ISSN: 2581 – 3633
PubMed - National Library of Medicine - ID: 101745081

International Journal of Medical Science and Advanced Clinical Research (IJMACR)

Available Online at: www.ijmacr.com

Volume – 3, Issue – 6, November - December - 2020, Page No. : 01 – 07

Dose Dependent Prophylactic Effect of Henna (Lawsonia inermis Linn) Leaf on Acetaminophen Induced Hepatotoxicity in Adult Albino Rats

[1]Dr Misbah Ishtiaq,[2]Muhammad Ishtiaq Rana, [3]Dr Saima Abdul Waheed ,[4] Dr Iqra Javed, [5]Dr Abdul Hanan Jawad,[6]Dr Haseeb Ahmed Awan.

[1]Demonstrator,Khwaja Muhammad Safdar Medical College,Sialkot.

[2]Lecturer,Allama Iqbal Postgraduate College,Sialkot.

[3]Demonstrator,Postgraduate Medical Institute.Lahore.

[4]Demonstrator,Sahiwal Medical College,Sahiwal.

[5]MO,DHQ Hospital Narowal.

[6]Demonstrator, Al Aleem Medical College,Lahore.

Corresponding Author: Dr Misbah Ishtiaq, Demonstrator, Khwaja Muhammad Safdar Medical College, Sialkot.

Type of Publication: Original Research Article

Conflicts of Interest: Nil

Abstract

The effect of Lawsonia is evaluated on rats. The rats were given the extract of Lawsonia Inermis and then were treated with acetaminophen. First, lawsonia inermis was given to rats and then its prophylactic effect on liver was observed after treating with Acetaminophen. A number of parameters are studied which includes gross as well as histological features. In the gross parameters the body weight, liver weight, percentage weight gain and relative tissue weight index was noted. In the histological parameters, the quantitative and qualitative analysis was performed. It is analyzed that this plant has considerable prophylactic effect on protection of hepatocytes.

Keywords: Lawsonia, parameters, Animals, Effects.

Introduction

Lawsonia inermis is a plant from a family Lythraceae.[1, 2, 3, 4, 5]It has numerous applications and uses in daily life. The medicinal purposes of this plant are mainly due to the active ingredients present in leaves. Henna is present in Tropical, subtropical and semi-arid areas[6, 7, 8, 9, 10] of Asia and Africa. It has a hepatoprotective effect on liver which is studied in this research. This study is conducted on the rat. The effect of this plant is analyzed by considering various parameters.

Parameters

Gross Parameters

1) Body Weight

Weight of animals was documented by analytical balance before and after the experimental study with the help of Sartorius Precision Balance, Germany.

2) Weight of Liver

After postmortem of body the mass of liver of was noted frequently

3) %age Weight gain

%age Weight gain was calculated from the following formula:

Mean wt. at end of experiment (g)−Mean wt. at start of experiment (g) x 100

Mean wt. at start of experiment (g)

4) Relative Tissue Weight Index (RTWI)

RTWI was calculated from the following formula:

RTWI =Weight of liver (g) X 100

Animal body weight (g)

Quantitative

1. Weight of animal at the start and end of experiment (g)
2. Animal liver weight (g)
3. Relative tissue weight index

RTWI = Weight (g) of liver X 100

Animal body weight (g)

Histological Parameters

Histologically, sections of the tissue were examined for:

Quantitative

1. No. of hepatocytes/mm².

Qualitative

1- Hepatocytes vacuolization (Present/Absent).

2- Nuclear changes (Present/ Absent)

Data was coded as

Present = 1

Absent = 0

Mild/Moderate/Severe = +/++/+++

Observations and Results

Test of Normality

Before analyzing of data, the variation in data was evaluated by using Shapiro-Walk test and empirical rule of normal distribution. The table below reveals that distribution of all quantitative parameters was normally distributed.

Table1: Normality of the Data Variables

Variables	Groups	Shapiro-Walk		
		Statistic	N-Value	P-Value
Initial weight	Group A	0.967	7	0.875
	Group B	0.877	7	0.213
	Group C	0.956	7	0.781
	Group D	0.959	7	0.813
Final weight (g)	Group A	0.959	7	0.810
	Group B	0.864	7	0.166
	Group C	0.936	7	0.600
	Group D	0.942	7	0.653
Weight gain (g)	Group A	0.858	7	0.144
	Group B	0.840	7	0.099
	Group C	0.915	7	0.432
	Group D	0.787	7	0.030
Liver weight (g)	Group A	0.926	7	0.515
	Group B	0.903	7	0.350
	Group C	0.919	7	0.463
	Group D	0.949	7	0.719
Relative tissue weight index	Group A	0.756	7	0.014
	Group B	0.758	7	0.015
	Group C	0.981	7	0.965

	Group D	0.912	7	0.411
Number of degenerated hepatocytes /mm²	Group A	0.600	7	0.000
	Group B	0.988	7	0.988
	Group C	0.948	7	0.711
	Group D	0.600	7	0.000

Weight of Rats

The rats were weighed before the experiment and at the end of it. During experiment they stayed healthy. As data was normally distributed, one way ANOVA test was applied for comparison of the initial bodyweight, final bodyweight and weight gain among the groups.

It was observed that there was no considerable association between **body weight** at the start and end of the experiment (p-value = 0.947, p-value = 0751 respectively). After the completion of the experiment, there was statistically considerable difference in **mean weight gain** of different groups (p-value < 0.001).

Table: 2 Groups and their Weight Change

Variable	Group A Mean ± SD	Group B Mean ± SD	Group C Mean ± SD	Group D Mean ±SD	p-value#
Initial body weight (g)	130.0 ± 16.4	131.1 ± 13.7	134.1 ± 16.4	129.6 ± 16.4	0.947
Final body weight (g)	135.0 ± 16.4	129.0 ± 13.6	137.7 ± 15.4	132.6 ± 16.0	0.751
Weight gain (g)	5.00 ± 0.82	-2.14 ± 0.69	3.57 ± 1.51	3.00 ± 1.00	< 0.001*

One way ANOVA

*p value ≤ 0.05 is regarded as significant statistically.

For more than two comparisons, post hoc Turkey test was used which showed that the "mean weight gain" in group B was considerably lower as compared to group A, C and D. Significant variation was also detected in the "mean weight gain" between group A and D. While, no significant association was seen between group A vs. C and C vs. D.

Table 3: Pair wise comparison of mean body weight and weight gain after experiment amongst groups

	Group	Group	Mean Difference	Std. Error	p-value
weight after experiment	B	A	7.14286	0.56243	< 0.001*
		C	1.4285	0.56243	0.079
		D	2.00000	0.56243	0.008*
Weight gain	C	B	-5.71429	0.56243	< 0.001*
		D	-5.14286	0.56243	< 0.001*
	D	C	0.57143	0.56243	0.742

Weight of Liver and Relative Tissue Weight Index (RTWI)

The mean liver weight and relative tissue weight index in all groups were observed.

One way ANOVA showed that there was great difference in mean liver weight and relative tissue weight index among groups (p value = 0.013, p-value < 0.001 respectively).

Table 4: Comparison of liver weight and relative tissue weight index between groups

Variable	Group A Mean ± SD	Group B Mean ± SD	Group C Mean ± SD	Group D Mean ± SD	p-value
Liver weight (g)	2.57 ± 0.40	3.43 ± 0.58	3.29 ± 0.60	2.69 ± 0.55	0.013*
Relative tissue weight index	1.90 ± 0.15	2.68 ± 0.53	2.37 ± 0.22	2.01 ± 0.21	< 0.001*

One way ANOVA

*p value ≤ 0.05 is regarded as significant statistically

For pair wise comparisons, post hoc Turkey test was used which indicated that liver weight of group B was significantly higher when compared with group A whereas no notable difference was detected in mean liver weight among group A, C and D. Similarly, mean relative tissue weight index of group B and C was significantly higher when compared group A. However, noteworthy difference was noticed between group B and D whereas no significant difference was observed in mean relative tissue weight index between group B vs. C and A vs. D

Table 5: Pair wise comparison of weight of liver and relative tissue weight index among groups

Variable	Group	Group	Mean Difference	Std. Error	p-value
Liver weight (g)	A	B	-0.85714	.28755	0.031*
		C	-0.71429	.28755	0.088
		D	-0.11429	.28755	0.978
	B	C	0.14286	.28755	0.959
		D	0.74286	.28755	0.072
	C	D	0.60000	.28755	0.186

Variable	Group	Group	Mean Difference	Std. Error	p-value
Relative tissue weight index	A	B	-0.77571	.16732	0.001*
		C	-0.47286	.16732	0.043*
		D	-0.11000	.16732	0.912
	B	C	0.30286	.16732	0.293
		D	0.66571	0.1673	0.003
	C	D	-0.36286	.16732	0.161

*p value ≤ 0.05 is regarded as significant statistically

Table 6: Number of Degenerated Hepatocytes

Variable	Group A Mean ± SD	Group B Mean ± SD	Group C Mean ± SD	Group D Mean ± SD	p-value
Number of degenerated hepatocytes /mm²	0.110 ± 0.010	2.090 ± 0.126	0.281 ± 0.077	0.110 ± 0.010	< 0.001*

For multiple comparisons, post hoc Tukey test was used which indicated that mean number of degenerated hepatocytes/mm² size in group B was significantly higher when compared with group A, C and D. However, no significant difference was observed in mean number of degenerated hepatocytes /mm² between group A and D.

Hepatocytes Vacuolization

Fisher's exact test showed that there was an association between hepatocytes vacuolization and groups. Hepatocytes vacuolization was absent in all rats of group A. In group B, hepatocytes vacuolization was observed in all rats. In group C, hepatocytes vacuolization was present in 4 (57.1%) rats whereas in group D, hepatocytes vacuolization was present in only 2 (28.6%) rats.

Table 7: Distribution of Hepatocyte Vacuolization among Groups

	Group A n (%)	Group B n (%)	Group C n (%)	Group D n (%)	P-Value
Absent	7 (100.0%)	0 (0.0%)	3 (42.9%)	5 (71.4%)	0.001*
Present	0 (0.0%)	7 (100.0%)	4 (57.1%)	2 (28.6%)	

Fisher's exact test

*p value ≤ 0.05 is considered statistically significant

Nuclear Changes

Fisher's exact test showed that there was an association between nuclear changes and groups. Nuclear changes in all rats of control group A were absent. In group B, nuclear changes were observed in all rats. In group C, nuclear changes were observed in 5 (71.4%) rats whereas in group D, nuclear changes were observed in only 1 (14.3%) rat.

Table 8: Distribution of Nuclear Changes among Groups

Nuclear Change	Group A n (%)	Group B n (%)	Group C n (%)	Group D n (%)	p-value
Absent	7 (100.0%)	0 (0.0%)	2 (28.6%)	6 (85.7%)	< 0.001*
Present	0 (0.0%)	7 (100.0%)	5 (71.4%)	1 (14.3%)	

Discussion

In the last years, research has been conducted on the use of traditional drugs for the treatment of the liver injury. Now, a study has been conducted to find out the prophylactic effects of the plant known as Lawsonia inermis Linn. The aqueous leaf extract of the drug is seen on the hepatotoxicity due to acetaminophen. The most commonly used drug for pain relief and fever is acetaminophen. The dose of any drug is very necessary because a high dose can lead to hepatotoxicity. When the dose of paracetamol is increased, it produces hepatotoxic effects. [11] The formation of reactive oxygen species is the reason for this harmful effect. These reactive species damage the cell membrane as well as nucleic acids. The effect is on the polyunsaturated fatty acids, DNA bases, and the protein groups. Normally there is a balance between ROS and antioxidant enzyme system. However, when a high dose of acetaminophen is taken, the balance disturbs leading to liver damage. [12]

A single-use of high-dose acetaminophen causes severe damaging effects on the structure of the liver. The high dose of APAP will cause the formation of excessive NAPQI (N-acetyl-p-benzoquinone imine). The NAPQI needs fast detoxification but because of the saturation of two pathways namely glucuronidation and sulfation pathway.

Albino rats were used for this research. These are easy to handle. They can bear a long duration of experimentation

as they lack-vomiting a center. “Mean body weight” gain or loss of rats was not considerably different before and after an experiment in all groups. Results were almost similar to those given by Mohammad et al., 2014.

“Liver weight” of toxic group B was a lot higher when compared with group A, C, and D. To standardize the weight of liver in each animal, “tissue body weight index (TBWI)” was calculated, MRTWI of group B significantly higher when compared with group A and D. No significant difference was observed in “mean relative tissue weight index” among A, C and D groups. This matches with the results given by Lakshami et al., in 2020. Acetaminophen caused a significant increase in no. of degenerated hepatocytes/mm2 so results showed that mean no. of degenerated hepatocytes/mm2 in toxic group B was significantly higher when compared with group A, C, and D. This death of liver cells due to apoptosis from free radicals initiated mitochondrial dysfunction. As an antioxidant, Lawsonia inermis might have decreased oxidative stress by stopping lipid peroxidation, reducing free radicals thus providing protective effects in groups C and D.

“Hepatocytes vacuolization” could be the signs of hepatotoxicity and cell degeneration in APAP treated group B which was less remarkable in henna (100mg/kg)+APAP treated group(C) while group D appeared more or less similar to control sections revealing the possible protective effects of henna leaves in my study. These results correlate with studies done by Sakran et al., 2014 and Mohammad et al., 2015. The results also correlate with that of a study done by Lakshmi et al., 2020

In the microscopic examination, a nucleus of control, toxic, and treated groups are examined. In the control group, the hepatocytes are hexagonal with centrally placed rounded nucleus.

While in toxic group B, the nucleus appeared pyknotic and karryorhectic and scattered throughout the degenerating hepatocytes. In toxic groups, nucleus of all rats showed degenerative changes while in groups of C & D, only two and only one out of seven rats in each group show nuclear changes respectively. These results are similar to results given by Mohammad et al., 2015 in which methanol extract of Lawsonia inermis was used as a protective agent at a dose of 100mg/kg and 200mg/kg against CCl4 induced hepatotoxicity.

Collectively, the current study demonstrated that aqueous extract of Lawsonia inermis Linn leaves showed a prophylactically protective effect against APAP induced hepatotoxicity in a dose-dependent manner as evident from results when compared to biochemistry in APAP-only treated group along with histopathological observations. This plant extract showed hepatoprotective activity due to its antioxidant property provided by its flavonoid contents.

Conclusion

Taking into account the above-mentioned observations and results; it gives strong support to the suspicion that Lawsonia inermis has significant prophylactic effects on the microarchitecture of the liver that would be destroyed by the toxic effect of acetaminophen. However, more long-term studies can be made to further explore the genetic effects of this drug therapy on the liver.

Reference

1. Borade, A.S.,. Kale B. N and Shete, R.V. A phytopharmacological review on Lawsonia inermis(Linn). Int. J. of Pharm. & Life Sci. (IJPLS). 2 (1), 536-541
2. Neera, J. Dalal N. Bisht, V and Dhakar, U. 2019. Review Article. Henna (Lawsonia inermis): from Plant to Palm. International Journal of Agriculture Sciences. 11(24), 9370-9372

3. Chaibi, R., Romdhane, M. Ferchichi, A. Bouajila, J. 2015. Assessment of antioxidant, anti-inflammatory, anti-cholinesterase and cytotoxic activities of Henna (Lawsonia inermis) flowers. 8, 85-92
4. Yadav, S. Kumar, A. Dora, J and Kumar, A.2013. Essential Perspectives of Lawsonia inermis. 2(2), 888-896
5. Upadhyay, B. Dhaker, A. K. Sing K.P. h, and Kumar, A. Phytochemical Analysis and Influence of Edaphic Factors on Lawsone Content of Lawsonia inermis L. J. Phytol. 2(6)47-54.
6. Yigit, D. 2017. Antifungal Activity of Lawsonia inermis (Henna) Against Clinical Candida Isolates. Erzincan Univ. J. Sci. Tech. 10 (2), 196-202.
7. Ramasubramaniam, P. Anandhavel. R. Nataraj.P. T.R. and Kumaran. B. 2016. Dyeing of Silk with Lawsonia inermis L. (Henna) Extract and Study on their Fasting Properties. International Journal of Research – Granthaalayah.4 (2), 101-106.
8. Mohamed, M.A. Taj Eldin, I.M. Mohammed,A.H., Hassan, H.M. 2015. Effects of Lawsonia inermis. (Henna) leaves' methanolic extract on carbon tetrachloride-induced hepatotoxicity in rats. Journal of Intercultural Ethnopharmacology. 5(1), 22-26.
9. Galloa, F.R. Multaria, G Palazzinoa, G Pagliucaa, G. Zadehb, Biapac, P. C. Nicolettid, M. 2014. Henna through the centuries: a quick HPTLC analysis proposal to check henna identity. Rev Bras Farmacogn 24,133-140.
10. Olawuyi, S.T., Paul, W.C. and Oladipo, G.S. 2018. Effects of Aqueous Leaf-extract of Lawsonia Inermis on Aluminum Induced Oxidative Stress on the Histology and Histopathology of the Testes of Adult Wistar. Rats. Indian J. Physiol. Pharm. 62(4), 468-478.
11. Mitchell R. Mcgill. (2013). Acetaminophen Hepatotoxicity In Humans And Mice.
12. Hartmut Jaeschke C. David Williams Anup Ramachandran Mary L. Bajt. (2014). Acetaminophen hepatotoxicity and repair: the role of sterile inflammation and innate immunity. 32(1), 8–20.
13. McGill, M. R., & Jaeschke, H. (2013). Metabolism and disposition of acetaminophen: recent advances in relation to hepatotoxicity and diagnosis. Pharmaceutical Research, 30(9), 2174–2187.

How to citation this article: Dr Misbah Ishtiaq, Muhammad Ishtiaq Rana, Dr Saima Abdul Waheed, Dr Iqra Javed, Dr Abdul Hanan Jawad, Dr Haseeb Ahmed Awan,"Dose Dependent Prophylactic Effect of Henna (Lawsonia inermis Linn) Leaf on Acetaminophen Induced Hepatotoxicity in Adult Albino Rats", IJMACR- November - December - 2020, Vol – 3, Issue -6, P. No. 01 –07.