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Research Article

Histopathological Alteration in Detoxifying Organs (Liver and Kidney) of Freshwater Fish *Ctenopharyngodon idella* Exposed to Lufenuron

Maria Saeed Khan[1], Abdul Ghaffar[1], Maryam Mukhtar[1]

Department of Zoology, The Islamia University of Bahawalpur, Pakistan.
Correspondence:
mariasaeed2122@gmail.com

Abstract

The experimentation was designed to investigate the harmful effects of the widely used insecticide lufenuron on freshwater fish Grass carp (*Ctenopharyngodon idella*). The objective of this experimental study is an "analysis" of histopathological abnormalities in the liver and kidney of Grass carp. The research was accomplished in the Aquaculture, genetic toxicity, and molecular biology laboratory, Department of Zoology at the Islamia University of Bahawalpur. A total of 40 fish, with an average weight of 50-100g, were isolated to lufenuron at concentrations of 0µg/L, 2µg/L, 3µg/L, and 4µg/L, respectively, for 33 days. Three samplings were done at 11, 22, and 33 days, respectively. The liver tissues of affected fish under the microscope show various alterations, including hepatocellular vacuolization, eccentric nuclei, severe vacuolation, vascular dilation, eccentric nuclei, and hepatocyte hypertrophy. At the same time, the tissues in the control group were unaffected. Light microscopic studies revealed various histological changes in the kidney, such as vacuolar degeneration, (MGC) mild glomerulus congestion, (TD) tubular degeneration, (MMC) melano macrophage centers, (DGC) degenerated glomerulus capillaries and necrosis.

Keywords: Aquaculture, Lufenuron, Grass Carp, Histopathology

1. Introduction

Aquaculture stands as a most rapidly expanding sector of global food production and has continued to grow steadily for the past three decades [1,2]. Knowledge of the dietary requirements of each species of fish under numerous environmental situations is needed to improve and achieve aquaculture business. To achieve the best growth, best water maintenance financial efficiency, it is important to know which phase of nutritional protein is critical [3]. Food rich in protein is supposed to serve as a store of essential and non-essential amino acids, energy for organic processes, equipment for tissue conservation and repair [4, 5]. The use of pesticides has become an integral part of modern agricultural systems. Pests and parasites have severely damaged Pakistan's agricultural industry, which has had a negative impact on the country's economy. Many corrective procedures, including the regular use of pesticides, insecticides, herbicides, and fungicides, have been encouraged throughout the nation to control pests, parasites, and to improve crop productivity [6]. In fields to control pests, agrochemicals are used, and almost 90% of chemicals remain in the environment without degradation. After being used, these pesticides, insecticides, herbicides, and fungicides are easily able to infiltrate surrounding surface water through runoff during the monsoon, which has a negative effect on freshwater species [7]. It is observed that perceived constituents respond with genetic material and lead to transformations at the cell level in uncovered species. A few irregularities in living beings like

apoptosis, hypospadias, formative testicular oddities, just as conceptive status, and organ capacities, primarily in fish, were seen because of pollution by buildups of pesticides utilized in agrarian items [8-10]. Health issues for both aquatic creatures and people are being caused by the global threat of aquatic ecosystem pollution brought on by numerous poisons [11]. To understand how aquatic organisms and people are exposed, the ecotoxicological study is crucial [12]. Histological damage to the liver and subsequent physiological disturbance may occur in the conclusion of pesticide exposure [13-15]. A subsequent transfer toward the food chain is the introduction of agricultural contaminants into water bodies, which become a significant threat to human and aquatic organisms [16]. Primarily, it is used in a variety of industries, including public health management, cereal crops, grapes, corn, citrus, sugar beetroot, potatoes, and decorative plants to eradicate pests, insects, and western flower thripids [17, 18]. Lufenuron is also available in the market and used as an agricultural insecticide against lepidopteron, thrips, and eriophyid mites[16]. Insects' digestive systems are affected by the uncontrolled usage of the benzoyl urea pesticide, lufenuron (LUF) [17]. Lufenuron, as classified by the European Food Safety Authority (EFSA), was considered highly toxic to aquatic life. Additionally, LUF causes tissue damage by altering the antioxidant system and fish's ability to adapt to saltwater [16, 18]. Lufenuron plays a substantial function in the contest against pests that afflict corn and vegetables, particularly those belonging to the Lepidoptera and Coleopteran families. Moreover, its remarkable efficacy has been exhibited in managing pests like the citrus rust mite *Helicoverpa armiger* and *Pectinophora gossypiella*. These pests are responsible for causing substantial harm to cotton cultivation in nations like India and Egypt [19]. Its (Match 10%) functions as a CSI insecticide, replicating juvenile hormones and ecdysteroid agonists to disrupt insect growth and cause their death. It also exhibits therapeutic potential in mammal cells. Lufenuron is at as a versatile tool in both insect control and animal health, with capable applications in agriculture and veterinary medicine [19, 20]. Various pollutants are mainly absorbed through the gills, skin, and digestive tract and spread to organs and tissues, changing biological and natural mechanisms [21]. Gills serve as the most polluted organ because they are completely exposed to water. Toxins come in contact with the body across the gills and increase oxygen consumption. Consequently, monitoring critical stress in the aquatic background is a valuable marker [22]. The assessment of DNA damage in aquatic creatures has stressed that genotoxic effects can cause genetic and reproductive changes if germ cells are impacted, as this can result in failure, and somatic cell effects can cause genotoxic effects to cause the onset of carcinogenesis. These things may cause infertility and subsequent demographic shifts [23, 24]. The grass carp belongs to the family Xenocypridinae and is native to the vast rivers of eastern Asia; introduced to various regions (Asia, North America, and nearly all of Europe) since 1945, primarily for aquaculture and management of aquatic vegetation [25]. A valuable species of fish for human consumption, carp rely heavily on gut health for growth and survival. Regardless of its economic importance, some scientists are investigating the impacts of micro plastics (MP) on grass carp guts. It was reported that MPs could adversely affect antioxidant enzyme activity, intestinal system, bacterial communal, and metabolism in grass carp intestines [26]. The primary diet of the grass carp consisted of streptophyta, including arthropods, rotifers, ascomycetes, and chlorophytes. The prevalence of Streptophyta increased progressively from tributaries to the central delta. Significantly positioned at the second trophic level, the grass carp displayed varying isotopic niche characteristics across regions [27].

The objective of this research was to see how lufenuron affects the survival of grass carp. The specific goal was to examine the histopathological changes in fish caused by the exposure of lufenuron. Furthermore, observe the lethal and sub-lethal effects of lufenuron in exposed fish (*Ctenopharyngodon idella*) and examine the activity of detoxifying organs (Liver & Kidney) in grass carp.

2. Materials and methods

2.1. Experimental location

The overall study "Effects of Lufenuron on Enzymatic Activity and Detoxifying Organs of *Ctenopharyngodon idella*" was completed in thirty-three days, which was conducted at the Aquaculture, Genetic Toxicity, and Molecular Biology laboratory in the Department of Zoology at The Islamia University of Bahawalpur, Pakistan.

2.2. Experimental fish

Grass carp, scientifically known as *Ctenopharyngodon idella*, was used as an experimental fish. It has significant importance in freshwater systems and displays a global distribution. Grass carp is a notable herbivorous commercial, and the largest freshwater fish species in China showed dose-dependent microscopic kidney changes.

2.3. Experimental chemical

Lufenuron was used to examine the histological alteration in detoxifying organs of *Ctenopharyngodon idella*. To attain the required concentrations of 2µg/l, 3µg/l, and 4µg/l, solutions were promptly prepared by dissolving a 5 percent emulsifiable lufenuron in distilled water, with a volume of 1 ml per solution.

2.4. Experimental design

After the acclimatization period, 40 experimental fish were evenly allocated among four experimental groups. Within individual groups consisting of 10 fish, random assignment (T1, T2, T3, and T0 control group) was performed, followed by the administration of the insecticide lufenuron once the fish had adapted to laboratory conditions. The control group was denoted as a group (T0), while groups T1-T3 were exposed to variable concentrations of lufenuron (Table: 1.1). Utilizing environmentally relevant levels (2, 3, and 4µg/L) based on previous research; the lufenuron treatment persisted for 33 days. In this period, samples of various tissues such as gills, kidneys, brain, liver, and heart were collected from each experimental fish on 11, 22, and 33 days of trial. These tissue samples were preserved for later analysis.

2.5. Appraisal of physicochemical facets

The temperature, electrical conductivity, and pH of each aquarium were monitored regularly. The usage and safety of laboratory organisms were addressed in all experimental methods in accordance with the guidelines published by the Islamia University Bahawalpur office of the Directorate of Research and Bioethics committee. The physicochemical parameters were tested after each sampling during the trial period.

2.6. Blood sampling

At the end of the trial period, four random samples of blood were taken from each pond to examine the health and anomalies of the reared fish. There will be less disruption in each pond during fish collecting, which will reduce stress on the species. Blood was removed from the caudal part of the vein of the fish via 3mL disposable plastic syringes containing a modest amount of EDTA and a 21-number gauge disposable syringe. Blood should be discarded if clotting occurs in the plastic syringe or if drawing blood is difficult. Blood was collected and placed in a BD vacutainer Plastic K3EDTA Tube. Then, for anticoagulant mixing, gently slide it back and forth. Blood samples are kept at 4°C temperature.

2.7. Biochemical analysis

Fish was dissected on days 11, 22, and 33 of the experiment for the separation of many internal organs (the kidneys, gills, brain, liver, and heart) for biochemical assessment.

2.8. Organ collection and necropsy

After dissecting vital organs including the heart, gills, brain, kidneys, and liver were extracted carefully, weighted, and preserved for further analysis. The relative and absolute weight of organ of whole groups fishes were measured and compared. Histopathological changes were detected in all including control and treatment groups. Organs tissues were preserved in 10 % formalin. A techniques paraffin–wax embedding was applied on 5µm thick section of tissue for histopathological analysis. Eosin and hematoxylin [28] stain was used on it.

2.9. Statistical analysis

To perform statistical analysis on normally distributed data within each group, a one-way investigation was conducted

using ANOVA statistical software. Post hoc Tukey's test with a consequence of beginning was employed to assess variation in mean values (mean ± SE) pertaining to oxidative stress and antioxidant enzymes in blood, as well as in certain internal organs of both experimental and control groups. Additionally, correlation analysis of Pearson was conducted to examine the relationships between various variables in the gills, kidney, heart, brain, and liver of the experimental fish.

3. Results

After 11, 22, and 33 days, samples were taken from the control and treatment groups, respectively. Dissection of fish was done, and the internal organs of fish were dissected. The relative and absolute weight of the organs of whole groups of fish was measured and compared. Tissue slices of required organs were preserved at 10 percent formalin and applied to examine the histopathology of tissue. For dehydration of slices of tissue, ethanol was used and further cleaned in xylene and fixed in paraffin. 5µm dense tissue pieces were obtained by cutting. The staining process was used to cut tissues. For tissue fixation, the cutting tissue samples were kept at 10 percent formalin. After this process, the solution tissue had moved into seventy percent ethanol. Now, the tissues were ready for histopathology.

3.1. Histopathology of kidney

The kidney of untreated group T0 of fish *Ctenopharyngodon idella* (Grass carp) did not show any microscopic deviation. Distal tubule (DT) and mitotic epithelial cell (MEC) were integral while proximal tubule (PT) and bowman's space (BS) were in normal pattern. Group T1 treated with 2µg/L of lethal concentration of chemical for 33 days showed only mild congestion of kidney. However, the kidney of group T2 and T3 treated with 3µg/L of lethal concentration of lufenuron, showed extensive microscopic abnormalities such as Enlarged bowman capsular space (EBCS). T2 group shows (VD) vacuolar degeneration, (TD) Tubular degeneration, and (MDG) Mild degeneration of glomerular capillaries. While T3 group shows (MMC) Melano macrophage centers, (VD) vacuolar degeneration, (TD) Tubular degeneration, and (DGC) Degenerated glomerulus capillaries. We observed that experimental group T1 show moderate abnormalities while group T2 and T3 shows sever irregularities.

3.2. Histopathology of liver

Microscopically, the tissue section of the liver of control group T1 of fish Grass carp showed standard patterns of all the cells, counting hepatocytes, containing one nucleus in each, squalors epithelial cells, pancreatic vein, and hepatocytes with pyknotic nuclei. While experimental group T1, treated with 2µg/L of lethal concentration of chemical for 33 days, showed the least alteration in the histology of the liver. Group T2 and T3 treated with 3µg/L and 4µg/L of lethal concentration of lufenuron, respectively, showed the following abnormalities: hepatocellular vacuolization, eccentric nuclei, vascular dilation, Esentric nuclei, and hepatocytes hypertrophy. We observed that experimental group T2 shows moderate abnormalities while group T3 shows severe abnormalities.

3.3. Histopathological aberration induced by Lufenuron

Histopathological alteration in blood parameters is supposed to be symbolic of environmental stress, tissue damage, and harmful conditions [29]. Pathophysiological conditions, blood biochemistry, and genetic toxicity calculation were regarded as dependable diagnostic methods for evaluating the health of fish in polluted systems [30-32]. All recovered tissues underwent processing and staining via hematoxylin and eosin staining procedures to document any histopathological anomalies [6]. The experimental fish exposed to various concentrations of lufenuron at 1.2µg/l and 1.7µg/l for 39 days showed histopathological modification in gills. Anomalies include disarray in lamellae arrangement, atrophy, congestion, telangiectasia, and disruption in secondary lamellae, causing severe damage [33]. In particular, previous research has also identified several histopathological irregularities in the gills of *Colossoma macropomum* [18]. When the concentration of lufenuron increases, it causes histological changes in the liver of tilapia fish. These changes include congestion, pyknosis, hemorrhages, karyolysis, and hepatocytes with eccentric nuclei. Similar anomalies were seen in *Cyprinus carpio* [34].

Table 1. Shows different histopathological changes in liver and kidney of *Ctenopharyngodon idella* treated with various concentrations of Lufenuron.

Histopathological alterations	Groups		
	T1 (2µg/L)	T2 (3µg/L)	T3 (4µg/L)
Liver			
Hepatocellular vacuolization	+	++	+++
Eccentric nuclei (ECC)	+	++	+++
Severe vacuolation	+	++	+++
Esentric nuclei	+	++	+++
Hepatocytes hypertrophy	+	+++	+++
Kidney			
Vacuolar degeneration (VD)	+	+	+++
Mild glomerulus congestion (MGC)	+	+	++
Mild tubular degeneration (MTD)	+	++	+++
Tubular degeneration (TD)	+	+++	++
Mild degeneration of glomerular capillaries (MDG)	-	+++	++
Melano macrophage centers (MMC)	+	+++	++++
Degenerated glomerulus capillaries (DG)	+	+	+++

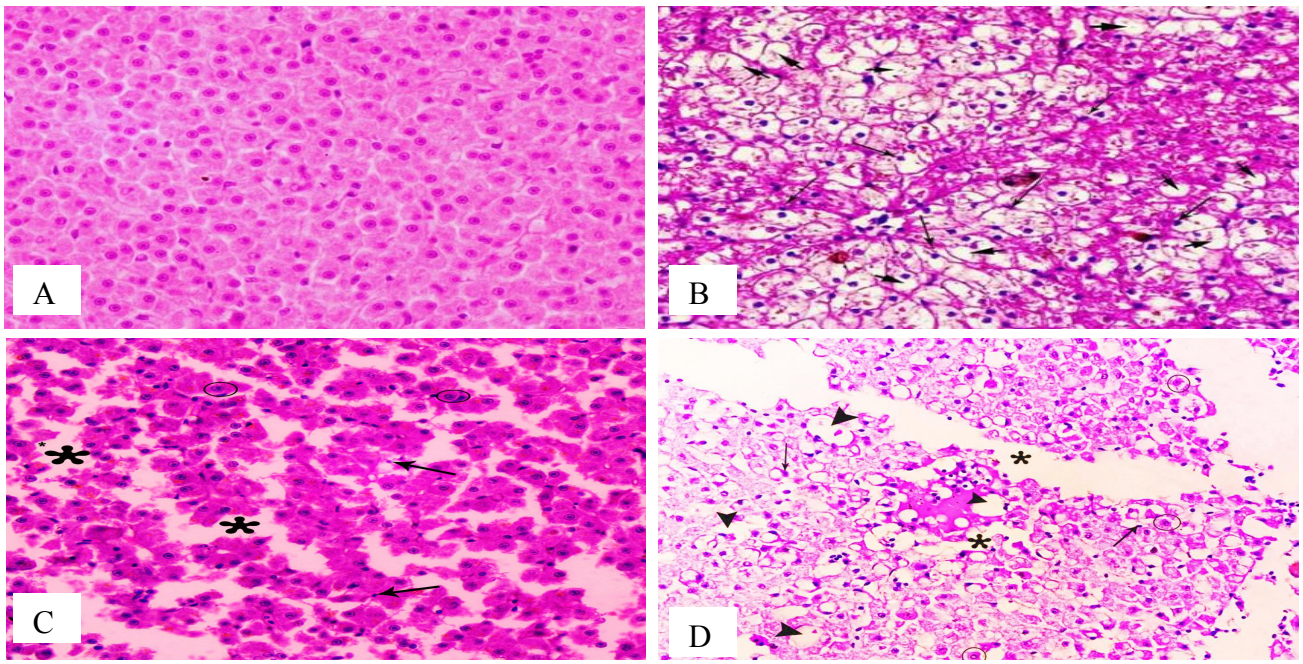

Figure 1. Liver tissues of Grass carp (Ctenopharyngodon idella) at different concentrations of toxicant (0 µg/L, 2µg/L, 3µg/L, and 4µg/L) at 40×, H&E staining showing showed pathological alterations. (A) Represent (T0) control group with no effect. (B-C) T1 to T3 show effects such as hepatocellular vacuolization (big arrows), (ECC) eccentric nuclei (short arrows), Severe vacuolation (arrowhead), vascular dilation (*), Esentric nuclei (thin arrows) and hepatocytes hypertrophy (round).

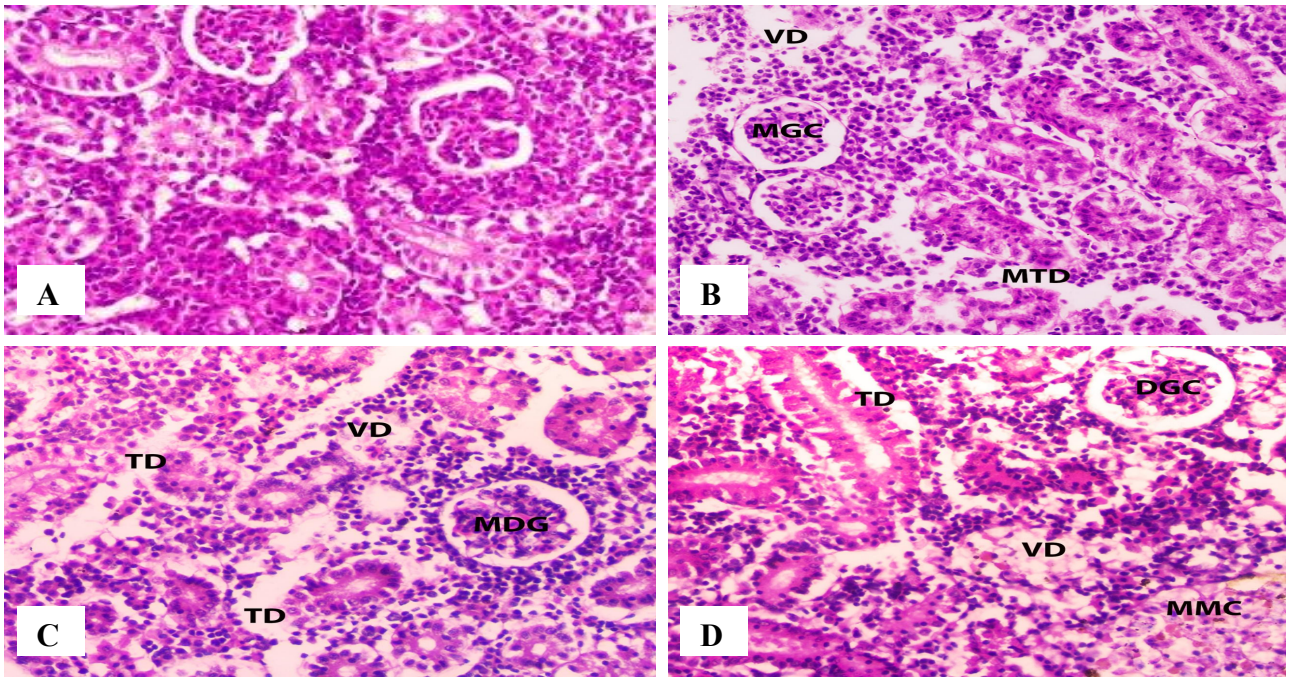

Figure 2. Micrograph of Grass carp (*Ctenopharyngodon idella*) renal sections at different concentrations of toxicant (0 µg/L, 2 µg/L 3µg/L, and 4µg/L) at 40×, H&E staining. (A) Represent T1, the control group with no effects, while T1 shows (VD) Vacuolar degeneration, (MGC) Mild glomerulus congestion, and (MTD) Mild tubular degeneration. (B) T2 shows (VD) vacuolar degeneration, (TD) Tubular degeneration and (MDG) Mild degeneration of glomerular capillaries. (C) T3 shows (MMC) Melano macrophage centers, (VD) vacuolar degeneration, (TD) Tubular degeneration, and (DGC) Degenerated glomerulus capillaries.

Lufenuron lowered reproductive levels, motility, sperm count, and viability while inducing adverse histological alterations in testicular increased luminal diameter with degenerated spermatogenesis [35]. These changes were ascribed to oxidative damage marked by increased MDA and NO levels, along with decreased antioxidant enzyme activity [36]. Table 1 shows the histopathological alteration in the liver and kidney of grass carp exposed to various concentrations of lufenuron.

4. Discussion

Investigation of diverse fish species reveals physiological challenges, histopathological issues, and changes in enzymatic activity during the acclimatization phase caused by exposure to multiple harmful pesticides. Therefore, this research explores the impact of lufenuron on histological illnesses in grass carp fish [13]. Lufenuron exposure at concentrations of 1.2µg/L and 1.7µg/L induced substantial histopathological changes in gills by day 39, including congestion in cartilaginous cores, lamellae disarray, atrophy, necrosis, telangiectasia, disruption, curling, uplifting, and lamellar disorganization. These findings highlight the significant impact of lufenuron on the gill structure and function in aquatic organisms [33]. On day 39 of the recent research, diverse microscopic abnormalities in gills were seen, ranging from mild to severe. The liver serves as the primary site for triglyceride and cholesterol metabolism. Experience with toxicants can reduce passing concentrations of cholesterol and triglyceride. The result is from both impaired absorption of cholesterol and triglycerides in the intestine, stemming from the toxicity of gut cells, and/or liver damage leading to compromised cholesterol as well as lipoprotein synthesis [13]. The gills are accountable for absorbing poisonous substances from water, and the liver is one of the vital organs of detoxification. As the gills are the primary source and are affected first, toxic substances enter the fish through them. The destructive effect of lufenuron on fish organs exhibits acute

lesions. There was a clear difference between doses with modest and high meal sizes [13]. The liver of Nile tilapia displayed histological variations caused by elevated concentration of lufenuron, including nuclear hypertrophy, congestion, karyolysis, and vacuolar deterioration, shrinkage of cells, bleedings, karyorrhexis, and hepatocytes with irregular nuclei. Comparable histological alterations were previously viewed in *Cyprinus carpio* in earlier studies [37]. Lufenuron introduction led to mobbing, neuronal necrosis, and intracellular edema in the brain, resulting in mild to moderate histological changes. Additionally, the exposure to lufenuron prompted histopathological alterations in the kidneys, such as Melano macrophage centers, vacuolar degeneration, Tubular degeneration, and degenerated glomerulus capillaries.

5. Conclusion

In conclusion, our findings suggested that lufenuron easily bioaccumulates in various tissues of different aquatic species, even at very low concentrations. It was observed that lufenuron affects the multiple tissues of the liver and kidney of grass carp. Histopathological analysis of liver and kidney of *Ctenopharyngodon idella* exposed to lufenuron at increased level induced severe alterations. This experiment also investigates the toxicity of lufenuron and problems regarding its use and improper discarding. And recommend the measurements to prevent contaminations in aquatic environments. The results of this research work were a guide for researchers who are engaged in scientific work, especially for the protection of aquatic systems. In the future, we can neutralize the contamination of lufenuron in marine ecosystems by developing natural agents such as biofilters, phytoremediation, and microbial degradation.

Data availability statement

The data supporting the results of this study can be obtained from the corresponding author upon request.

Conflicts of interest

All authors declare that they have no conflicts of interest.

Ethical approval

Not applicable (N/A)

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Authors Contribution

Maria Saeed Khan conducted the research, collected and analyzed the data and wrote the manuscript. Abdul Ghaffar supervised and reviewed. Maryam Mukhtar reviews the manuscript.

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