Pak. J. Agri. Sci., Vol. 54(1), 181-188; 2017
ISSN (Print) 0552-9034, ISSN (Online) 2076-0906
DOI: 10.21162/PAKJAS/17.4939
http://www.pakjas.com.pk

AMELIORATIVE EFFECTS OF QURS-E-AFSANTEEN ON GENTAMICIN INDUCED HEPATOTOXICITY AND OXIDATIVE STRESS IN RABBITS

Ahsan Aziz[1], Tanweer Khaliq[1], Junaid Ali Khan[1], Amer Jamil[2], Wafa Majeed[1,*], Muhammad Naeem Faisal[1], Bilal Aslam[1] and Komal Atta[1]

[1]Institute of Pharmacy, Physiology and Pharmacology, University of Agriculture, Faisalabad-38000, Pakistan;
[2]Department of Biochemistry, University of Agriculture, Faisalabad-38000, Pakistan.
[*]Corresponding author's e-mail: wafamajeed@hotmail.com

Qurs-e-afsanteen® has been reported to possess hepatoprotective potential, contains *Artemisia absinthium* (Afsanteen), *Valerianaofficinalis* (Sumbal-ut-tayyab), *Rheum emodi* (Ravendcheni), potassium nitrate and ammonium chloride. The present study was conducted to assess the protective effects of Qurs-e-afsanteen against gentamicin-induced liver toxicity in rabbits. Thirty rabbits were divided into five groups having six rabbits in each. Group 1: *Normal control* on routine diet; Group 2: *Untreated control* on Gentasym® injection (gentamicin 80mg/kg I/P); Group 3: *Treated control* on Gentasym® injection (gentamicin 80mg/kg I/P) + tablet Silliver® (silymarin 200mg/kg orally); Group 4: *Treated group I* on Gentasym® injection (gentamicin 80mg/kg I/P) + tablet Qurs-e-afsanteen® (50mg/kg orally); Group 5: *Treated group II* on Gentasym® injection (gentamicin 80mg/kg I/P) + tablet Qurs-e-afsanteen® (100mg/kg orally). Qurs-e-afsanteen® showed high percentage of flavonoid and phenolic components upon phytochemical analysis. Combination of Qurs-e-afsanteen® with Gentamicin restored the liver function parameters towards normal in a dose dependent manner. Qurs-e-afsanteen® significantly increased hepatic Total Antioxidant Capacity (TAC), Catalase (CAT), Superoxide dismutase (SOD) and significantly decreased the Total Oxidant Status (TOS) and Malondialdehyde (MDA) levels. Antimicrobial assay of Qurs-e-afsanteen® exhibited significant antimicrobial activity and DNA damage protection properties. Histopathological analysis also supported the defensive nature of Qurs-e-afsanteen® against gentamicin induced liver damage.
Keywords: Liver injury, polyherbal medicines, silymarin, hepatoprotetive, phenolic components, antimicrobial assay

INTRODUCTION

Drug induced liver injury (DILI) is a major problem for public health and medicine manufacturing industries. All kind of remedies which are used to treat different diseases including allopathic drugs, herbal formulations and nutrition supplements cause DILI that leads to hepatitis and jaundice. Free radical production during drug metabolism also causes DILI (Chen *et al*., 2011).

Aminoglycoside, a class of antibiotics, has been used as antibacterial therapy for a long time. It produces toxicity at slightly high doses. Following aminoglycosides treatment approximately 5-10% patients have to face adverse effects like hepatotoxicity, nephrotoxicity and ototoxicity due to production of free oxygen radicals. Gentamicin produces free oxygen radicals by acting on mitochondria of hepatocytes and accelerates the lipid peroxidation process (Alarifi *et al*., 2012).

From the history of human being polyherbal formulations are in use to cure different ailments. Approximately 80% population around the world is using polyherbal medicines (Ademiluyi *et al*., 2013). The usage of plant derived medicines is still quite frequent. *Silybum marianum*, belongs to *Asteraceae* family, is also known as milk thistle and silymarin. The seeds of silymarin contain flavonoids and lignins like silybine, silychristine, silydianine, tyramines, essential oils and bitter substances (Huma *et al*., 2016). Silymarin has hepatoprotective activity due to its antioxidant and anti-inflammatory properties (Karkanis *et al*., 2011; Kaur *et al*., 2011).

The formulation under study is Qurs-e- afsanteen® manufactured by Ashraf laboratories. This product consists of following components *Artemisia absinthium* (Afsanteen), *Valeriana officinalis* (Sumbal-ut-tayyab), *Rheum emodi* (Ravend cheni), potassium nitrate and ammonium chloride. *Artemisia absinthium* (Afsanteen) commonly known as Wormwood, belongs to *Compositae* family, contains flavonoids, carotenoids, tannins and lignins as its major constituents. It has antibacterial, antifungal, anthelmintic, antimalarial and antioxidant activities (Amrollahi *et al*., 2014). It shows hepatoprotective activity by scavenging free radicals and lipid peroxidation inhibiting mechanism (Pirbalouti *et al*., 2013). *Valeriana officinalis* (Sumbal-ut-tayyab) commonly known as valerian and belongs to *Valerianaceae* family. Its main constituents are valepotriates, isoborneol, baldrinals, valerenic acid, valerenals and valeranone. Antioxidant, sedative, spasmolytic and anxiolytic are major pharmacological effects of valerian (Bhatt *et al*.,

2012). *Rheum emodi* (Ravend cheni) belongs to family *Polygonaceae*. Its main constituents are anthraquinones, emodine, chrysophenol and beta-asarone. Mostly, it is used as purgative, diuretic, appetite stimulant, hypoglycaemic and hepatoprotective agent. It shows antioxidant property due to scavenging a stable radical 1, 1-diphenyl-2-picrylhydrazyl (DPPH) that forms complexes with the reduced metals (Mehmet *et al*., 2007).

By considering above literature, the present study was intended to check the hepatoprotective effect of Qurs-e-afsanteen against hepatotoxicity induced by gentamicin in rabbits.

MATERIALS AND METHODS

***Drugs and chemicals used*:** Injection Gentasym® (gentamicin sulphate) was obtained from Symans Pharmaceuticals. Tablet Siliver ® (Silymarin) was obtained from Abbott laboratories, Karachi. Qurs-e-afsanteen®tablets were obtained from Ashraf Laboratories, Faisalabad. Diagnostic kits (AST, ALT, Bilirubin, Total oxidant status, Catalase) were purchased from Randox Laboratories Ltd, United Kingdom.

***Test animals used*:** Thirty healthy, adult rabbits weighing 1500g (1.50±0.07) were procured from local market of Faisalabad and kept in animal room of the Institute of Pharmacy, Physiology and Pharmacology, University of Agriculture, Faisalabad, Pakistan. The rabbits were housed in individual iron cages with a period of light and dark alternatively, at ambient temperature (22±2°C) with proper ventilation facility. They were acclimatized for 1 week. Rabbits were fed with seasonal fodder (lucerne) and water ad-libitum till completion of experiment.

Study protocol:

***Hepatic toxicity induction*:** Hepatic toxicity was induced in the rabbits by administration of Gentamicin (80mg/kg) intra peritoneal. Induction of hepatotoxicity was confirmed by the elevated levels of liver function markers. Total 30 rabbits were used in whole experiment which were divided into 5 equal groups (n=6) and the following protocol (Table 1) was followed for 15 days.

***Sampling*:** Blood samples were collected on 0, 7th and 14th day. Approximately 5-7ml of blood was collected with the help of syringe from jugular vein of each rabbit in tubes.

***Hematological analysis*:** Blood samples were collected in EDTA tubes at day 0, 7th, 14th for evaluation of hematological parameters including Hb, RBCs, WBCs, Platelet, (PCV)/ Hematocrit (Hct), MCV, MCH and MCHC.

Serum Analysis

***Liver function tests*:** Levels of Alanine transaminase (ALT; U/L), Aspartate transaminase (AST; U/L), Alkaline Phosphatase (ALP;U/L) and Total bilirubin (mg/dl) were measured by the use of commercially available kits. ALT (reference # BT294QY) and AST (Reference # BT294Q) kits were purchased from Randox Laboratories Ltd., Ardmore, Diamond Road, Crumlin, CO. Antrim, United Kingdom (Vansoling and Bias, 2006).

Table 1. Feeding and drug administration schedule in rabbits during the experimental period for 0 to 14 days.

Groups	Treatment
Group 1 Normal control (CTRL)	Routine diet + water *ad-libitum* daily
Group 2 Untreated control (GMC)	Routine diet + inj. Gentasym® (gentamicin 80mg/kg I/P daily)
Group 3 Treated control (GMC+SMN)	Routine diet + inj. Gentasym® (gentamicin 80mg/kg I/P daily) + tablet Silliver® (silymarin 200mg/kg orally daily)
Group 4 Treated group I (GMC+LQA)	Routine diet + inj. Gentasym® (gentamicin 80mg/kg I/P daily) + tablet Qurs-e-afsanteen® (50mg/kg orally daily)
Group 5Treated group II (GMC+HQA)	Routine diet + inj. Gentasym® (gentamicin 80mg/kg I/P daily) + tablet Qurs-e-afsanteen® (100mg/kg orally daily)

***Phytochemical analysis*:** For phytochemical screening total flavonoids and phenols were determined (Eric and Gill, 2013; Ben *et al*., 2013).

***Serum health biomarkers*:** Total Antioxidant Capacity (TAC) (Erel, 2004), Total Oxidant Status (TOS) (Erel, 2005), Malondialdehyde (MDA) (Ohkawa *et al*., 1979) and Catalase (Goth, 1991) levels were measured.

Biochemical Assays

***Antibacterial assay by disc diffusion method*:** Antibacterial assay of plant extract was performed and zone of inhibition was measured (Sawai, 2003).

***Determination of cytotoxicity of sample and its polar fractions*:** Cytotoxicity of the sample was determined by Hemolytic Activity (Kang *et al*., 2009).

***Determination of DNA damaging protection activity*:** DNA damaging protection activity of sample and its polar fractions was determined (Kumar and Chattopadhyay, 2007).

***Histopathological analysis*:** At the end of experimental trial, all rabbits were euthanized by cervical dislocation and the abdomen was opened and viscera exposed. Then the liver was removed and washed with normal saline to remove the blood completely. Then the liver tissues were subjected to histopathological investigations (Bancroft and Gamble, 2007).

RESULTS

***Liver function markers*:** Levels of ALT, AST, ALP and Bilirubin were elevated significantly ($P<0.05$) in rabbits

Table 2. Effect of Qurs-e-afsanteen® and silymarin on serum ALT, AST, ALP and bilirubin levels (mean± SE) in gentamicin induced hepatic damage in rabbits.

Groups	ALT (U/L)	AST (U/L)	ALP (U/L)	Bilirubin (mg/dl)
CTRL	32.50±0.76C	76.50±1.14E	12.66±0.71D	0.65±0.06C
GMC	55.00±1.98A	134.33±1.58A	32.00±2.73A	1.80±0.07A
GMC+SMN	33.50±0.92C	85.66±2.02D	16.16±0.60C	0.95±0.07B
GMC+LQA	38.66±0.70B	92.66±2.91B	21.00±0.89B	1.10±0.06B
GMC+HQA	35.16±0.88C	90.16±2.46C	18.33±1.28C	1.00±0.07B

Mean values in a column sharing similar alphabets do not differ significantly (P>0.05)

Table 3. Effect of Qurs-e-afsanteen® and silymarin on Hb, RBCs, Hct and platelet count (mean± SE) in gentamicin induced hepatic damage in rabbits.

Groups	Hb (g/dl)	RBC (10^6/ µl)	Hct (%)	PLT (10^3/ µl)
CTRL	11.05±0.26A	4.90±0.14A	31.60±0.79A	242.83±7.26A
GMC	8.91±0.39B	3.86±0.18B	29.60±0.21B	193.83±5.33B
GMC+SMN	11.45±0.22A	4.96±0.13A	33.78±0.77A	247.83±3.48A
GMC+LQA	10.75±0.12A	5.01±0.18A	32.10±0.77A	222.50±6.73A
GMC+HQA	11.38±0.17A	5.21±0.17A	33.26±0.74A	223.50±9.11A

Mean values in a column sharing similar alphabets do not differ significantly (P>0.05)

Table 4. Effect of Qurs-e-afsanteen® and silymarin on TLC, MCV, MCH and MCHC (mean± SE) in gentamicin induced hepatic damage in rabbits.

Groups	TLC (10^3/µl)	MCV (µm^3)	MCH (pg)	MCHC (g/dl)
CTRL	8.68±0.32B	65.66±1.24A	22.08±0.39A	29.78±0.25A
GMC	10.90±0.24A	64.71±1.47A	20.96±0.36A	29.03±0.54A
GMC+SMN	8.58±0.30B	65.70±1.03A	22.36±0.67A	29.50±0.31A
GMC+LQA	9.05±0.19B	60.26±0.42B	21.33±0.64A	29.01±0.38A
GMC+HQA	8.56±0.29B	61.05±0.58B	21.20±0.68A	29.38±0.43A

Mean values in a column sharing similar alphabets do not differ significantly (P>0.05)

Table 5. Effect of Qurs-e-afsanteen® and silymarin on catalase, MDA, TOS and TAC (mean± SE) levels in gentamicin induced hepatic damage in rabbits.

Groups	Catalase (Ku/L)	MDA (nmol/L)	TOS (µmol/L)	TAC (mmol/L)
CTRL	35.76±1.49AB	5.58±0.15D	3.83±0.14B	0.53±0.06B
GMC	29.89±1.11C	9.45±0.42A	4.93±0.20A	0.3±0.03C
GMC+SMN	36.05±0.88AB	6.23±0.12C	3.50±0.07B	0.66±0.05A
GMC+LQA	34.13±1.02B	6.91±0.12B	3.70±0.09B	0.6±0.05A
GMC+HQA	39.24±0.95A	6.68±0.11B	3.60±0.3B	0.68±0.04B

Mean values in a column sharing similar alphabets do not differ significantly (P>0.05)

treated with gentamicin (80mg/kg) indicated gentamicin-induced hepatotoxicity. The administration of silymarin (200mg/kg) along with gentamicin (80mg/kg) caused significant restoration of ALT, AST, ALP and bilirubin normal levels. Qurs-e-afsanteen® (50mg/kg or 100mg/kg) along with gentamicin (80mg/kg) restored the ALT, AST, ALP and bilirubin levels towards normal in dose-dependent manner.

***Hematological analysis*:** Hemoglobin (Hb), Red blood cells (RBCs), Hematocrit (Hct) and Platelet count (PLT) were reduced significantly (P<0.05) in rabbits treated with gentamicin (80mg/kg) indicated gentamicin-induced hepatotoxicity. The administration of silymarin (200mg/kg) along with gentamicin (80mg/kg) caused significant restoration of Hb, RBCs, Hct and PLT count towards normal level. Qurs-e-afsanteen® (50mg/kg &100mg/kg) along with gentamicin (80mg/kg) raised Hb, RBCs, Hct and PLT count towards normal in dose-dependent manner.

Total leukocyte count was increased significantly (P<0.05) in rabbits treated with gentamicin (80mg/kg). The administration of silymarin (200mg/kg) along with gentamicin (80mg/kg) restored total leukocyte count significantly towards normal level. Qurs-e-afsanteen® (50mg/kg or 100mg/kg) along with gentamicin (80mg/kg) restored total leukocyte count towards normal in dose-dependent manner. Current study also revealed that there was

no significant difference in mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), and mean corpuscular hemoglobin concentration (MCHC) between control and experimental groups.

***Oxidative stress markers*:** Gentamicin induced oxidative stress in the liver, was indirectly evaluated by serum MDA level (nmol/L) and CAT activity (kU/L) compared to normal control group. Qurs-e-afsanteen® (50mg/kg &100mg/kg) significantly ($P<0.05$) reduced the level of MDA in dose dependant manner. CAT activity was increased compared to normal control group resulting in increase in antioxidant activity. Results have also shown that use of gentamicin increased the TOS and decreased the TAC. But use of silymarin and Qurs-e-afsanteen® along with gentamicin significantly ($P<0.05$) reduced TOS and produced an increase in TAC as indicated in the results.

Bioactivity assay

***Cytotoxicity*:** The results of cytotoxicity assay showed that Qurs-e-afsanteen® has less hemolytic activity (Table 6).

Table 6. Bioactivity assay

Parameters	Results (Mean ± S.E)
Cytotoxicity	12.89± 0.434
Antibacterial (zone of Inhibition in millimeter)	
Bacillus subtillus	21.38 ± 1.38
Escherichia coli	15.93 ± 0.92
DPPH	73.96%± 0.176
DNA damage	DNA was protected
Total phenolic contents (Gallic acid equivalent)	290.654 ± 1.24
Total flavonoid contents (Gallic acid equivalent)	253.557± 2.81

***Antibacterial assay*:** Qurs-e-afsanteen®showed significant zone of inhibition against the bacterial strains *Bacillus subtillus* and *Escherichia coli*. Mean and S.E values of zone of inhibition are given in the Table 6.

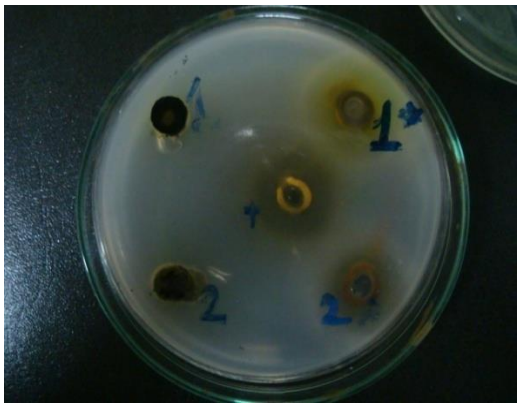

Figure 1. Antimicrobial assay

***DPPH assay*:** Qurs-e-afsanteen®showed significant DPPH radical scavenging activity and the result is shown in Table 6.

***DNA damage protection assay*:** Qurs-e-afsanteen® significantly protected the DNA in DNA damage assay.

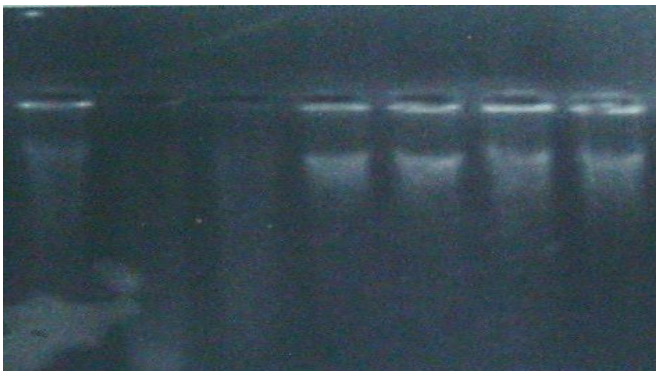

Figure 2. DNA damage protection assay.

***Total phenolic and flavonoid contents*:** Qurs-e-afsanteen® showed high phenolic and flavonoid contents which are given in Table 6.

***Histopathological examination*:** The protective effect of Qurs-e-afsanteen® against gentamicin-induced hepatotoxicity was also confirmed through histopathological examination. Light microscope evaluation of liver from control group showed normal morphology of the hepatocytes, normal sinusoidal spaces and nuclei were also normal in appearance (Fig. 3). The treatment with gentamicin (80mg/kg) for 14 days caused severe inflammation indicated cellular infiltration of portal area and massive hepatocytes necrosis, with pyknosis and karyolitic changes in the nucleus. Cytoplasm of the hepatocytes was hazy in appearance (Fig. 4).

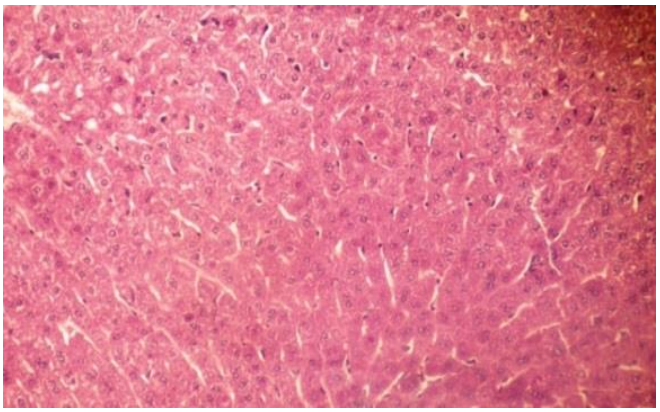

Figure 3. Photomicrograph of liver of rabbit of CTRL GMC (control) group showing normal nuclei and sinusoidal Spaces.

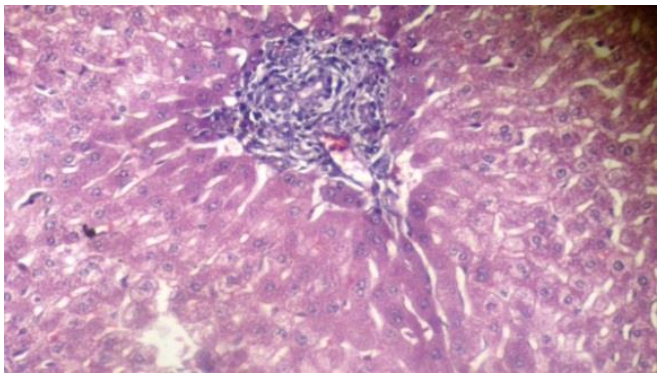

Figure 4. Photomicrograph of liver of rabbit of (gentamicin) group showing severe inflamemation and massive hepatocytes necrosis.

Liver tissues of the rabbits that were received silymarin along with gentamicin exhibited normal hepatic parenchyma, normal nucleolus and chromatin material of hepatocytes although cytoplasm was little hazy in appearance at few places (Fig. 5). However, liver tissues from the group received low dose of Qurs-e-afsanteen® along with gentamicin (80mg/kg) showed a slight decrease in the gentamicin-induced cellular necrosis of hepatocytes, mild vacuolar degeneration was also present in the cytoplasm. Mild ameliorative effect was observed in these tissues (Fig. 6). Liver tissues from the group received high dose of Qurs-e-afsanteen® along with gentamicin (80mg/kg) showed significant ameliorative effect on gentamicin-induced necrosis of the hepatocytes, sinusoidal spaces are normal and cytoplasm is also clear (Fig. 7).

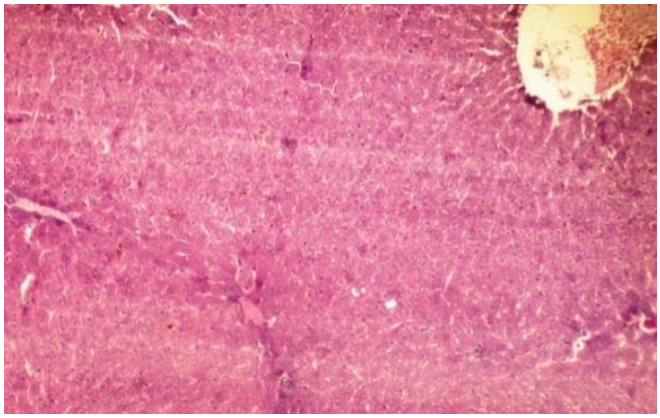

Figure 5. Photomicrograph of liver of rabbit GMC+SMN (gentamicin and silymarin) group showing normal hepatic parenchyma, normal nucleolus and chromatin material of hepatocytes.

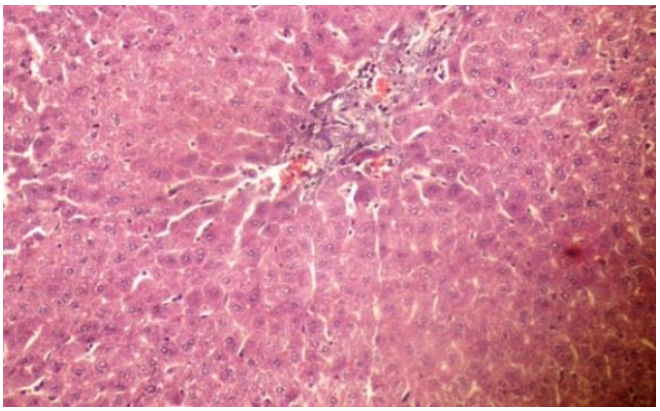

Figure 6. Photomicrograph of liver of rabbit GMC+SMN (gentamicin + low dose Qurs-e-afsanteen®) group showing mild cellular necrosis of hepatocytes, vacuolar degeneration is present in the cytoplasm.

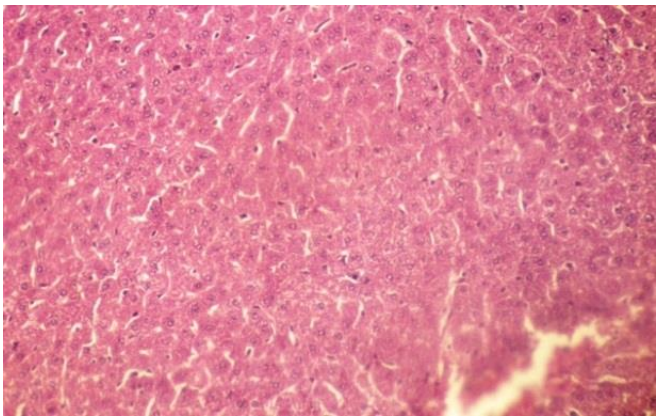

Figure 7. Photomicrograph of liver of rabbit GMC+HQA (gentamicin+ high dose of Qurs-e-afsanteen®) group showing normal hepatocytes and sinusoidal spaces.

DISCUSSION

Gentamicin is an important aminoglycoside antibiotic that is efficacious against gram negative bacteria in human and animals. The exact mechanism of hepatotoxicity is not well understood yet but it is believed that it increases the production of free radicals which lead to create oxidative stress in the body (Alarifi *et al*., 2012). Silymarin was chosen for the present study as a standard because of its high antioxidant property and its free radicals scavenging activity (Mosallanejad *et al*., 2012). Silyamrin enhances the gene expression of the antioxidant enzymes like catalase, superoxide dismutase and glutathione peroxidase (Karimi *et al*., 2011).

Qurs-e-afsanteen® tablets are composed of three plants and two synthetic compounds, among them all the plants have antioxidant property. The flavonoids and phenolic contents present in these plants are important for protection of liver against drug induced liver damage (Uorakkottil *et al*., 2016). Individually, all the plants of this product were studied by different researchers (Syed *et al*., 2014; Nurmuhammat *et al*., 2010).

The reduction in the increased levels of ALT, AST and ALP in the groups treated with high and low doses of the formulation was due to their scavenging activity against free radicals present in the chemical constituents of the plants that led to decrease the oxidative stress produced by gentamicin. High doses showed significant effect against oxidative stress than the low doses of Qurs-e-afsanteen®. The results of current study are in accordance with previous research studies (Girish *et al*., 2009; Syed *et al*., 2014; Mohammad *et al*., 2015).

Toxic effects on hematological parameters are secondary to the harmful effects of xenobiotics on the organs of hematopoiesis in the body which are liver and kidneys (Adeneye *et al*., 2008). Therefore, the present study was conducted to check the hematological parameters. Qurs-e-afsanteen®, showed hematopoietic potential at different doses (100mg/kg or 50mg/kg), increased RBCs, hematocrit and hemoglobin level towards the normal values in the dose dependent manner. Results of the current study were similar to the previous findings that the increase level of blood parameters might be due the fact that polyherbal formulation possesses active biological constituents and phytochemical components (phenolics, flavonoids, lignins and tannins) which help to reverse the gentamicin induced toxicity and also increase the process of hematopoiesis. These constituents could have the hematopoietin like effect and increase the release of hematopoietin from the kidneys (Adeneye *et al*., 2008). Hepatoprotective effect of Qurs-e-afsanteen® is supported by the previous research studies which have shown that herbal plants possess antioxidant potential that causes increase in RBCs count and hemoglobin level (Sharma *et al*., 2010). Qurs-e-afsanteen® also restored the platelet count because the medicinal plants present in the product have ability to inhibit the platelet aggregation and the values could be restored to the normal (Dinev *et al*., 2007).

In the current study, there was a clear decrease in the catalase activity and significant increase in total oxidant status in the group treated with gentamicin. Significant improvement was seen in the catalase activity and reduction in total oxidant status in the group treated with high dose of Qurs-e-afsanteen® rather than the group treated with lower dose of Qurs-e-afsanteen® because presence of flavonoids and phenolics are key constituents which have antioxidant activity (Anilkumar *et al*., 2009).

The herbal product used in study has good antimicrobial activity as found in the previous findings that the crude plant extract showed high antimicrobial activity because they contain alkaloids, phenols, saponins, flavonoids, tannins, salicylic acid and terpenes which have strong antimicrobial activity against different pathogens (Joy and Raja, 2008).

The 1, 1-diphenyl-2-picrylhydrazyl (DPPH) is a stable free radical which is used to check *in vitro* antioxidant activity of the different experimental compounds and is being used all over the world to measure the radical scavenging capability. Antioxidant activity can be calculated by the biological agent capability of scavenging free radicals. The product under investigation showed better antioxidant activity due to its phenolics and flavonoids. Hydrogen peroxide is used in the DNA damage protection assay which is scavenged by the experimental drug and DNA was protected because the presence of antioxidant compounds (Molyneux, 2004).

The protective effect of Qurs-e-afsanteen® against gentamicin-induced hepatotoxicity was also confirmed through histopathological examination. Liver tissues of the rabbits received silymarin along with gentamicin exhibited normal hepatic parenchyma, normal nucleolus and chromatin material of hepatocytes, although, cytoplasm was little hazy in appearance at few places. However, liver tissues from the group received low dose of Qurs-e-afsanteen® along with gentamicin (80mg/kg) showed a slight decrease in the gentamicin-induced cellular necrosis of hepatocytes, mild vacuolar degeneration was also present in the cytoplasm. Mild ameliorative effect was observed in these tissues. Liver tissues from the group received high dose of Qurs-e-afsanteen® along with gentamicin (80mg/kg) showed significant ameliorative effect on gentamicin-induced necrosis of the hepatocytes, sinusoidal spaces were normal and cytoplasm was also clear.

***Conclusion*:** In conclusion, the overall results of study have shown that Qurs-e-afsanteen® offers hepatoprotective potential against the deleterious effects of gentamicin in dose dependent manner.The current study has revealed that the hepatoprotective potential of Qurs-e-afsanteen® was mainly because of its antioxidant activity. However further studies are also required in order to isolate and characterize the bioactive constituents of Qurs-e-afsanteen® to elucidate the possible mechanisms behind hepatoprotection and therapeutic applications of Qurs-e-afsanteen®.

***Acknowledgements*:** Research facility and help provided by Prof. Dr. Ijaz Javed Hassan and Prof. Dr. Tanweer Khaliq (Director of Institute of Pharmacy, Physiology and Pharmacology) for the completion of this project is highly acknowledged.

***Author's contributions*:** All authors contributed equally in the completion of project.

REFERENCES

Ademiluyi, A.O., G. Oboh, T.R. Owoloye and O.J. Agbebi. 2013. Modulatory effects of dietary inclusion of garlic (*Allium sativum*) on gentamycin-induced hepatotoxicity and oxidative stress in rats. Asian Pac. J. Trop. Biomed. 3:470-475.

Adeneye, A.A., J.A. Olgunju, A.S. Beneho, S.O. Elias, A.O. Adisa, B.O. Idowu, M.O. Oyedeji, E.O. Isioye, O.B. Braimoh and O.O. Oladejo. 2008. Nephroprotective effects of the aqueous root extract of *Harungana madagascariensis* (L.) in acute and repeated dose of acetaminophen renal injured rats. Int. J. Appl. Res. Nat. Prod. 1:6-14.

Alarifi, S., A. Al-Doaiss, S. Alkahtani, S.A. Al-Farraj, M.S. Al-Eissa, B. Al-Dahmash, H. Al-Yahya and M. Mubarak. 2012. Blood chemical changes and renal histological alterations induced by gentamicin in rats. Sau. J. Biol. Sci. 19:103-110.

Amrollahi, H., N. Hossein, P. Shirin, N. Najmeh and M. Abolfazl. 2014. Anti-inflammatory and analgesic activities of *Artemisia absinthium* and chemical composition of its essential oil. Int. J. Pharm. Sci. Rev. Res. 24:237-244.

Anilkumar, K., V. Saritha, F. khanum and A. Bawa. 2009. Amreliorative effect of ajwain extract on hexachlorocyclohexane-induced lipid peroxidation in rat liver. Food. Chem. Toxicol. 47:279-282.

Bancroft, J.D. and M. Gamble. 2007. Theory and Practice of Histological Techniques. Philadelphia, Churchill Livingstone. pp.131-133.

Ben, I.O., E. Woode, W.K.M. Abotsi and E.B. Gyasi. 2013. Preliminary phytochemical screening and *in vitro* antioxidant properties of *Trichiliamonadelpha* (Thonn). J. Med. Biomed. Sci. 2:6-15.

Bhatt, I.D., P. Dauthala, S. Rawata, K.S. Gairaa, A. Jugrana, R.S. Rawala and U. Dhar. 2012. Characterization of essential oil composition, phenolic content and antioxidant properties in wild and planted individuals of *Valeriana jatamansi*. Sci. Hortic. 136:61-68.

Chen, M., V. Vijay, Q. Shi, Z. Liu, H. Fang and W. Tong. 2011. FDA-approved drug labeling for study of drug-induce liver injury. Drug. Discov. Today. 16:697-703.

Dinev, T., D. Zapryanova and L. Lashev. 2007. Changes in some blood biochemical and hematological parameters in goats after aminoglycosides and aminocyclitol treatment at therapeutic doses. Turk. J. Vet. Anim. Sci. 31:179-188.

Erel, O. 2004. A novel automated direct measurement method for total antioxidant capacity using a new generation, more stable ABTS radical cation. Clin. Biochem. 37:277-285.

Erel, O. 2005. A new automated colorimetric method for measuring total oxidant status. Clin. Biochem. 38:1103-1111.

Eric, S. and R.O.C. Gil. 2013. Flavonoid electrochemistry: a review on the electroanalytical applications. Rev. Bras. Farmacogn. 23:542-558.

Girish, C., B.C. Koner, S. Jayanthi, K.R. Rao, B. Rajesh and S.C. Pradhan. 2009. Hepatoprotective activity of six polyherbal formulations in paracetamol induced liver toxicity in mice. Ind. J. Med. Res. 129:569-78.

Goth, L. 1991. A simple method for determination of serum catalase activity and revision of reference range. Clin. Chim. Acta 196:143-151.

Huma, W., M.K. Humaira and A.A. Anjum. 2016. Antiepileptic potential of *Silybum marianum* seeds in pentylenetetrazol-induced kindled mice. Bangladesh J. Pharmacol. 11:603-609.

Joy, P. and D.P. Raja. 2008. Anti-bacterial activity studies of *Jasminum grandiflorum* and Jasminum sambac. Ethnobot. Leaflet 7:87-95.

Kang, C., , A. Munawir, M. Cha, E.T. Sohn, H. Lee, J.S. Kim, W.D. Yoon, D. Lim and E. Kim. 2009. Cytotoxicity and hemolytic activity of jellyfish *Nemopilema nomurai* (*Scyphozoa: Rhizostomeae*) venom. Comp. Biochem. Physiol. 150:85-90.

Karkanis, A., D. Bilalis and A. Efthimiadou. 2011. Cultivation of milk thistle (*Silybum marianum* L. Gaertn), a medicinal weed. Ind. Crops. Prod. 34:825-830.

Karimi, G., M. Vahabzadeh, P. Lari, M. Rashedinia and M. Moshiri. 2011. "Silymarin", a promising pharmaceutical agent for the treatment of disease. Iran. J. Basic. Med. Sci. 14:308-317.

Kaur, A.K., A.K. Wahi, B. Kumar, A. Bhandari and N. Prasad. 2011. Milk thistle (*Silybum marianum)*. Int. J. Pharm. Res. Dev. 3:1-10.

Kumar, A. and S. Chattopadhyay. 2007. DNA damage protecting activity and antioxidant potential of pudina extract. Food Chem. 100:137-1384.

Mehmet, O.Z., A.M. Fatma, E.D. Mehmet and G. Topc. 2007. Antioxidant activity of stem and root extracts of Rhubarb (*Rheum ribes*), an edible medicinal plant. Food Chem. 103:623-630.

Mohammad, A.K., A. Gupta, S. Kumar, S. Ahmad and J.L.N. Sastry. 2015. Hepatoprotective activity of a new polyherbal formulation against paracetamol and D-galactosamine induced hepatic toxicity. J. Pharm. Bioallied. Sci. 7:246249.

Mosallanejad, B., R. Avizeh, H.N. Varzi and M. Pourmahdi. 2012. Evaluation of prophylactic and therapeutic effects of silymarin on acute toxicity due to tetracycline severe overdose in cats: a preliminary study. Iran. J. Vet. Res. 13:16-22.

Molyneux, P. 2004. The use of stable free radical I, I-diphenyl-2-picrylhydrazyl (DPPH) for estimating antioxidant activity. J. Sci. Technol. 25:114:121.

Nurmuhammat, A., U. Halmurat, B. Bla and Z. Ovi. 2010. *In vivo* hepatoprotective activity of the aqueous extract of

Artemisia absinthium L. against chemically and immunologically induced liver injuries in mice. J. Ethnopharmacol. 131:478-484.

Ohkawa, H., N. Ohishi and K. Yagi. 1979. Assay for lipid peroxides in animal tissues by thiobarbituric acid reaction. Anal. Biochem. 95:351-358.

Pirbalouti, G., M. Firoznezhad, L. Craker and M. Akbarzadeh. 2013. Essential oil compositions, antibacterial and antioxidant activities of various populations of *Artemisia chamaemelifolia* at two phenological stages. Rev. Bras. Farmacogn. 23:861-869.

Sawai, J. 2003. Quantitative evaluation of antibacterial activities of metallic oxide powders (ZnO, MgO and CaO) by conductimetric assay. J. Microbiol. Methods. 54:177-182.

Sharma, V., A. Sharma and L. Kansal. 2010. The effect of oral administration of *Allium sativum* extracts on lead induced toxicity on male mice. Food Chem. Toxicol. 48:928-936.

Syed, S.N., W. Rizvi, A. Kumar, A.A. Khan and A. Ahsan. 2014. Antioxidant and Hepatoprotective activity of ethanol extract of *Valeriana wallichii* in CCl4 treated rats. British J. Pharma. Res. 4:1004-1013.

Uorakkottil, I., D.P. Katare, V. Aeri and P.P. Naseef. 2016. A review on hepatoprotective and immunomodulatory herbal plants. Pharmacog. Rev. 10:66-70.

Vansoling, W.W. and R. Bias. 2006. Teitz Textbook of Clinical Chemistry and Molecular Diagnosis. Missouri, Saunders.