Diyala Journal for Pure Science

Protective Effect of Aqueous – Methanol Extract of *Melia azedarach* Against Paracetamol – Induced Hepatitis in Rabbits

Mayada Nazar AL-Khafaji*, Estipraq Yunus Shlash, Amal Ismael Nayef and Noor Salah Shaker

Department of Biology – College of Sciences – University of Diyala – Diyala – Iraq

mamnazar83@gmail.com

Received: 1 March 2019 **Accepted: 29 July 2019**

DOI: https://dx.doi.org/10.24237/djps.15.04.500D

Abstract

The objective of the present study was to investigate the efficiency of aqueous – methanol (30:70%) extract of *Melia azedarach* L. fruit against paracetamol – induced hepatitis in rabbits. This research was conducted at, University of Diyala, College of Veterinary Medicine. Twenty mature, 1-2 years old male rabbits, were kept in a room at 20- 27ºC. After 2 weeks of adaptation, they were divided into four groups of, 5 animals each. Rabbits of the first group were left without any treatment (without extract and without treatment with paracetamol) to serve as a (negative control group). The rabbits in the second group, were treated orally (PO) with aqueous – methanol (30:70%) extract of *M. azedarach* fruit (300 mg / kg body weight) dissolved in 2 ml saline, daily for 9 days. On the 10th day, hepatitis was induced via treatment with paracetamol (250 mg / kg body weight (b.w) dissolved in 2 ml saline) intraperitoneally (IP), daily for 9 days, the treatment with extract was continued till the end of the study on day 18th (hepatoprotective group). Meanwhile, rabbits of the third group were left without treatment during the first 9 days, then hepatitis was induced on 10th day, as described previously, accompanied by treatment with the same dosage of the extract, dissolved in 2 ml saline for 9 days (PO) (hepatocurative group). Rabbits in the fourth group were not treated with extract during the study, but on the 10th day they were treated with paracetamol, 250 mg / kg b. wt.

dissolved in 2 ml saline (I. P.) for 9 days (control group). All animals, were euthanized on day 18 and, blood was collected and serum was obtained to estimate levels of serum enzymes (RMS, ALT, AST, TSB, TSP, BUN, and Creatinine). The dependent parameters were heart rates, respiratory rates, body weight and body temperature, in addition to some hematological parameters, clotting and bleeding times, total and differential leucocytes count. The results revealed that the levels of biochemical parameters increased in paracetamol- treated rabbits in comparison with the non- treated groups, while the total protein content decreased. The extract exhibited a significant reduction in biochemical parameters ($P<0.05$). In conclusion, it is obvious that Sibahbah aqueous alcohol extract possesses a significant hepatoprotective effect against paracetamol – induced hepatitis.

Keywords: Hepatitis, Melia azedarach, Aqueous – methanol extract, Rabbits.

التأثير الوقائي للمستخلص المائي – الميثانولي للسبحبح (*Melia azedarach*) ضد التهاب الكبد المحدث بالبر سيتمول في الارانب

ميادة نزار جبار الخفاجي، استبرق يونس شلش، امل اسماعيل نايف و نور صلاح شكر

قسم علوم الحياة – كلية العلوم – جامعة ديالى – العراق

الخلاصة

الغرض من الدراسة الحالية كان التحري عن التأثير الحامي للمستخلص المائي – الميثانول (70:30%) للميليا ازيدراج ضد التهاب الكبد المحدث بالبرسيتمول في الارانب. انجز البحث في جامعة ديالى كلية الطب البيطري. عشرون من ذكور الارانب الناضجة بعمر 1-2 سنة تركت للتكيف بغرفة ذا 20- 27 درجة مئوية. بعد اسبوعين من التكيف قسمت الى 4 مجموعات كل منها تحتوي 5 حيوانات. تركت ارانب المجموعة الاولى بدون علاج بالمستخلص النباتي ولم تتعرض للبرسيتمول (مجموعة سيطرة سالبة). بينما عولجت حيوانات المجموعة الثانية عن طريق الفم بالمستخلص المائي – الميثانول لثمار المليا ازيدراج 300 ملغم \ كغم من وزن الجسم مذابة في 2 مل عالق ملح الطعام الطبيعي (سلاين) يوميا لمدة 9 ايام وفي اليوم العاشر احدث التهاب الكبد من خلال المعالجة بالبرسيتمول بجرعة 250 ملغم \ كغم معلق بالسائل الملحي الطبيعي حقننا في الصفاق لمدة 9 ايام متتالية واستمر العلاج بالمستخلص لغاية نهاية الدراسة في اليوم 18 (مجموعة حماية الكبد). بينما تركت ارانب

Diyala Journal for Pure Science

Protective Effect of Aqueous – Methanol Extract of *Melia azedarach* Against Paracetamol – Induced Hepatitis in Rabbits

Mayada Nazar AL-Khafaji, Estipraq Yunus Shlash, Amal Ismael Nayef and Noor Salah Shake

المجموعة الثالثة بدون علاج خلال 9 ايام الاولى، ثم احدث التهاب الكبد في اليوم العاشر، كما مذكور اعلاها رافقها العلاج بنفس الجرعة من المستخلص مذاب في 2 مل سلاين ولمدة 9 ايام عن طريق الفم (مجموعة علاجية الكبد). لم تعالج ارانب المجموعة الرابعة بالمستخلص خلال الدراسة لكن عولجت في اليوم العاشر بالبر سيتمول 250 ملغم \ كغم مذاب بالسائل الملحي الطبيعي حقنا في الصفاق لمدة 9 ايام (مجموعة سيطرة). كل الحيوانات تم ذبحها في اليوم 18، وجمع الدم وتم الحصول على المصل لتحديد مستويات انزيمات المصل (سكر الدم العشوائي، ناقل الامينيز الالنين، ناقل الامينيز الاسبرتيت، البليروبين الكلي في المصل، البروتين الكلي في المصل، يوريا نيتروجين الدم، الكرياتنين). من المعايير الاخرى التي اعتمدت معدل التنفس، سرعة ضربات القلب، حرارة الجسم و وزن الجسم فضلا عن بعض الفحوصات الدموية وايجاد زمن التخثر و زمن النزف وعد الخلايا البيض التفريقي والكلي. اظهرت النتائج ان مستويات المعايير الكيميوحيوية ارتفعت في الارانب المعالجة بالبرسيتمول مقارنة بتلك التي لم تتعالج بالبرسيتمول. بينما محتوى البروتين الكلي قل. اظهر المستخلص تأثير خافض وبشكل معنوي في المعايير الكيميوحيوية ($P<0.05$). الاستنتاج، من الواضح ان مستخلص السبحبح المائي الكحولي يمتلك تأثيرا حامي للكبد ضد التهاب الكبد المحدث بالبرستمول.

الكلمات المفتاحية: تسمم الكبد، ميليا ازيدراج (السبحبح)، المستخلص المائي – الميثانول، الارانب.

Introduction

Liver performs many physiological functions such as, protection from hazards of harmful drugs and chemicals. Hepatic disorders account for a high mortality rate, Jaundice and hepatitis are major manifestations [1]. Liver is an important organ, which plays essential roles, including; metabolic activities, removal of toxicity of many internal and external harmful agents, e.g. alcoholism and drugs [2].

Recently, plant – derived products received considerable attention because of their diverse pharmacological actions such as antioxidant and hepatoprotective activities [3 and 4].

Various chemical ingredients present in leaf, root, and stem of *M. azedarach*, such as meliacarpin, limonoids, sendanins, trichlins and azedarachins [5]. Leaves extracts contain many phytochemical bioactive contents [6].

Paracetamol is one of typical examples of dose- related toxicity [7]. It is the etiological agent of liver dysfunction, as it exhibits an injurious effect on liver [8 and 9]. In contrast, it is a useful

counter analgesic and antipyretic agent [10]. *M. azedarach* is without risk, in general, in human uses, if used at lower, mild levels, but in higher doses causes a harmful effect if it is taken > 1000 mg in one dose, or > 400mg in a day, by adult human [11], or above 2000 mg per day, in alcoholism [12]. At these conditions' paracetamol can effectively destruct liver. And in few cases, even ordinary non - harmful quantities of plant, may lead to some extent of harm, in normal persons.

Paracetamol has no danger, at a dose which depends in treatment, but at high level, it is one of famous poisonous products, as it may cause liver injury. It is regarded as a product of choice against pain, for many individuals suffering from chronic hepatic disturbances [13 and 14].

Acetaminophen (Paracetamol) may cause liver necrosis [15]. Acute liver injury may involve the parenchyma, bile secretory function or both. Some drugs including acetaminophen have been reported to cause hepatic injury that is cytotoxic in nature [16].

The current research was planned to study the efficiency of water alcohol extract of Sibahbah as preventing agent of liver from injurious effects resulted from treatment with paracetamol in rabbits.

Materials and Methods

Preparation of extract

Mature sibahbah fruits were collected from local gardens of Baquba city, Diyala, Iraq. The fruits were washed under running tap water, then crushed, and ground to fine powder, by a mortar and pestle, then by electric grinder. The grounded fruits, were macerated in aqueous – methanol (30:70) mixture for 3 days with continuous shaking using magnetic stirrer. Then filtered, concentrated using Rotary evaporator, till a semisolid residue was obtained. This extract was used in this study as hepatoprotective agent.

Preparation of paracetamol

Five % solution was prepared, by dissolving 5 g of acetaminophen in 100 ml, distilled water, freshly whenever treatment was applied.

Experimental animals

Twenty healthy mature 1-2 years old male rabbits weighing from 1-1.9 kg were obtained from the Department of Medicine, College of Veterinary Medicine, University of Diyala. The animals were maintained at standard housing conditions and fed green and concentrated diet and water ad libitum [17].

Acute toxicity studies

The acute toxicity of 30: 70% aqueous – methanol *M. azedarach* extract, was initiated in rabbits by staircase method [18]. The LD_{50} was determined as per the OECD guide line no. 423 (acute toxic class method) [19].

Six rabbits were used for determination of acute toxicity of *M. azedarach*. They were grouped in three groups of two rabbits and kept in separated cages. The first group was treated with 250 mg / kg, monitored for 48 hours, if no death occurs, the second group was exposed to 500 mg / kg, while the third group was given 1000 mg / kg and waited for 48 hours to see whether they die or not. All the animals in this section of the study were observed for 14 days.

It was observed that the tested extract was not fatal, even at 1000 mg / kg, hence 300 mg / kg was selected for this research.

Experimental design

After 2 weeks of acclimatization, 20 rabbits were selected, and grouped into four groups of 5 each.

Groups treatment

Gr. I---------- not treated with extract nor exposed to paracetamol.

Gr. II--------- Hepato-protective: treated with extract then induced hepatitis.

Gr. III---------- Hepato- curative: Induced hepatitis then treated by the extract

Gr. IV-------- Control positive: Hepatitis induced without treatment with extract.

Control Negative: Rabbits of 1st group did not treat with the extract and not exposed to paracetamol.

Hepato-protective: rabbits of 2nd group were treated with aqueous – methanol (30:70%) of *M. azedarach* fruit extract, 300 mg / kg b. wt. in 2 ml saline, for 9 days, then hepatitis, was induced, through exposure to paracetamol, 250 mg / kg b. wt. in 2 ml saline, (IP) for 9 days, the treatment with extract was continued till the end of the experiment (in day 18th).

Hepato- curative: Rabbits of 3rd group, were left without treatment, in 1st, 9 days, but hepatitis was induced, as in previous group, accompanied by, treatment with the extract, 300 mg /kg b. wt. in 2 ml saline for 9 days.

Control positive: those of 4th group, were exposed to paracetamol, 250 mg / kg b. wt. in 2 ml saline, for 9 days, without treatment with the extract.

In day 18 all animals, were euthanized, for serum collection, to estimate, enzymes [20] (RMS, ALT, AST, TSB, TSP, BUN, and Creatinine) using standard assay kits [21 and 22]. Hepatic samples for histopathological examination were obtained, stained, using hematoxylin- eosin stain [23 and 24].

The dependent parameters; were, clinical, included, heart rates, respiratory rates, body temperature, body weight, with some hematological examination (total red blood cell count, Hb% and PCV%), clotting and bleeding times, total and differential leucocytes count, three times [25].

Statistical analysis

Values were represented as Mean ± SE. Students T- test was used for statistical analysis. Duncan test, was used to compare the level of significance. Values of probability over 0.05 were considered significant [26].

Results

Acute plant toxicity test

Only signs of mild toxicity were monitored. No death in animals was recorded so the extract was considered non- toxic at concentrations more than 1000 mg / kg body weight.

Sub-acute toxicity studies

The results did not show any significant difference between the groups of animals regarding the clinical parameters table 1.

Table 1: The clinical picture (Body weight / kg, body temperature ^{o}C, respiratory rates / minute, and heart rates / minute) of rabbits used in the study

Parameter	Group	Time	
		Pretreated	Post treated
Body weight Kg	I	1.50 ± 0.10a	1.52 ± 0.20a
	II	1.47 ± 0.03a	1.46 ± 0.04a
	III	1.48 ± 0.02a	1.50 ± 0.30a
	IV	1.46 ± 0.15a	1.44 ± 0.25a
Body temp. ^{o}C	I	39.0 ± 0.15a	38.9 ± 0.12a
	II	38.60 ± 0.29a	38.90 ± 0.17a
	III	38.8 ± 0.15a	38.8 ± 0.20a
	IV	39.1 ± 0.10a	39.3 ± 0.25a
Resp. rate / min.	I	125.0 ± 5.50a	125.0 ± 4.0a
	II	120.0 ± 9.54a	122.25 ± 8.60a
	III	130.0 ± 8.0a	128.0 ± 6.0a
	IV	128.0 ± 2.50a	131.0± 3.0a
Heart rate/ min.	I	171.5 ± 8.0a	175.5 ± 6.0a
	II	170.0 ± 7.91a	173.75 ± 9.35a

	III	175.0 ± 5.0a	178.0 ± 6.50a
	IV	180.0 6.00a	185.0 3.50a

Values are M ±SE: a, non- significant differences between pre and post treatment.

The result revealed that prolongation of both bleeding and clotting times in group exposed to paracetamol in comparison with the non - treated and those exposed to paracetamol and treated with the extract of *M. azedarach* table 2.

Table 2: Showing bleeding and clotting times (seconds) of rabbits used in the study

Parameter	Group	Time	
		Pretreated	Post treated
Bleeding / sec.	I	45.0 ± 0.5aA	47.0 ± 0.6aA
	II	43.0 ± 1.07aA	45.0± 3.54aA
	III	45.4 ± 2.50aA	50.5 ± 0.25bA
	IV	46.0 ± 1.50aA	52.5 ± 1.50bB
Clotting / sec.	I	45.20 ± 3.29aA	46.0 ± 3.69aA
	II	47.50 ± 3.0aA	47.0 ± 6.0aA
	III	49.5 ± 4.0aB	55.0 ± 4.0bB
	IV	50.5 ± 2.50aB	59.0 ± 1.50bB

Values are M ±SE: a, b refer to the significant differences between pre and post treatment values; A, B significant differences between groups.

The results showed that TEC, Hb, PCV, MCV, MCH and MCHC, did not change table 3.

Table 3: Showing total erythrocytes count, Hb, PCV and erythrocyte indices (MCV, MCH, MCHC) of rabbits used in the study.

Parameter	Group	Time	
		Pretreated	Post treated
Erythrocytes $X10^6$/cmm	I	5.70 ± 0.35a	5.40 ± 0.08a
	II	6.08 ± 0.27a	6.36 ± 0.51a
	III	6.25 ± 0.25a	5.90 ± 0.15a
	IV	5.8 ± 0.15a	6.00 ± 0.35a
Hb g%	I	9.20 ± 0.25a	8.85 ± 3.15a
	II	10.00 ± 0.17a	9.98 ± 0.27a
	III	9.60 ± 0.30a	9.00 ± 0.28a
	IV	9.80 ± 0.25a	9.20 ± 0.30a
PCV %	I	35.7 ± 0.65a	36.0 ± 9.0a
	II	36.0 ± 0.82a	33.5 ± 0.58a
	III	33.0 ± 0.6a	36.2 ± 0.80a
	IV	32.5 ± 0.15a	35.5 ± 1.00a
MCV ft	I	55.40 ± 3.15a	49.6 ± 14.91a

	II	59.46 ± 2.37a	48.95 ± 4.10a
	III	50.9 ± 2.00a	50.25 ± 1.85a
	IV	49.5 ± 1.25a	51.9 ± 1.00a
MCH	I	19.5 ± 0.90a	18.19 ± 5.23a
	II	20.28 ± 0.87a	16.64 ± 1.38a
	III	18.4 ± 0.9a	19.0± 0.85a
	IV	19.5 ± 0.6a	20.5 ± 0.70a
MCHC	I	35.9 ± 0.25a	34.9 ± 0.38a
	II	34.09 ± 0.12a	34.01 ± 0.21a
	III	35.1 ± 0.20a	35.0 ± 0.18a
	IV	33.9 ± 0.6a	36.6 ± 0.25a

Values are M ±SE: a refer to non- significant differences between groups and pre and post treatment values.

Results showed that TLC, lymphocytes and heterophils % changed significantly, while eosinophils, basophils and monocytes % did not change, in treated group in comparison with the control group table 4.

Table 4: Showing total and differential leucocyte counts of rabbits suffered from induced hepatitis

Parameter	Group	Time	
		Pretreated	Post treated
Total leucocytes x10^3/cmm	I	20.0 ± 1.50aB	19.8 ± 0.6aB
	II	19.45 ± 2.22aB	18.48 ± 2.05aA
	III	18.2 ± 3.0aA	18.8 ± 0.5aA
	IV	18.8 ± 1.50bA	17.0 ± 0.25aA
Heterophil%	I	48.0 ± 2.5aA	50.5 ± 12.60bA
	II	46.67 ± 6.01aA	49.0 ± 8.66bA
	III	52.5 ± 3.5aB	53.0 ± 2.5aB
	IV	50.0 ± 2.5aB	51.0 ± 3.0aB
Lymphocyte%	I	46.8 ± 3.5aA	48.0 ± 4.0bB
	II	47.0 ± 5.77aA	49.0 ± 1.22bB
	III	44.4 ± 2.0bA	40.0 ± 2.80aA
	IV	47.0 ± 2.0bA	42.0 ± 1.50aA
Eosinophil%	I	3.9 ± 0.7aA	3.5 ± 0.5aA
	II	4.0 ± 0.1aA	4.33 ± 1.20aA
	III	3.0 ± 1.0aA	3.45 ± 0.5aA
	IV	3.15 ± 0.1aA	3.20 ± 0.15aA
Basophil%	I	2.8 ± 0.25bA	1.50 ± 0.5aA
	II	2.0 ± 0.58bA	1.33 ± 0.33aA
	III	2.0 ± 0.35aA	1.8 ± 0.15aA
	IV	2.5 ± 0.05aA	1.8 ± 0.09aA
Monocyte%	I	1.5 ± 0.5aA	1.5 ± 0.5aA
	II	1.33 ± 0.88aA	1.0 ± 0.53aA

Protective Effect of Aqueous – Methanol Extract of *Melia azedarach* Against Paracetamol – Induced Hepatitis in Rabbits

Mayada Nazar AL-Khafaji, Estipraq Yunus Shlash, Amal Ismael Nayef and Noor Salah Shake

	III	1.5 ± 0.9aA	1.8 ± 0.5aA
	IV	1.0 ± 0.09aA	2.5 ± 0.15bA

Values are M ±SE: a, b refer to the significant differences between pre and post treatment values; A, B significant differences between groups.

Bilirubin, ALT, AST, RBS, BUN values showed significant changes in group treated with paracetamol, while total protein decreased, in comparison with those of non- treated rabbits table 5.

Table 5: Levels of, RBS, ALT, AST, TB, TP and BUN of rabbits

Parameter	Groups			
	1st	2nd	3rd	4th
RMS mg / dl	115 ± 4.85a	125.47 ± 7.04b	130.6 ± 3.5b	150.13 ± 2.0c
ALT U/L	61.65 ± 5.55a	90.85 ± 1.2b	140.8 ± 3.0c	160.7 ± 2.5d
AST U/L	74.85 ± 17.96a	80.86 ± 7.74a	100.1 ± 8.0b	119.2 ± 5.6c
TSB mg / dl	0.55 ± 0.15a	0.7 ± 0.03a	0.7 ± 0.2a	0.9 ± 0.03b
TSP g/ dl	8.0 ± 1.5b	6.8 ± 0.8b	5.8 ± 1.0a	4.5 ± 0.9a
BUN mg / dl	18.72 ± 0.42a	32.2 ± 0.2b	40.0 ± 0.1b	45.6 ± 0.2c
Creatinine mg / dl	1.25 ± 9.85a	0.9 ± 5.8a	0.8 ± 0.5a	1.2 ± 0.4a

Values are M ± SE: a, b, c, d refer to the significant changes in values.

The grossly appearance showed hepatomegaly, congestion, fibrin formation on the surface of liver in exposure to paracetamol in comparison with those of control non - exposed, and those treated with aqueous – methanol extract of *M. azedarach* fruit figure 1.

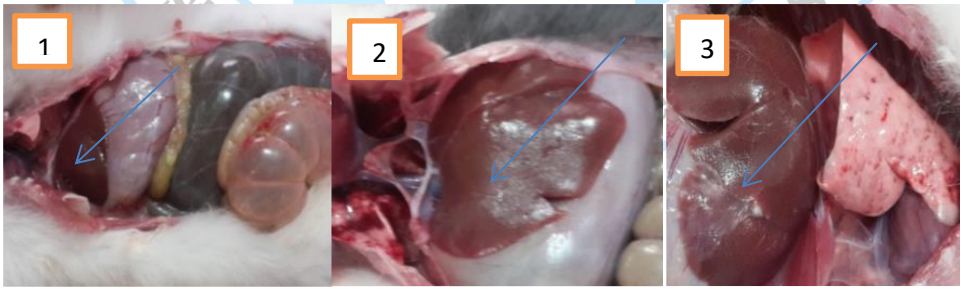

Figure 1: Panel 1: showed Normal liver from the control group; Panel 2: Liver showing fibrin on the surface and congestion together with lung congestion. Panel. 3: Hepatomegaly, congestion, fibrin formation on the liver and congestion of the lung

The histological changes showed there is hydropic degeneration in the hepatocytes and there is infiltration of monocyte cells in the portal areas of liver from rabbits exposed to paracetamol. While in liver from those treated with aqueous – methanol extract of *M. azedarach* there are congestion of blood vessels with mild infiltration of mononuclear cells figure 2.

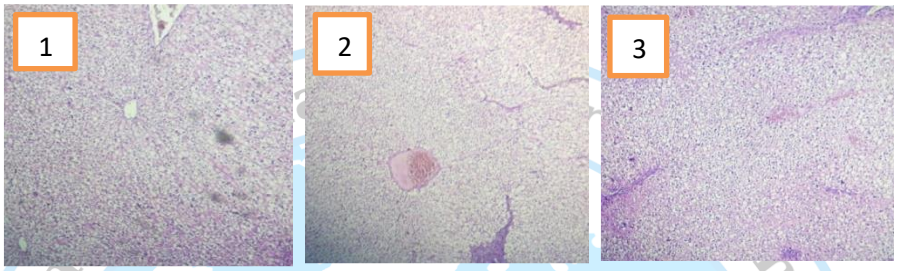

Figure 2: Panel 1: showing hydropic degeneration in the hepatocytes and infiltration of monocytes in the portal areas. Panel 2: showing hydropic degeneration, congestion of blood vessels with infiltration of monocytes (group treated with aqueous – methanol extract of *M. azedarach*). Panel 3: Normal liver, from control group non- exposed to paracetamol

Discussion

The results of current study showed that the harmful dose of *M. azedarach* fruit extract was found to be greater than 1000 mg / kg. The signs observed on animals during the study may be due to physiological reactions to the administered extract. Results revealed a dose of 1000 mg was save and fit to be used in the next section of the study and accordingly, the study was completed without any mortality during the 14 days of monitoring.

Current study showed significant increases in bilirubin, ALT, AST, RBS, BUN, in group treated with paracetamol in comparison with non – treated group. The elevated levels of these enzymes may contribute to hepatic injuries caused by toxins.

Catechins which are one of the phytochemical content of plants used in this study, may be responsible for lowering the levels of (ALT and AST) in treated animals [27].

In our study, the extract of *M. azedarach* fruits kept the levels of serum enzymes (ALT and AST), and (TB). Similarly, elevated levels of bilirubin, in serum, may be due to the hepatitis caused by administration of acetaminophen which may led to the production of abnormal amounts of bilirubin by the liver. In addition, AST and ALT enzymes, present in hepatocytes, they are also found in lesser amounts in kidneys, heart and skeletal muscles.

High levels of serum liver enzymes, ALT, AST, and serum bilirubin, in current study, pointed out to the fact that administration of paracetamol produces a hepatotoxic effect. These findings are in agreement with the results of other studies [28 - 31], as they attributed the rising of liver markers to hepatic injuries.

Elevated levels of serum enzymes can be attributed to loss of hepatocytes integrity, that lead to releasing of enzymes from hepatocytes, this was cleared, in our study from the histopathological changes, as there were degenerative and inflammatory changes, with congestion of blood vessels. These findings were in agreement with [32-35].

Muhammad *et al.*, [36] referred to the fact that in normal rabbits, suffering from paracetamol intoxication, high levels of AST, ALT, ALP, total bilirubin, and direct bilirubin, indicating acute centrilobular necrosis. Flavonoids and glycosides are known strong antioxidants [37].

Conclusions

On basis of obtained results, aqueous – methanol extract of Sibahbah fruit, exerts a protective effect via lowering the damage, induced by paracetamol.

Administration of 250 mg / kg of paracetamol, to rabbits induced a hepatic damage which revealed by changes in liver markers, in addition to the histopathological changes.

References

1. S. Pang, S. Lerone, M. Sandrini, A. Ferrari, A. Bertolini, Annual review of pharmacology and toxicology, 32, p. 625 (1992)
2. A. Bhardwaj, P. Khatri, P.M.L. Soni, D. J. Ali, Journal of Advanced Applied Scientific Research, 2(2), pp.15-20 (2011).
3. F. V. DeFeudis, V. Papadopoulos, K. Drieu, Fundam. Fundamental & clinical pharmacology, 17(4), pp.405-417 (2003)
4. B. J. Wang, C.T. Liu, C.Y. Tseng, C.P. Wu, Z. R. Yu, Food and Chemical Toxicology, 42(4), pp.609-617. (2004).
5. D. N. Choudhary, J. N. Singh, S. K. Verma, B. P. Singh, Indian journal of experimental biology, 28(8), pp.714-716 (1990)
6. F. A. Mohammed, R. Srinivasa, R. A. Shaik, I. Mohammed Journal of Pharmacy Research, 5(5), pp.2664-2667(2012).
7. L. P. James, P. R. Mayeux, J. A. Hinson, Drug metabolism and disposition, 31(12), pp.1499-1506 (2003).
8. T. Kelava, I. Cavar, F. Culo, Canadian journal of physiology and pharmacology, 88(10), pp.960-967(2010).
9. D. S. El- Agamy, M. N. Makled, N. M. Gamil, Journal of physiology and biochemistry, 70(1), pp.141-149 (2014).
10. S. G. Nelson, Z. Wan, M. A. Stan, The Journal of organic chemistry, 67(14), pp.4680-4683(2002).
11. S. Ohki, N. Ogino, S. Yasmamoto, O. Hayaishi, Journal of Biological Chemistry, 254(3), pp.829-836(1979).
12. A. Ottani, S. Leone, M. Sandrini, A. Ferrari, A. Bertolini, European journal of pharmacology, 531(1-3), pp.280-281(2006).
13. J. P. Dwyer, C. Jayasekera, A. Nicoll, Journal of gastroenterology and hepatology, 29(7), pp.1356-1360(2014).

14. F. Imani, M. Motavaf, S. Safari, S. M. Alavian, Hepatitis monthly, 14, e23539 (2014).

15. K. J. Simpson, N.W. Lukacs, A. H. McGregor, D. H. Harrison, R. M. Striter, S. L. Kunkel, Journal of the Pathological Society of Great Britain and Ireland, 190(4), pp.489-494 (2000).

16. R. Q. Gill, R. K. Sterling, Journal of clinical gastroenterology, 33(3), pp.191-198(2001)

17. National Institute of Health. Guide for the care and use of laboratory animals. NH contact no. NOI- RR- 2-2135. NIH, Bethesda, MD, 11-28 (1985).

18. M. N. Ghosh, Fundamentals of experimental pharmacology, 2nd ed. (Scientific Book Agency Calcutta, India, 1984) p.154

19. OECD, (2002). Acute oral toxicity. Acute oral toxic class method guideline 423 adopted 23.03. 1996.In: Eleventh Addendum to the, OECD, guidelines for the testing of chemicals organization for economical co-operation and development, Paris, June, 2000.

20. E. Porchezhian, S.H. Ansari, Phytomedicine,12(1-2), pp.62-64 (2005).

21. S. Eminzade, F. Uras, F. V. Izzettin, Nutrition & Metabolism, 5(1), p.18 (2008).

22. P. R. N. Kind, E. J. King, Journal of clinical Pathology ,7(4), p.322 (2005).

23. L. G. Luna, Manual of Histology, Staining Methods of Armed Forces, Institute of Pathology, 3rd ed. (McGraw Hill Book Co., New York,1996), p. 43.

24. S. Yasmin, M. A. Kashmiri, K. Anwar, Journal of Medicinal Plants Research, 5(3), pp. 349-353 (2011).

25. E. H. Coles, Veterinary Clinical Pathology, 4th ed. (W. B. Saunders Company, Philadelphia, USA, 1986), pp. 10-75.

26. R. G. D. Steel, G. H. Torrie, D. A. Dickey, Principles and procedures of statistics, 3rd Ed. (McGraw Hill, New York, 2007), pp. 1746-1762.

27. C. Z. Nkosi, A. R. Opoku, S. E. Terblanche, Phytotherapy Research, 19(4), pp.341-345 (2005).

28. M. Mazer, J. Perrone, Journal of Medical Toxicology ,4 (1), pp.2-6 (2008).

29. A. Zira, E. Mikros, K. Giannoiti, P. Galanopoulou, A. Papalois, C. Liapi, S. Theocharis, Journal of Applied Toxicology, 29(5), pp.395-402. (2009).

30. C. I. Ghanem, M. L. Ruiz, S. S. Villaneuva, M. Luquita, S. Llesuy, V. A. Catania, L. A. Bengochea, A. D. Mottino, Biochemical pharmacology, 77(10), pp.1621-1628 (2009).

31. M. S. Makoshi, M. I. Adanyegh, L. I. Nwatu, Journal of Veterinary Medicine and Animal Health, 5(1), pp.8-15 (2013).

32. S. K. Ramaiah, Food and chemical toxicology, 45(9), pp.1551-1557(2007).

33. S. Sreelatha, P. R. Padma, M. Umadevi, Food and Chemical Toxicology, 47(4), pp.702-708(2009).

34. G. S. Aseervatham, T. Sivasudha, J. M. Sasikumar, P. H. Christabel, R. Jeyadevi, D. A. Ananth, Journal of physiology and biochemistry, 70(1), pp.1-14 (2014).

35. T. Kiichiro, I. Yoshio, Biochemical pharmacology, 17(10), pp.2023-2032(1968).

36. M. S. Akhtar, M. I. Qayyum, N. Irshad, M. Yaseen, A. Hussain, H. Altaf, S. Rehman, N. Sulema, International Journal of Innovation and Applied Studies, 10(2), p.701(2015).

37. K. S. Natarajan, M. Narasimhan, K. R. Shanmugasundaram, E. R. B. Shanmugasundaram, Journal of ethnopharmacology, 105, pp.76-83(2006).