implementation of the globally harmonized classification system (GHS) p 18-21. http://iccvam.niehs.nih.gov/SuppDocs/FedDocs/OECD/OECD_GL423.pdf

**Qu L, Xin H, Zheng G, Su Y, Ling C.** 2012. Hepatoprotective Activity of the Total Saponins from *Actinidia valvata* Dunn Root against Carbon Tetrachloride-Induced Liver Damage in Mice. Evidence-based Complementary and Alternative Medicine. PMID: 23243434; PMCID: PMC3518303. http://dx.doi.org/10.1155/2012/216061.

**Rajasekar N, Sivanantham A, Ravikumar V, Rajasekaran S.** 2021. An overview on the role of plant-derived tannins for the treatment of lung cancer. Phytochemistry **188,** 112799. http://dx.doi.org/10.1016/j.phytochem.2021.1127

**Raju R, Prakash T, Rahul R, Poonangadu SS, Kumar SS, Sonaimuthu P, Capili JT.** 2021. Phytochemical Analysis of Three Common Medicinal Plants (Gliricidia sepium, Melothria pendula, and Pithecellobium dulce) in the Philippines. Scholars Academic Journal of Biosciences **3,** 84-88.

**Rayfield B.** 2013. Why we need extra liver protection. Sinclair Method [Online]. Available from: http://www.sinclairmethod.com/index

**Timbrell JA.** 2009. Principles of Biochemical Toxicology. Retrieved from https://www.researchgate.net/publication/238301066_Principles_of_Biochemical_Toxicology

**Vian Jethro T, Bariuan Chasey T, Mabborang Sheila Mae G, Macanas Kenneth Christian O. Santiago Jayvee B, Salomon Jinky Marie T, Chua Ramelo B, Ramirez Jr.** 2020. *Mollugo oppositifolia* leaf tea formulation and its effect against alloxan-induced hyperglycemic male sprague dawley rats. International Journal of Biosciences **16(4),** 71-78.

**Wang H, Liu S, Cui Y, Wang Y, Guo Y, Wang X, Piao C.** 2021. Hepatoprotective effects of flavonoids from common buckwheat hulls in type 2 diabetic rats and HepG2 cells. Food Science & Nutrition **9(9),** 4793–4802. http://dx.doi.org/10.1002/fsn3.2390.

**Zhu XA, Yang WO, Lan YQ, Xiao H, Tang L, Liu G, Feng KL, Zhang LQ, Song MY, Cao Y.** 2020. Anti-hyperglycemic and liver protective effects of flavonoids from Psidium guajava L. (guava) leaf in diabetic mice. Food Bioscience **35,** 100574. http://dx.doi.org/10.1016/j.fbio.2020.100574