Research Article

Regulatory Effects of Garlic Extract (*Allium sativum* L.) on Hematobiochemical Markers in Rabbits Intoxicated with Paracetamol

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Abstract | The current research was aimed to examine the effects of garlic aqueous extract on the levels of hematobiochemical parameters and several liver related serum parameters in paracetamol-intoxicated rabbits. Moreover, the amount of (DPPH) free radicals that garlic aqueous extract scavenged at various doses was used to determine its antioxidant capacity. There were five groups each of which contains eight rabbits as experimental animals. These groups were, group A (control animals), group B (intoxicated animals), group C (Animals treated with standard drug), group D and group E (extract was given at doses of 100 and 300 mg/kg body weight after paracetamol intoxication) retrospectively. The results were contrasted with those of the common hepatoprotective medication silymarine (50 mg/kg body weight). When contrasted to toxic control rabbits, the highest dose of garlic aqueous extract i.e. 300 mg/kg b.w excellently decreased the high serum rates of alkaline phosphatase (ALP), alanine transaminase (ALT), and aspartate transaminase (AST). The outcomes of the extract-treated rabbits were comparable to those of the group of rabbits given silymarine. RBC, platelets, haemoglobin, MCH concentration, and HCT levels did not significantly alter after treatment with garlic aqueous extract and silymarine. However, a substantial (P 0.05) rise in the overall WBC count was seen. The current research findings revealed that silymarine, a common drug for hepatoprotection, might be replaced by garlic aqueous extract as a herbal remedy. However, more research is needed to identify the bioactive ingredient and determine the effectiveness of this herbal substitute.

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Introduction

The human liver is a reddish-brown organ that is situated in the upper right corner of the belly. The liver contains two lobes, one on each side, and carries out several essential metabolic processes (Naruse *et al.*, 2017). Furthermore, the levels of many biological indicators such as glutamic oxaloacetic transaminase (SGOT), aspartate amino transaminase, glutamic pyruvic transaminase (SGPT), alanine amino transaminase, total bilirubin (TB), and total cholesterol are measured (Rajesh

and Latha, 2004). Hepatitis is one of the potential features of liver damage brought on by hepatotoxicity. (Murray *et al*., 2018). Jaundice, which is brought on by an accumulation of bilirubin in the extracellular fluid and manifests as lightheadedness, acute exhaustion, black urine, and dark stools, is the main sign of hepatotoxicity (Bleibel *et al*., 2007). Animals may become toxic either by producing secondary metabolites or by being hosts to other species such as bacteria, plants, or other animals (Mebs, 2001). The modern pharmacological age has produced over a thousand medications that have been found to cause hepatotoxicity with a variety of clinical manifestations (Biour *et al*., 2004). Several pharmacological medications that cause lipid membrane peroxidation and damage to hepatocytes are employed in medical therapy (Gupta and Lewis, 2008). Several anaesthetic medications, including chloroform, halothane, isoflurane, desflurane, and nitrous oxide, directly harm the hepatocytes by interfering with the metabolism of bilirubin (Fallahian, 2009). There have also been reports of a variety of chemical toxins being toxic to both people and animals. One of the analgesic drugs is paracetamol. The amount of dosage and length of exposure to this substance affect the degree of tissue damage. The ideologies of toxicity is based on peroxidation of membrane lipid and the production of the very lethal trichlomethyl radical ($\bullet CCl_3$) (Gupta and Lewis, 2008). Numerous researchers have shown that biological substances found in plants; including flavonoids, phenolic acids, procyanidins, tannins, and anthocyanins, have heart, liver, and nephroprotective properties (Oliboni *et al*., 2011). According to numerous research, plant extracts with antioxidant activity reduce lipid peroxidation and boost antioxidant enzyme activity to protect against the hepatotoxicity caused by paracetamol (Shahjahan *et al*., 2004). Various free radicals, such as hydrogen peroxide, nitric oxide, DPPH, trichloromethyl, and super oxide, are used in vitro antioxidant activations (CCl_3) (Abalaka *et al*., 2011). DPPH for other radicals is a well-known scavenger (Ionita *et al*., 2004). Hepatic disorders are among the many ailments that are frequently treated using herbal remedies manufactured from plant extracts (Darbar *et al*., 2011). Alkaloids, steroids, organic acids, lignins, xanthenes, carotenoids, lipids, essential oils, phenols, flavonoids and monoterpines are among the chemical constituents of hepatoprotective plants (Radhika *et al*., 2014). In poor nations, looking for herbal treatments is a prevalent activity. These treatments are frequently utilized for a variety of ailments and operate as conventional healers, providing the majority of people with just basic health care in a preventative rather than curative manner. Plants are more enticing as a direct curative agent than modern medications since they are more readily available and affordable.

The present experiment will use garlic crude extract. The plant belonged to the family Rhamnaceae. 58 genera and almost 900 species are included (Qaiser *et al*., 1984). Garlic is a popular cash crop in Pakistan, Australia, tropical America, and Africa (Pawlowska *et al*., 2009) Several reports showed that that a number of species are used against ulcer of different kinds (Wahida *et al*., 2007). The plant's aqueous extract indicated above, garlic, was employed in the current research since it has historically been used to treat a variety of liver problems.

Materials and Methods

Extract preparation

The shade-dried bulbs of Garlic had been were crushed into a powder using an electric chopper machine and dissolved in 1500 ml of deionized water. Next to this the liquid was clean and filtered with a muslin cloth and later using a special filter paper made for the syntheses of herbal extracts. The filtrate was evaporated using a rotatory evaporator (Heidolph Laborta 400 efficient). To minimize fungus attack, the final extract solution was moved to flasks from rotary evaporator and then passed through a lipoholizer machine to produce a dense paste of reddish-black crude extract (Altemimi *et al*., 2017).

Experimental animal grouping

Forty (40) adult male domestic rabbits (*Oryctoagus cuniculus*) was obtained from the Rifah Institute of Pharmaceutical Sciences in Islamabad, which ranges from 700 to 1000 g. The animals were accustomed for a week to the laboratory animal housing. The animals were fed consistently and provided unrestricted access to fresh water. The temperature in the animal enclosures ranged from 22 to 25 °C (ambient temperature) with a 12-hour cycle of light and darkness. According to the research guiding principles of the animal's committee for animals care and use, all operations involving animals were carried out, and approval from animal care and ethical committee was also obtained (Griffin and Locke, 2016; EANO 34-2).

The experiment was conducted on forty adult male rabbits for 21 days duration, with eight animals in each of the five groups. The descriptions of the groups are mentioned in (Figure 1).

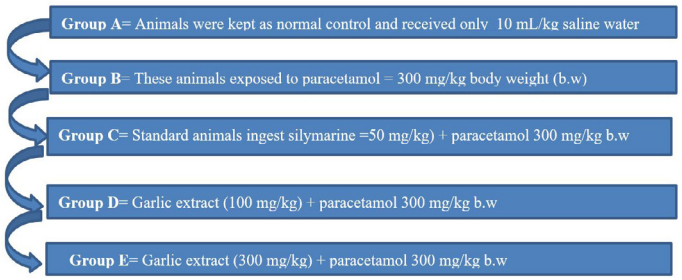

Figure 1: *Showing animal grouping and treatment pattern.*

- Group A: Received pre-oral normal saline (10 ml/kg body weight (b.w).
- Group B: (toxic control group) received 300 mg/kg b.w paracetamol once regularly for 21 days
- Group C: (standard control group): Animals in this group received silymarine 50 mg/kg + paracetamol 300 mg/kg one time daily for 21 days following paracetamol intoxication.
- Group D: (extract low dosage group): Animals in this group were given 100 mg/kg of garlic aqueous extract and 300 mg/kg of paracetamol once daily for 21 days following paracetamol intoxication.
- Animals in group E (extract high dosage group) received paracetamol 300 mg/kg once daily for 21 days following paracetamol intoxication along with garlic extract at a dose rate of mg/kg b. w.

Acute toxicity test

The metabolic extract of garlic at various dosages i.e. 500, 1000, and 2000 in milligram underwent an acute toxicity investigation. Organization for Economic Co-operation and Development (OECD) guideline number 420 and the Litchfield and Wilcoxon (1949) approach were used to determine acute toxicity (Bassanini, 2001). Since no fatality occurred in this experiment, the maximum dose of the extract (3000 mg/kg body weight) was deemed to be safe.

Dissection of animals and blood sampling for hematological and biochemical research. All of the animals were taken one by one to the lab for dissection and blood collection when the experiment was completed. According to (Bădărău, 2013), all animals were given 35 mg/kg of pentobarbital sodium as anaesthesia before being cervically decapitated to death. For the purpose of examining the biochemical parameters, blood was obtained using a heart puncture. The serum was separated from the blood samples by centrifuging them for 10 minutes at 3400 rpm (Centurion Scientific Pvt., Ltd., and UK). For the examination of biological markers such as serum glutamate oxaloacetate transaminase (SGOT), serum alkaline phosphatase (ALP) and serum glutamate pyruvate transaminase (SGPT), via (Human kit; Germany) using UV-Spectrophotometer, the serum was analysed using a spectrophotometer (Perkin Elmer; Germany).

Histological research

Liver samples were removed from the matching group of animals and promptly preserved in a solution comprising 0.9% sodium chloride and 10% formalin for histopathological investigation. The tissues were then paraffin-embedded, cut into thin sections by means of a microtome, marked with dye eosin for accurate shape and texture evaluation, and examined beneath a light microscope with fastened digital camera via which all photos were taken. Each slide of liver and kidney had a minimum of five fields that were observed and reported semi-quantitatively for the severity of alterations.

Radical scavenging activity in the DPPH

The 2, 2 diphenyl-1-picrylhydrazyl (DPPH) scavenging experiment was used to investigate the in vitro antioxidant properties of aqueous extract of garlic. Parts per million (ppm), or mg/L, is the unit of measurement used to represent the concentration of solutions produced for the activity. 500 ppm of the extract was produced as a 25 mL stock solution in methanol. From this solution, five different 5ml solutions at 20, 40, 80, 100, and 200 ppm were created. Three samples of each concentration were obtained. The same process was performed via UV-1700 spectrophotometer (Shimadzu Japan) at 517 nm for absorbance to measure ascorbic acid, which was utilised as the standard. The formula utilised was as follows: Ac is the absorbance of the control, whereas As is the absorbance of the sample, and (Ac - As/Ac) 100 determines the percentage of radical scavenging activity.

Statistic evaluation

The results were analysed using Tukey test and ANOVA keeping P value 0.05 as statistically significant.

Results and Discussion

Liver parameters

The impact of the methanolic extract of garlic root bark on liver enzymes level including ALT, AST, and ALP is described in (Table 1). The findings show that as compared to control group A, paracetamol treatment substantially (P 0.05) raised the blood levels of ALT, AST, and ALP. The concentration of ALT, AST, and ALP (group D and group E) considerably (P 0.05) decreased after treatment with garlic aqueous extract at 100 to 200mg/kg body weight, leading to a subsequent normalization. In particular, a high dose appears to be better at healing liver damage when compared to the outcomes of the toxic control group of rabbits (group B), which ingest paracetamol alone. The outcomes of the extract-treated groups were analogous to those of the rabbits (group C) given silymarin (50 mg/kg body weight), demonstrating that the effectiveness of the extract therapy depends on its dose. This discovery lends credence to the medicinal benefits of garlic aqueous extract.

Histopathological analyses

All experimental animals' liver slices were used to create photomicrographs for Figures 2, 3, 4, 5, and 6, respectively. Normal liver possessed healthy cellular architecture with dynamic cytoplasm, a conspicuous nuclei and nucleoplasm and a tight organization of hepatocytes were seen in hepatic slices from control rabbits (group A) (Figure 2). Contrarily, animals (group B) given paracetamol had hydropic change and injuries in their central lobular cells, sinusoids, and central vein. Blockage highlighted severe damaged cells invading (Figure 3) correspondingly. Silymarin-treated animals (group C) display histoarchitecture with a minimal infiltration of inflammatory cells (Figure 4). Tissue damage and necrosis were less severe in the rabbit in group D treated with garlic aqueous extract at doses of 100 mg/kg body weight and 200 mg/kg body weight (Figure 5). The hepatocyte cords of the animals in Group E were free of any problems (Figure 6). The evaluation of histological harm is shown in (Table 2).

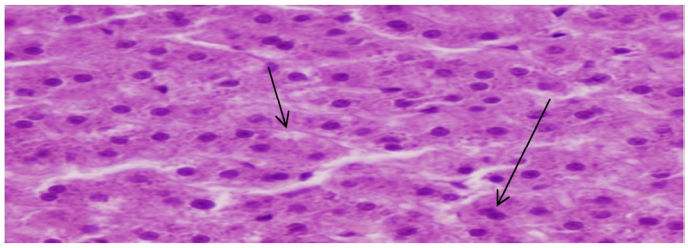

Figure 2: *Hepatic micrograph from normal rabbit (group A) suggest healthy cellular structure with well-developed mitochondria and nuclei.*

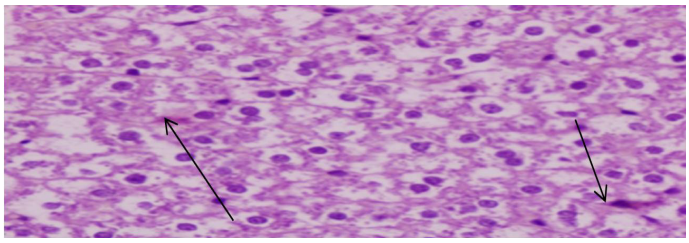

Figure 3: *Liver sections from intoxicated rabbits (group B) having necrosis and intrusion of liver cells, arrows indicating damage area.*

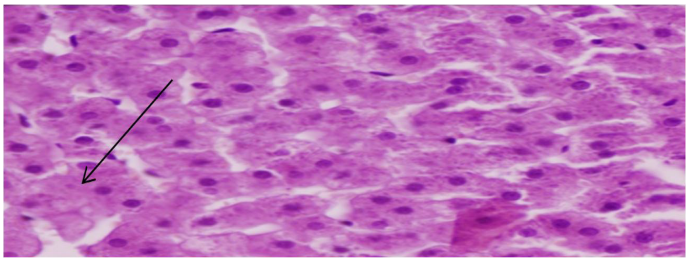

Figure 4: *Histological image from rabbit liver (group C), arrows signifies slight hepatocellular organization and healing of necrosis and restoration of cellular stability.*

Hematological properties

The blood hematology examination includes measurements of red blood cells, white blood cells, platelets, hemoglobin, hematocrit and mean corpuscular haemoglobin level. The mean values of RBC, WBC, PLT, and Hb levels of the animals in group A, who fed only with standard saline solution, were

Table 1: *Effects of garlic extract on liver function enzymes and blood parameters in CCL4 intoxicated rabbits (P < 0.05) variance.*

Liver and hematological parameters		Serum ALT (IU/L	Serum AST (IU/L)	Serum ALP (IU/L)	RBCs levels x103/µL	WBC levels x103/µL	Platelets levels G/dL	Hemoglobin levels G/dL	Hematocrit values %	(MCH) values G/dL
Control (n=8)		36.2±3.7	41.5±5.8	43.8±3.56	5.7±0.09	6.2±0.34	1.39±0.64	12.47±0.45	39±2.0	32.60±0.79
Control (PCM) (n=8)		168.5±3.42	291.5±2.6	133±3.7	5.1±0.09	3.97±0.08	1.41±1.49	10.87±0.63	24.20±0.31	20.50±0.84
Experimental PCM+ Extract (n=8)	Low dose	65.7±3.7	95.5±4.9	45.2±2.23	5.2±0.13	5.07±0.11	1.27±0.85	11.00±0.27	26.12±2.3	22.12±1.05
	High dose	43.7±7.0	47.8±7.6	45.2±2.65	5.5±0.04	5.75±0.06	1.28±0.85	11.14±0.41	34.11±0.21	23.54±0.85
	Silymarine	41.5±3.27	40.2±0.85	89.5±3.06	5.4±0.04	5.90±0.25	1.38±1.19	11.22±0.51	38±1.41	26±0.74

Table 2: *Semiquantitive score of histopathological findings.*

Groups	Group A	Group B	Group C	Group D	Group E
Hydropic degeneration	0	+++	+	++	+
Liver steatosis	0	++	0	0	0
Inflammatory cell infiltration	0	+++	0	+	0
Necrosis	0	+++	+	+	+

Damage grade are as follow: 0 denotes no abnormality, + denotes mild injury, ++ denotes moderate injury and +++ denotes severe injury.

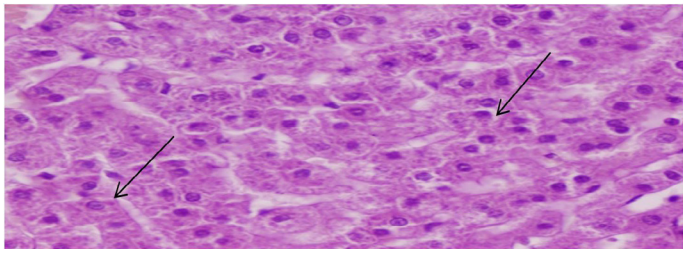

Figure 5: *Section of rabbit liver treated with 100 mg of Garlic extract (group D), arrows representing minor infiltration of inflammatory cells along with mild necrotic areas.*

all within the normal limit. When compared to rabbits receiving a normal control dose of paracetamol, there was a substantial decrease in the levels of RBC, WBC, platelets, and haemoglobin in rabbits receiving paracetamol alone (group B). Table 1 demonstrated that there was no change in haemoglobin, RBC, and platelet concentration between group D and group E rabbits given with different dosages of 100 mg/kg and 200 mg/kg ($p>0.05$). In contrast to group A (normal control) rabbits, garlic aqueous extracts Additionally, when their haemoglobin, RBC, and platelet levels were tested, silymarine-treated group C (standard control) rabbits did not differ substantially from group B (toxic control group). However, treatment with 100 mg/kg and 200 mg/kg body weight doses of aqueous extract of garlic raised the WBC concentration. The results of the rabbits treated with extract (groups D and E) were very similar to those of the extract, even though silymarine is somewhat superior (Table 1). Additionally, (Table 1) demonstrated that the treatment of aqueous extracts of garlic at doses of 100 mg/kg and 200 mg/kg did not substantially improve the HCT and MCHC values of rabbits that had been drunk with paracetamol prior to the administration of the extracts. No notable alterations in haematological markers were seen with the administration of extract and Silymarine, with the exception of WBC, which was improved (Table 1).

DPPH activity

The outcomes of the garlic root bark methanolic extract's antioxidant activity against DPPH at several dosages are displayed in (Table 3). Levels of inhibition were found to be 45.66 percent, 48.96 percent, 53.71 percent, 72.70 percent, and 76.55 percent. The lowest extract concentration (20 ppm) inhibited 45.66%, followed by 60 ppm, 80 ppm, 100 ppm, and 200 ppm, in that order. As the extract concentration increased, the percent inhibition grew. The increase in inhibition percentage is a sign of garlic's antioxidant activity (Figure 7).

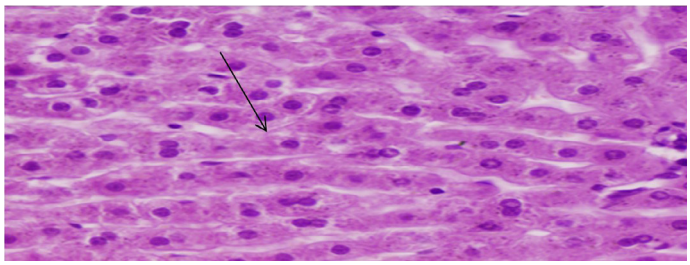

Figure 6: *Histopathological photo of rabbit liver treated with 300mg of Garlic extract (group E) arrows suggest progression in the hepatic tissue and upsurges of hepatocytes morphology.*

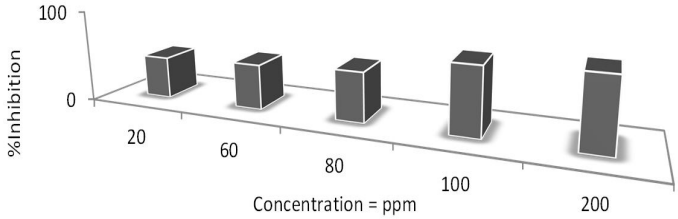

Figure 7: *Linearity between increase in concentration (ppm) of garlic extract and increase in % inhibition of stable DPPH radical.*

Identifying the hepatoprotective, therapeutic, and antioxidant properties of aqueous extract of garlic against paracetamol-persuade liver damages was the goal of the current study. For the potential hepatoprotective medications, paracetamol is routinely utilized as a hepatotoxic agent. Paracetamol is transformed by the cytochrome P 450 2EI enzyme into two radicals, which are trimethyl radical (CCl_3) and an alternative trichlomethyl radical ($OOCCl_3$) (Jia *et al.*, 2011). These radicals are the major factor for lipid peroxidation, which target the polyunsaturated fatty acids component of the membrane, this result in the oxidative stress-induced liver damage. The macromolecules and these radicals form a covalent connection (Daniel *et al.*, 2013). The damage of the liver is generally assessed by the presence of transaminases such AST, ALP, and ALT in the blood (Agarwal *et al.*, 2006). The outcomes from the current research showed that carbon tetra chloride

Table 3: *The antioxidant activity of garlic extract at various concentrations on the basis of percent inhibition of DPPH free radical (P < 0.05) of variance.*

Garlic extract concentration	20 ppm	60 ppm	80 ppm	100 ppm	200 ppm
Percent inhibition	45.66%	48.96%	53.71%	72.70%	76.55%
Mean ± SE	42.70±4.38	46.70±3.49	53.70±4.20	69.76±1.21	74.7±0.89

(Paracetamol)-treated rabbits had higher blood levels of AST, ALP, and ALT than control animals (P>0.05). An immediate rise of AST, ALP and ALT in blood is considered as key markers of significant liver injury caused by paracetamol (Vishwanth *et al*., 2012). It is believed that some pharmaceutical medications used in medical therapy, such as rifampicin, isoniazid, paracetamol, and others, are hepatotoxic, that generate free radicals which promote the lipid membrane peroxidation inducing hepatocytes damage. Necrosis and inflammatory cell infiltration seen during histological analyses of microphotographs of liver sections supported the claim (Gupta and Lewis, 2008). In the current study, silymarine and garlic aqueous extract were administered to rabbits that had ingested paracetamol to various degrees of intoxication. Silymarine, a common antioxidant medication that is widely used to protect the liver, is extracted from the *Silybum marianum* plant (Samudram *et al*., 2008). Present research investigation, the treatment of garlic aqueous extract to rabbits that had been overdosed on paracetamol reduced the paracetamol-persuade rise of serum AST, ALP and ALT toward normal or just slightly raised, indicating protection against liver injury. This indicates that garlic extract, which is almost identical to the common medication silymarine, displays strong hepatoprotective activity against paracetamol (Latif *et al*., 2021). They tested if the alcohol extract of *Capparis sepiaria* stem might protect the liver from paracetamol intoxicated albino rats. Following Paracetamol intoxication, liver sections from rabbits treated with garlic root bark metabolic extract showed altered cellular membrane structure or fewer injury to the liver cells contrasted to animals treated with paracetamol. The findings of the biochemical parameters are supported by the enhanced histoarchitecture, which further validates the liver protective capability of the garlic aqueous extract. Alkaloids, flavonoids, and saponins, which are components of garlic aqueous extract, are responsible for the extract's ability to scavenge free radicals and for its therapeutic benefits. This study shows that garlic aqueous extract has a considerable hepatoprotective potential against paracetamol at 200 mg/kg body weight and 100 mg/kg body weight, which is almost similar to the normal dose of silymarine, which is 50 mg/kg. However, the amount of a bioactive substance in an extract may be as little as a few milligrams. In the past, researchers looked at the phenolic and antioxidant content of garlic's fruit, stem, and leaves (Waqar *et al*., 2013; Mughal *et al*., 2014; Rizwan *et al*., 2013). The conclusion was made that the garlic root bark methanolic extract displayed antioxidant potential against DPPH scavenging action, and the fact that the percentage inhibition increased with the rise of extract volume additionally supported this finding.

Conclusions and Recommendations

The outcomes from the current investigation showed that garlic aqueous extract is hepatoprotective and may replace silymarine for the majority of haematological measures that display bitterness (assuming the phytochemical components of the studied plant were identified and further investigated using scientific principles).

Novelty Statement

The current study showed thatthe garlic extract has immnese regulatory effets on emtobiochemical indices even in its crude form.

Author's Contribution

Bashir Ahmad: Presented the idea.
Ali Muhammad: Did supervision.
Waqar Ali: Helped during experimental works.
Ikram Ilahi: Provided research materials.
Farman Ullah: Cut off the plagiarism.
Saeed Ahmad: Helped in article writing.
Ayaz Ali Khan: Reviewed the article.
Umair Ahmad and Hafsa Maria: Wrote the article and corrected english grammar.

Conflict of interest
The authors have declared no conflict of interest.

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