International Journal of ChemTech Research
CODEN (USA): IJCRGG ISSN: 0974-4290
Vol.8, No.11 pp 617-623, 2015

Study of the Protective Effect of Red Mulberry (*Morus Rubra L*)Against Paracetmol-Induced Liver Injury on Oxidative Damage Antioxidant Enzymes and Biochemical Parameters in Rats

[1]Rehab Ibrahim Tag Al Deen and [2]Hany M.A.Wahba

[1]Home Economics Dept., Faculty of Specific Education, Zagazig University, Egypt.
[2]Nutrition and Food science Dept., National Research Center, Egypt

Abstract: In this study hepatoprotective and antioxidant properties of red mulberry (Morus rubra L) was investigated against liver injury caused by paracetmol in rats. thirty-two male Sprague-Dawley rats weighing (120 ± 5) were used for sixty days. Rats classified into to four groups (8 rats). The first group was used as a negative control and fed on the basal diet only. Other three groups had given were administered paracetamol drug at a single dose of 2 g/kg by stomach tube to induce liver injury. One of these groups left as positive control (second group). The third group was treated with fortified cake 5% red mulberry powder. The fourth group was treated with fortified cake 10% red mulberry powder.
Plasma and hepatic content analysis showed that red mulberry inhibited the levels of liver injury biomarkers (AST, ALT and ALP), triglyceride (TG) and cholesterol (TC). These results suggested that red mulberry prevents paracetamol-induced liver injury. This may be mediated by multiple pathways, including reduced lipid accumulation and decreased oxidative stress.
Keywords: Morus rubra-Oxidative-Paracetamol- Hepatotoxicity- Fortified.

Introduction

The liver is one of the vital organs of the body and the largest glandular organ that secretes chemicals—because it producesbile, a substance needed to digest fats. It performs multiple critical functions to keep the body pure of toxins and harmful substances[1].

The metabolism of drugs is done by the liver, the mechanisms by which drugs might injure the liver, Drug-induced liver injury is a major health problem that challenges health care professionals[2]. Paracetamol, also known as acetaminophen or APAP, is classified as a mild analgesic. It is commonly used for the relief of headaches and other minor aches and pains and is a major ingredient in numerous cold and flu remedies. It is important to remember that, when used at therapeutic levels, paracetamol is usually safe and effective. However taking 4 g per day (or slightly more) for a few days, has been known to result in hepatotoxicity[3]. Now days, the world towards for Traditional plant medicines or herbal formulations might offer a natural key to hepatoprotective effect against xenobiotic/drug[4].

Red mulberry *(Morus rubra),* is a species of mulberry native to eastern and central North America. It is belong to the family Moraceae, comprises 10–16 species of deciduous trees commonly known as mulberries

which are growing wild and under cultivation in many temperate world regions**.** The fruit is a compound cluster of several small achenes surrounded by a fleshy calyx, similar in appearance to a blackberry, 2–3 cm long, when it is ripening it is red or dark purple, edible and very sweet with a good flavor[5]**.** The most of modern medical systems has been reported mulberries as antioxidant and has protective action against oxidative damage to membranes and biomolecules this is due to it contains important phyto-chemical constituents e.g. flavonoids, alkaloids and phenols. The flavonoids compound has shown to have hepatoprotective activity as reported previously by[6]**.** In china, they are using both the red and white berry to nourish the blood as it consider as blood tonic, treat weakness, anemia, treat urinary incontinence and cleanse the liver from toxins[7]**.**

The main objective of the present study is to evaluate the protective effect of red mulberry through fortifying the cake by different concentrations for hepatic injury induced by paracetamol in Albino rats.

Material and methods

A-Materials:

Red mulberry (*Morus nigra*) was obtained from local market in Cairo, Egypt.

Paracetamol drug ©: was obtained from Kahira Pharm and Chem. Ind. Co., Cairo- Egypt.

Kits: For biochemical analysis were purchased from the Gamma Trade Company for Pharmaceutical and Chemicals, Dokki, Egypt.

Rats: Thirty-two male Sprague-Dawley rats weighing (120 ± 5) were purchased from Farm of experimental animals in Helwan, Egypt. The basal diet consisted of protein (13%), fat (4%), salt mixture (3.5%), vitamin mixture (1%), choline (0.2%), cellulose (5%) and the remainder was starch[8]**.**

Methods:

Preparation of red mulberry: Red mulberry fruits were washed with tap water, chopped into small pieces, dried with hot air oven (40-60°C) for approximately 6 – 7 hours[9]**.** The dried samples obtained as flakes were powdered in a lab scale homogenizer and sieved using 20mm mesh sieve. Wheat flour of (72% extraction) and sugar were replaced by red mulberry powder to produce mixtures containing (5, 10, and 15%).

Production of red mulberry fortified cake

Cakes with the substituted levels (0, 5 and 10 %) of papaya powder with wheat flour. Cakes was prepared and processed according to the common method described by[10]**.**

Proximate Composition:

Red mulberry powder was analyzed for moisture, fat, protein, ash, and crude fiber contents according to[11]**.** While total carbohydrates were calculated by difference as following: Carbohydrates % = 100 - (moisture % + fat % +protein % + ash) according to the methods of the[12]**.**

Preliminary phytochemical screening of Red mulberry

Detection of tannins: Tannins was detected in the plant sample according to the method of[13]**.**

Detection of saponins: Saponins substances were detected in different crude extracts under investigation according to the method of[14]**.**

Detection of flavonoids: Flavonoids substances were detected in extracts of different samples using the method of [13]**.**

Detection of carbohydrates and glycosides: Carbohydrates and glycosides were treated by Molish test according to the method of[13]**.**

Experimental design:

The experimental rats were divided into five groups after adaptation period (7day). The first group which kept as normal control (-ve) group (8 rats) which fed on basal diet only. The rest of rats were administered paracetamol drug at a single dose of 2 g/kg [15] by stomach tube to induce liver injury then classified into three groups. One of them acted as control (+ve) and the other two treated groups were cake with 5% red mulberry powder, cake with 10% red mulberry powder Feeding and growth performance were carried out by determination of daily food intake, body weight gain and feed efficiency ratio (FE:,R) according to[16].

Biochemical analysis:

The rats were sacrificed at the end of the experiment (60 days) for collection of blood samples which centrifuged at 3000 rpm/ 15 minutes to obtain serum.

Determination of liver function parameters:

The livers of rats were also collected for some biochemical analysis. Serum aspartate and alanine ainino transferase, alkaline phosphatase and gamma glutamyle transferase (AST, ALT, AI'& yGT) enzymes activity, were estimated according to [17,18,9] respectively. Also, serum total bilirubin and total protein were determined according to[20,21], respectively.

Determination of serum lipids:-

Serum cholesterol, triglycerides (TG) and high density lipoprotein cholesterol (HDL-c) were determined by using enzymatic colorimetric methods [22,23,24]. Low density lipoprotein cholesterol (LDL-c), very low density lipoprotein cholesterol (VLDL-c) and cholesterol/ HDL-c were calculated according to [25, 26], a total lipid was determined according to[27].

Assay of hepatic antioxidants parameters and nitric oxide:

Liver content of superoxide dismutase (SOD) activity and total antioxidants capacity (TAC), were determined according to [28,29], NO was measured by modified Griess reaction[30].

Statistical analysis:

The obtained data were statistically analyzed using computerized SPSS (Statistic Program Sigmastat, Statistical Soft-Ware, SAS Institute, Cary, NC). Effects of different treatments were analyzed by one way ANOVA (Analysis of variance) test using Duncan's multiple range test and $p<0.05$ was used to indicate significance between different groups according to[31].

Results and Discussion

The chemical composition of the red mulberry was represented in table (1); it contains (81.35, 7.96g, 7.09 and 1.54g) of moisture, ash, carbohydrates and protein respectively. Table (2) showing the Preliminary phytochemical screening of red mulberry as our results indicate that it contains tannins, saponins, glycoside, flavanoid, carbohydrate.

Table 1: Chemical composition of red mulberry g/100g:

Sample	Moisture	Protein	Fat	Ash	Fiber	Carbohydrates
Red mulberry	81.35	1.54	0.62	7.96	1.43	7.09

Each value represents the average of three determinations.

Table 2: Preliminary phytochemical screening of red mulberry:

Sample	Tannins	Saponins	Flavonoids	glycoside	*Carbohydrates*
Red mulberry	+	+	+	+	+

Table (3): Body weight gain, food intake and FER of the experimental rat groups

Groups / Variables	Normal control	Positive control	Red mulberry fortified cake 5%	Red mulberry fortified cake 10%
Body weight gain (g)	95.72± 8.42 [a]	41.88± 5.1 [d]	66.65± 5.57 [c]	74.21± 8.21 [b]
Feed intake g/day	16.85± 1.84 [a]	11.73± 1.17 [c]	15.21± 1.22 [a]	16.81± 1.71 [a]
FER	0.0894± 0.001[a]	0.059± 0.003 [d]	0.073± 0.004 [b]	0.073± 0.002 [b]

Comparing to negative normal group, the positive group showed a significant increase in body weight gain, feed intake and FER respectively which was represented on table (3). While the fortified cake with red mulberry at different ratios revealed the impact of the positive group as there was a significant decrease in those parameters.

This is due to the red mulberry compose of health promoting phyto-nutrient compounds like polyphenol pigment antioxidants, minerals, and vitamins that are essential for optimum health beside They are rich in B-complex group of vitamins B-6, niacin, riboflavin and folic acid. These vitamins are function as co-factors and help body in the metabolism of carbohydrates, protein, and fats. Also, the fibrous fruit boosts the digestive function of the body by burning additional calories, helping in weight loss. So our results were matched with[32].

The positive control group showed significant increase in AST, ALT and ALP activities while significant decrease in total bilirubin and total protein compared to normal control group which were represented in table (4). These results were matched with [33] who revealed that Damage to the liver, results not from paracetamol itself, but from one of its metabolites, *N*-acetyl-*p*-benzoquinoneimine (NAPQI). NAPQI depletes the liver's natural antioxidant glutathione and directly damages cells in the liver, leading to liver failure.

Table (4): Serum amino transferase (AST & ALT), alkaline phosphotase enzymes (Alk –Pho), total bilirubin and total protein of the experimental rats groups

Groups / Variables	Normal control	Positive control	Red mulberry fortified cake 5%	Red mulberry fortified cake 10%
AST (µ /ml)	41.17± 5.81[d]	72.39± 9.61 [a]	51.14± 8.10 [b]	48.21± 6.15 [c]
ALT (µ /ml)	13.35± 1.12[e]	28.55± 3.35 [a]	16.28± 2.01 [c]	18.13± 3.51 [b]
Alk –Pho (µ /ml)	30.17± 5.66 [d]	50.38± 5.81 [a]	38.73± 4.37 [b]	38.34± 5.01 [b]
total bilirubin (mg/dl)	0.77± 0.01 [d]	1.95± 0.11 [a]	0.88± 0.12 [c]	0.75± 0.13 [d]
total protein (g/dl)	6.83± 0.26 [c]	4.41± 1.01 [d]	5.41± 0.77 [c]	6.17± 0.67 [b]

Mean values in each column having different superscript (a, b, c) denote significant difference

On other hand, the other treated groups showed significant decrease in AST, ALT and ALP activities while significant increase in total bilirubin and total protein compared to positive control group. The fortification of cake by red mulberry enhanced the hepatosomatic index of paracetamol treated rats and brought this parameter back to values very similar to that observed in the control group. Also, the fortification with red mulberry given during the paracetamol application provided significant protection from the hepatotoxicity of

paracetamol. Recently [6] reported the hepatoprotective role of red mulberry on paracetamol induced liver injury in mice.

These studies showed that black mulberry administration prevented ALT and AST increase and improved liver function in rats. Moreover, [34] concluded that red mulberry juice possess antioxidant properties which could prevent liver dysfunction induced by CCl4. Thus, it may be due to free radical scavenger and antioxidant activities of red mulberry constituents, especially quercetin, isoquercitrin and anthocyanins**.**

Data presented in Table (6) showed that the effect of cake fortified by red mulberry on Total antioxidants (TAO), superoxide dismutase (SOD) enzymes and nitric oxide (NO), as the positive control group showed significant decrease in total antioxidant (TAO) and superoxide dismutase (SOD) while showed significant increase in nitric oxide (NO) compared to normal control group. This results were matched with [35] who demonstrated that toxication with paracetamol can induce lipid peroxidation and might cause change in the activity of some antioxidant enzymes in liver. While the other treated groups showed significant increase in total antioxidant (TAO) and superoxide dismutase (SOD) while showed significant decrease in nitric oxide (NO) compared with positive control group. The berries contain resveratrol which is a polyphenol flavonoid antioxidant. Resveratrol protects against free radical molecules by altering molecular mechanisms in the blood vessels; reducing their susceptibility to damage through reduced activity of angiotensin that causing blood vessel constriction that would elevate blood pressure**.** Red mulberry is a good source of very important vitamins and minerals such as vit C, vit E, selenium and zinc which they are acting as a free radical scavengers that protect the body from the oxidative stress and free radicals which can cause a wide damage of the organs [36]**.**

Table (5): Serum Total antioxidants, superoxide dismutase (SOD) enzymes and nitric oxide (NO) of the experimental rats groups.

Groups / Variables	Normal control	Positive control	Red mulberry fortified cake 5%	Red mulberry fortified cake 10%
Total antioxidants mmol/L	3.55± 0.22 ^{a}	1.67± 0.15 ^{c}	2.74± 0.06 ^{b}	2.92± 0.06 ^{b}
Superoxide dismutase U/Ml	70.13± 5.22 ^{a}	21.25± 3.47 ^{c}	55.87± 6.35 ^{c}	62.29± 7.23 ^{b}
NO (µmol /l)	2.17± 0.33 ^{d}	13.99± 1.44 ^{a}	3.22± 1.03 ^{c}	3.11± 1.05 ^{c}

Mean values in each column having different superscript (a, b, c) denote significant difference.

Table (6): Serum lipid profile of the experimental rat groups.

Groups / Variables	Normal control	Positive control	Red mulberry fortified cake 5%	Red mulberry fortified cake 10%
TC mg/dl	82.27± 4.16 ^{d}	128.57± 4.71 ^{a}	97.80± 2.44 ^{c}	92.27± 2.89 ^{c}
TG mg/dl	68.13± 2.97 ^{d}	68.13± 2.97^{d}	79.47± 5.22^{bc}	73.27± 1.42^{cd}
HDL-c mg/dl	39.50± 2.29^{a}	28.23± 1.37^{c}	33.53± 3.02^{b}	37.70± 2.07^{a}
LDL-c mg/dl	29.14± 5.99^{e}	78.87± 3.55^{a}	48.37± 1.34^{c}	39.91± 3.66^{d}
VLDL-c mg/dl	13.63± 0.59^{d}	21.47± 2.13^{a}	15.89± 1.04^{bc}	14.65± 0.28^{cd}

Mean values in each column having different superscript (a, b, c) denote significant difference.

Comparing to negative normal group, the positive group showed a significant increase in TC, TG, LDL, V-LDL and significant decrease in HDL and that was represented in table (5). On the other hand, the results of the other treated groups provided significant protection from the hepatotoxicity of paracetamol on lipid profile as there was significant decrease in TC, TG, LDL, V-LDL and significant increase in HDL compared to positive control group. This result was agreed with [37] who reported that red mulberry are plenty of nutrients and minerals. There is some evidence that red mulberry are a weight control herb as it is high in fiber, very low in carbs and fats, two attributes that should also help promote weight control.

Conclusions

In conclusion, the present study clearly demonstrates that cake fortified by different concentration of red mulberry can protect the liver from the injury caused by paracetamol intoxication, probably due to its antioxidant and cytoprotective characteristics. In addition, The results showed that all the red mulberry fruit was nutritionally rich and may be useful in a balanced diet. Higher phenolic contents with antioxidant activity further increase their nutritive as well as phytomedicinal potentials.

References

1. Canadian Cancer Society (2015): Anatomy and physiology of the liver. Cancer.ca. Retrieved. 06-26.
2. Yuan L, Kaplowitz N. (2013): Mechanisms of drug-induced liver injury. Clin Liver Dis. 17(4):507–18.
3. Larson, A.M., Polson, J., Fontana, R.J., Davern, T.J., Lalani, E., Hynan, L.S., Reisch, J.S., Schiødt, F.V., Ostapowicz, G., Shakil, A.O., and Lee, W.M. (2005): Acute liver failure study group. Acetaminophen-induced acute liver failure: results of a United States multicenter, prospective study. *Hepatology (Baltimore,Md.)* 42 (6):136472.
4. Mukazayire, M.J., Minani, V., Ruffo, C.K., Bizuru, E., Stévigny, C., and Duez, P. (2011): Traditional phytotherapy remedies used in Southern Rwanda for the treatment of liver diseases. J Ethnopharmacol.138 (2):415–31).
5. Northeastern Area State and Private Forestry (2011): Red Mulberry - USDA Forest Service. Retrieved 1.
6. Mallhi, T.H., Qadir, M.I., Khan, Y.H., and Ali, M. (2014): Hepatoprotective activity of aqueous methanolic extract of Morus nigra against paracetamol-induced hepatotoxicity in mice. Bangladesh J Pharmacol. 2014;9:60–6.
7. Memon, A.A., Memon, N., Luthria, D.L., Bhanger, M.I., and Pitafi, A.A. (2010): Phenolic acid profiling and antioxidant potential of mulberry (Morus laevigata, Morus nigra and Morus alba L.) leaves and fruits grown in Pakistan. Pol J Food Nutr Sci. 60(1):25–32 .
8. Reeves , P.G; Nielsen , P.M. and Fahmy , G.G. (1993): AIN-93. Purified diets for laboratory rodents : I inal report of the American Institute of Nutrition adhoc wriling committee on the reformulation of the AIN-76 A Rodent diet. J. Nutrition, 123: 1939-151.
9. A.O.A.C., (2005): Official methods for Analysis of the Association of Official Analytical Chemists. A.O.A.C.. 12th Hd. Washington, D.C.
10. Penfield, M.P. and A.M. Campbell, (1990a): Evaluating food by objective methods. In "Experimental Food Science," 3rd ed. pp. 23-45. Academic Press, Inc. San Diego, CA.
11. A.O.A.C. (2002): International. 17th ed., Gaithersburg, Maryland, USA. Chap. 4. pp: 21-39.
12. A.O.A.C. (2000): Official Methods of Analysis of tea polyphenols. Mol. Nutr. and Food Res.
13. Kahkonen, M.P., Hopia, A.L., Vuorela, H.J., Rauha,J.P., Pihlaja, K., Kujala, T.S., and Heinonen, M. (1999): Antioxidant activity of plant extracts containing phenolic compounds, J. Agr. Food Chem., (47): 3954-3962.
14. Trease, G.E. (1961): Textbook of pharmacognosybillier , 8th edn. Tindall and Cox. London, 596. Thymoquinone supplementation reverses acetaminophen-induced oxidative stress, nitric oxide production and energy decline in mice liver. Food Chem Toxicol, 48:2361-2365.
15. Rafael, B., Daniela, F.H., Haini, S., Hussein, A., Zipora, M, Moshe,P., Liliana, Z.,Yon a, A., Ran and Zjann'rir, J.,(1999):Hypothyroidism protects rat liver from acetaminophen hepatotoxicity. Digestive Diseases and Sciences. 44(6): 1228-1235.
16. Chapman, D.G., Gastilla, R. and Campbell, T.A. (1950): Evaluation of protein in food. I. A. Method for the determination of protein efficiency ratio. Can. J. Bioehem. Physio. I (37) 679-686.

17. Reitman, S. and Frankel, S. (1957): Determination of glutamate pyruvat transaminase and glutamate oxaloacetate transaminase. Amer. J. Clin. Path... 28:56-63.
18. Draper, H .H and Hadley, M. (1990): Malondialdehyde determination as index of lipid peroxideation. Methods Enzymol. I 86, 421 -43 I.
19. Kind, P.R and King, E.J. (1954): Estimation of alkaline phosphatase activity by determination of hydrolyzed phenorwith aminoantipyrene. J. Clin.Path.7, 322.
20. Jendrassik, L. (1938): Colorimetric determination of bilirubin. Bioehem., 97:72-81.
21. Weichselbaum, T.F. (1946): An accurate and rapid method for the determination of protein in small amount of blood scrum and plasma. Am ..I Clin Path (16):40.
22. Abell, L.L., Levy, B.B., Brodie, B.B and Kendal, R. (1952): A simplified method for the estimation of total cholesterol in serum and demonstration of its specificity. .1 .Biol .Chem, 357-366.
23. Buccolo, G. and David, H. (1973): Quantitative delerminarion of serum triglyeerides by use enzymes. Clin. Chem.,19: 419-32.
24. Kostener, C. M. (1977): Enzymatic determination of cholesterol high density lipoprotein fraction prepared by polyanion precipitation. J. Clin. Chem., 22:695.
25. Fruchart, G.C. (1982): LDL-Cholesterol Determination after Separation of Low Density Lipoprotein. Rev. Fr. Des. Laboratories. 103: 7-1 17.
26. Castelli ,T. and Levilar, Y. (1977): Atherogenic ,index Curr Presc p39.
27. Folch, J., lees, M., and Stanley, G.H. (1957): A simple method for isolation and purification of total lipid from animal tissue. J Biol Chem 266: 497-509.
28. Oyanagui, Y. (1984): Reevaluation of assay methods and establishment of kits for superoxide dismutase activity. Anal.Biochem; 42(2):290-296.
29. Cao G., Alessio H. M., and Cutler R. G.(1993): Oxygen-radical absorbance capacity assay for antioxidants. Free Radical Biol. Med. 14:303–311.
30. Nagi MN, Almakki HA, Sayed-Ahmed MM, and Al-Bekairi A. (2010): Thymoquinone supplementation reverses acetaminophen-induced oxidative stress, nitric oxide production and energy decline in mice liver. Food Chem Toxicol. Sep;48(8-9):2361-5.
31. Snedecor, G.W. and Cochran, W.G. (1967): Statistical Methods; 7th Ed., The Lowa State University Press., Ames, Lowa, U.S.A.
32. Adedosu, O.T., Oyedeji, A.T., Iwaku, T., Ehigie, A.F., and Olorunsogo, O.O. (2014): Hepatoprotective activity and inhibitory effect of flavonoid–rich extract of *Brysocarpus coccineus* leaves on mitochondrial membrane permeability transition pore. Asian Journal of Natural & Applied Sciences. 3(3):92–100.
33. Boutis, K., and Shannon, M., (2001). Nephrotoxicity after acute severe acetaminophen poisoning in adolescents. Journal of toxicology. Clinical toxicology 39 (5): 441–5.
34. Agha, F.G., Fyiad, A.A., and El Safi, H. (2012): Comparative study of the protective role of black berry juice and silymarin against liver damage induced by carbon tetrachloride in rats. J Appl Sci Res. 8(2):727–38.
35. Dart, R.C., Erdman, A.R., Olson, K.R., Christianson, G., Manoguerra, A.S., Chyka, P.A., Caravati, E.M., Wax, P.M., Keyes, D.C., Woolf, A.D., Scharman, E.J., Booze, L.L., and Troutman, W.G.(2006): American Association of Poison Control Centers Acetaminophen poisoning: an evidence-based consensus guideline for out-of-hospital management". Clinical toxicology (Philadelphia, Pa.) 44 (1): 1–18.
36. Mustafa, O., Zgen, A., Sedat Serc, E.B., and Cemal Kaya, C. (2008): Phytochemical and antioxidant properties of anthocyanin-rich Morus nigra and Morus rubra. Food Chem. 12-23.
37. Lin, J.Y., and Tang, C.Y. (2007): Determination of total phenolic and flavonoid contents in selected fruits and vegetables, as well as their stimulatory effects on mouse splenocyte proliferation. Food Chem.101 (1):140–147.

*****