(Research Article)

Effect of Sub Lethal Doses of Nickel Chloride on CBC and Histology of *Cirrhinus mrigala*

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Abstract: The present study aims to investigate the histological and CBC parameters on *Cirrhinus marigala* after the exposure of Nickel Chloride. The first group of fish was taken as control group and placed in normal water without given any treatment. The second and third groups of fish was administered with sub-lethal doses of nickel chloride (0.937 and 1.25 ppm) for 30 days. The study showed that the number of white blood cells, platelets and haemoglobin level were significantly increased where simultaneously the number of red blood cells was significantly decreased due to exposure of nickel chloride. It was also observed that nickel chloride accumulated in organs (Kidneys < Gills < Liver < Heart) of *Cirrhinus marigala*. Results of histopathology showed the heavy damage of vital organs in fish due to nickel chloride exposure. One-way ANOVA test was performed in SPSS package volume 16. This study concluded that nickel chloride effects the CBCs and histopathological parameters of fish *Cirrhinus marigala*.

Keywords: Fish, Heavy metal, CBC, Histology

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1. Introduction

Fish is a healthy diet even in a small portion because it contains lots of nutrients like calcium, iron, vitamins A, B, D and iodine [1]. Carps play important role in aquaculture like *Cirrhinus mrigala* or commonly known as Mori which is a significant for carp culture in Pakistan, Nepal, Bangladesh, Thailand, and Myanmar [2]. Adults of *Cirrhinus mrigala* are considered as bottom feeders and can tolerate wide range of temperature [3].21st century brings the issue of availability of clean water for all life forms The problem is more sever in the developing countries with the contamination of heavy metals. Heavy metals do not decompose and breakdown easily and cause serious problems in water reservoirs [4]. The most significant heavy metals in water contamination are zinc, copper, lead, cadmium, mercury, nickel and chromium [5]. Nickel (Ni) is considered as 24th important component present outside the surface of earth, which comprises of the 3% of earth structure [6]. The primary sources of nickel originate from hydrogenation of oil industry, paint factories, engine vehicle, airplane industry, and printing [7]. Nickel harmfulness is

commonly low, however continuous accumulation cause sub deadly impacts like malignant growths of the nasal cavity, paranasal sinuses and lungs [8].

The major objective of the present study was the study of the effect of the nickel chloride on the organs and blood of the *Cirrhinus mrigala* by using the method of histology and CBC. Histopathological studies are used to express the health condition of exposed tissue from pollution present in water [9]. Parameters of blood are important in diagnosing the functional properties of organism exposed to pollutants [10].

2. Materials and Methods

This experimental study was conducted in Fisheries lab of IMBB (Institute of Molecular Biology and Biotechnology) department of The University of Lahore. *Cirrhinus marigala* was acclimatized for laboratory condition for one week and examined thoroughly for disease conditions as well as for injuries. After examination healthy fish was utilized for experimental study. Fish was separated into three groups; each group contain 15 fish. All the groups were kept in their separate trough. A stock solution of $NiCl_2$ was prepared by dissolving it in distilled water. The nominal concentrations used for this study were $1/3^{rd}$ and $1/4^{rth}$ of LC_{50} (3.75ppm) of $NiCl_2$ [11]. The first group was a control group and placed in normal conditions without any treatment. The second group (165 ml) and third groups (124.24 ml) of Nickel chloride were administered for 30 days. The temperature of aquarium was checked and regulated at 22-24^{o}C with 8 pH. The fish from the respective experiments as well as controlled groups was dissected and the vital organs were isolated from the fish to perform the histopathological tests and the blood was collected for CBC studies by following standard methods [12]. Histopathological studies were done by using histopathological procedures used by [13].

All research work in the submitted paper had been conducted in an ethical and responsible manner, and is in full compliance with all relevant codes of experimentation and legislations. This study is approved by ethical committee of the University of Lahore. The meeting held at 12-03-2020 with approval number UOL-IMBB-2346.

3. Results

3.1. Blood parameters

The CBC results of *Cirrhinus marigala* after the exposure of nickel chloride shown in Table 1. Toxicity of nickel chloride increased with increasing the concentration of doses. Red blood cell count was normal in control group but after the exposure of nickel chloride RBC count are decreasing significantly (p = 0.002). Similarly, the white blood cell counts are increased significantly (p = 0.004) in *Cirrhinus mrigala*. Platelets cell counts are also increased significantly (p = 0.001). Hemoglobin counts was increased in second treatment group (T2) but decrease in First treatment group (T1) and values are statistically significant (p = 0.005).

3.2. Histology Results

3.2.1. Histology of Liver

Histopathological studies of Liver of control group revealed normal hepatic architecture, normal size sinusoids and normal morphology of hepatocytes and porta hepatis. No evidence of any inflammatory disease, granuloma or malignancy was observed as

shown in Figure 1a. Liver examination of both treatment group exhibit markedly congested central veins and sinusoids. The hepatocytes show vacuolar degeneration.

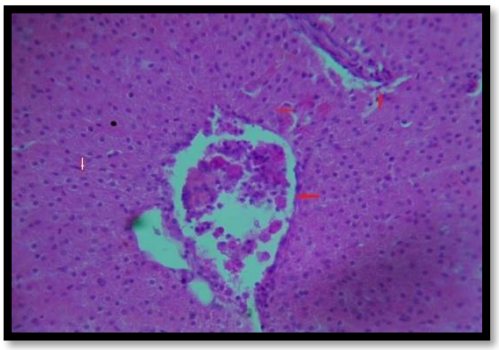
Parameters	Control Mean± St. Error	Treated Group (1) Mean ± St. Error	Treated Group (2) Mean ± St. Error	P value
WBC	1121.6 ± 0.3	1137 ± 1.7	1189 ± 6.07	0.004**
RBC	0.84 ± 0.002	0.68 ± 0.05	-1.98 ± 0.08	0.002**
HGB	3.56 ± 0.04	3.0 ± 0.2	6.78 ± 0.2	0.005**
PLT	21.00 ± 0.00	47.00 ± 1.8	38.80 ± 1.0	0.001***

Periductal fibrosis is present in some places. There is neo-generation of bile ductules. No evidence of granuloma or malignancy seen as shown in Figure 1b and 1c.

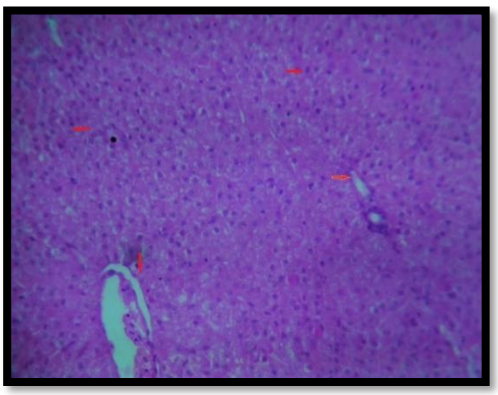
Table 1: Effect of nickel chloride in blood samples (RBC, WBC, HGB, and PLT)* of *Cirrhinus marigala* were given in table.

P < 0.001 = Highly significant (***), P < 0.01 = Highly significant (**)

- WBC (White blood cells), RBC (Red blood cells), HGB (Hemoglobin), PLT (platelets)

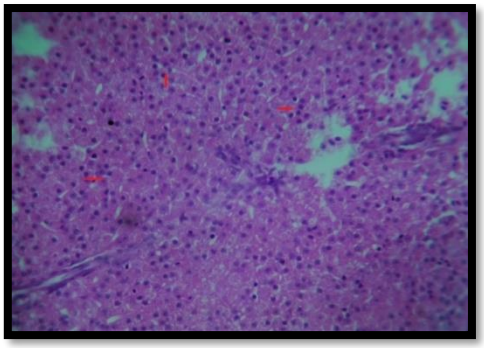


(a) **(b)**



(c)

Figure 1: (a) Histological Features of Control Liver. (b) Histological Features of Liver of T1 Group (c)



Histological Features of Liver of T2 Group

3.2.3. Histology of Kidney

Kidney histological examination of control group revealed normal looking renal tissue with normal nephrons. The tubules and ducts are normally functioning. The vasculature ratio is uniform. No evidence of any inflammatory disease, granuloma or malignancy as shown in Figure 2a. Kidney specimens from both treatment groups shown markedly congested vessels and presence of focal haemorrhages. The renal tubular epithelium shown hydropic degeneration. The glomerular Bowmman's spaces were oedematous, suggesting reduced glomerular filtration rate (GFR). No evidence of granuloma or malignancy seen in Figure 2b and 2c.

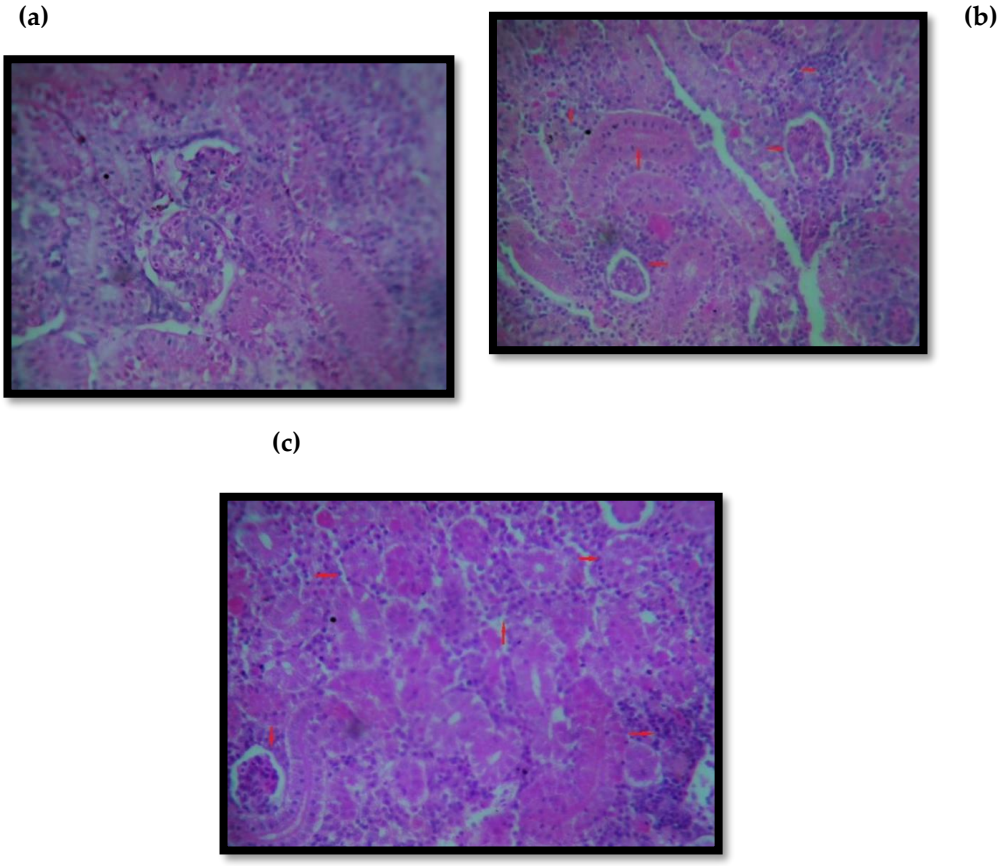
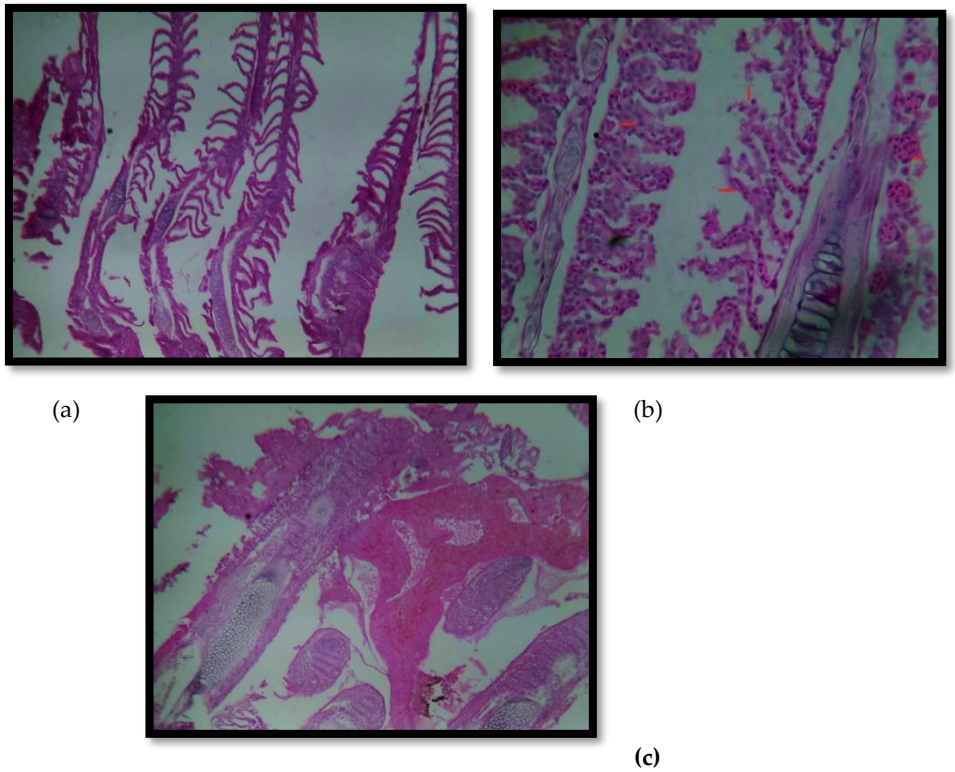


Figure 2: Microphotograph of the Kidney compared with control and treatment groups (a) Kidney Control (b) Kidney (T1) (c) Kidney (T2) 40X

3.1.4. Histology of Gills

Control group Gills of *Cirrhinus mrigala* were normal in histological studies as shown in Figure 3a. No evidence of any inflammatory disease, granuloma or malignancy seen. Histological examination of the treated group 1 and 2 gills specimen revealed presence of hemorrhages and rupture of lamellar epithelium. The blood vessels are congested and exhibit micro-aneurysms. No evidence of granuloma or malignancy observed as shown in Figure 3b and 3c.



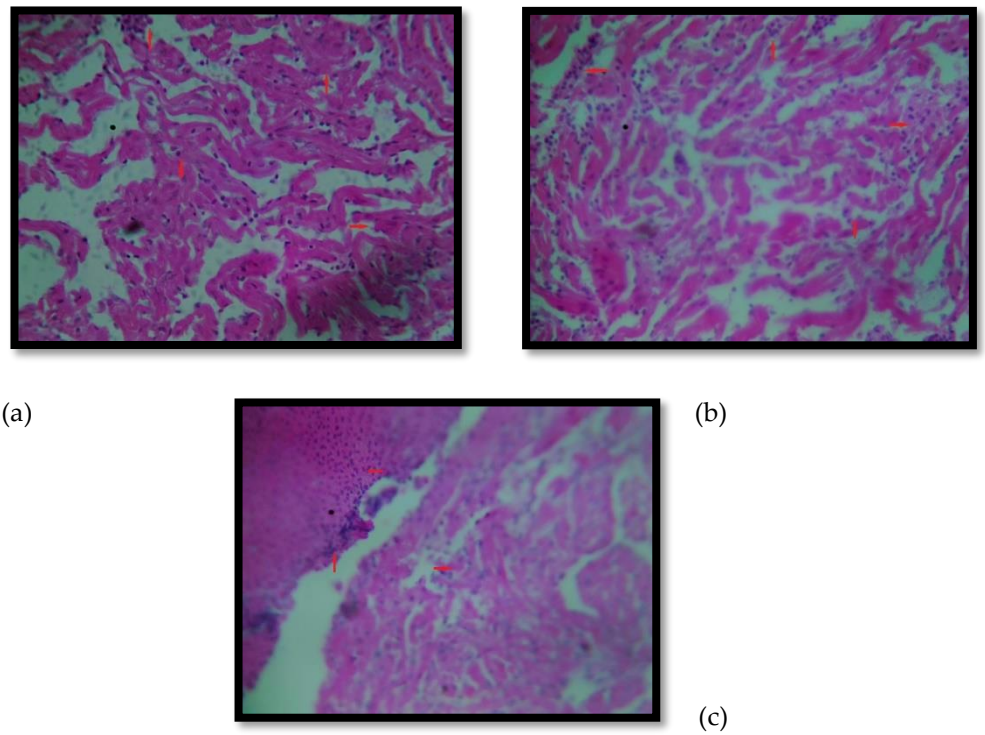
(a) (b)

(c)

Figure 3: (a) Histological Features of Control Gill. (b) Histological Features of Gill of T1 Group (c) Histological Features of Gill of T2 Group

3.1.5. Histology of Heart

Heart of *Cirrhinus mrigala* of the control group reveals normal looking vascular supply, neural bundles and heart muscles with no evidence of any inflammatory disease,



(a) (b)

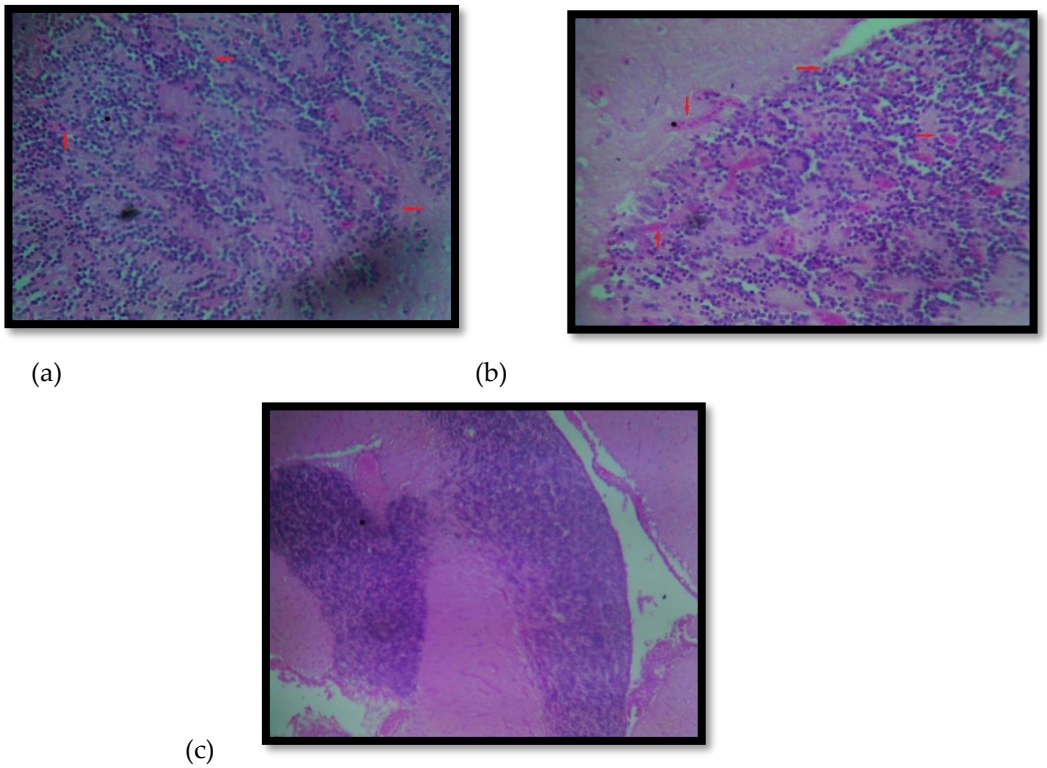
(c)

Figure 4: Comparision of histology of heart (a) Histological Features of Control Heart. (b) Histological Features of Heart of T1 Group (c) Histological Features of Heart of T2 Group

granuloma or malignancy (Figure 4a). Histological examination of heart (treated group 1and 2) revealed elongation of blood vessels of heart. The muscle fibers appear to be flaccid. No evidence of granuloma or malignancy seen (Figure 4b, 4c).

3.1.6. Histology of brain

Histology of control group brain of *Cirrhinus mrigala* shown in Figure 9a with normal looking brain tissue. The astrocytes, neurons, ganglions and vasculature were in normal ratio No evidence of any inflammatory disease, granuloma or malignancy was observed. Histological examination of the T1 and T2 group brain specimen revealed demyelination of neurons. There were areas of necrosis of brain tissue. Mild brain tissue oedema is also evident. No evidence of granuloma or malignancy also observed (Figure 9b, 9c).



(a) (b)

(c)

Figure 5: Comparison of histology of brain (a) Histological Features of Control Brain. (b) Histological Features of Brain of T1 Group (c) Histological Features of Brain of T2 Group

4. Discussion

During recent studies red blood cells (RBC) was significantly decreased because the nickel chloride exposure led to anemia according to [14] Sinha who also studied the hematological parameters of *Channa punctatus* exposed to nickel chloride, and the reduction level of RBC and HGB in fish when treated with heavy metal [15]. The results are supported by previous research work that many heavy metals salts and toxicants entering into aquatic system which exerts a toxic effect on fish blood [16]. Researchers explained that decrease of RBC is due to erythropoiesis or by diminishing of red cells [17]. Heavy metals exposure can lead to anemia and hemolysis which may be due to hypocromic

anemia in which deficiency of iron takes place or it diminished the use of erythrogenesis [18]. Izah and Angaye reported that significant decrease in Red Blood Cell count, Hemoglobin level, and increase in White blood cell count in *Channa puntatus* fish can related with effluents and heavy metal pollution [19]. The increase of WBC is because of lymphoiesis stimulation and its release from lymphoid to tissues [10]. Lymphocyte increases at faster rate and it remove cell debris present in necrotic tissue. Parrino and his colleagues stated that white blood cell count was significantly increased due to exposure of selenium [12]. Kapil have observed the hematological effect on *Cyprinus carpio* when exposed to sub lethal doses of sodium nitrate [20].

Decrease of RBC and HGB are similar with previous studies in *Oreochromis mossambicus* fish expose to zinc and copper [21]. Decrease in HGB content is due to inhibitory effect of heavy metals toxins in enzymes which synthesize the hemoglobin cells [14]. Ullah *et al*. studied that due to exposure of heavy metal RBC and HGB are decreased due to impaired iron absorption in intestine [22]. Sinha reported that decrease of HGB and RBC is due to exposure of cadmium in *Channa punctatus* [14]. Decrease in HGB count and Red Blood Cell count, reflects that fish is in anemic state [23].

Hyperalbuminaemia, hyperproteinnemia and hypercholesterolaemia was observed in ***Oreochromis niloticus*** fish after exposure of nickel chloride treatment is due to injury of liver [24]. Liver injury is characterized by degeneration, elongations of blood vessels and necrosis by lesions [16]. Qadir and Iqbal reported that hemorrhage and congestion in the liver sinusoids and hepatic vessels dilate degeneration and vacuolization of liver cells exposed to contaminated water having heavy metal salts [13]. Salts of heavy metal present in domestic sewage, agricultural runoff or industrial effluent may cause adverse effect on vital organs especially kidneys which filter urine and on gills and scales, but also on organs like ovary, testis, and liver because salts of heavy metal enter into the cells by crossing of the membranes of cells where they can interfere with enzyme into cells result into structural damage and vital functions [11]. Study showed that exposure of nickel chloride to ***Oreochromis niloticus*** gills presented a high damage to gill structure i.e. presence of histopathological lesions such as shortening of secondary lamellae, adjacent of filaments hypertrophy and fusion of adjacent lamellae and hyperplasia observed in gill epithelia of ***Oreochromis niloticus*** [21].

Cadmium exposure to shark significantly decreased ventricular developed pressure of hearts in reversible manner [25]. Nickel exposure decrease the activity in the brain of *rainbow trout* [26]. Present studies are similar with other studies performed in fish brain with other heavy metals causing necrotic change and demyelination were observed in brain tissues.

5. Conclusions

In the present research *Cirrhinus marigala* administered with two concentrations of sub-lethal doses of nickel chloride for 30 days, showed significant alteration in blood parameters (WBC, RBC, HGB, PLT) of fish. Results confirmed the sub lethal effect of nickel chloride on *Cirrhinus marigala*. The increase in the level of white blood cell, platelets and hemoglobin and decrease in the level of red blood cells showed the hematoxic effect of

nickel chloride. Histopathological alternation in different organs (Kidneys < Gills < Liver < Heart) of *Cirrhinus marigala* were also observed in this study. Nickel damaged liver, gills, kidneys, and heart of fish after the exposure of sub lethal doses.

Author Contributions: "Conceptualization, Dr. Quratulane Gillani.; methodology, Nida saleem and Saim Haroon software, Dr. Quratulane Gillani and Dr. Skinder Hayat; validation, Dr. Quratulane Gillani and Dr. Skinder Hayat; formal analysis, Dr. Uzma Yasmeen; investigation, Nida saleem and Saim Haroon; resources, Nida saleem and Saim Haroon; data curation, Nida saleem and Saim Haroon; writing—original draft preparation, Nida saleem and Saim Haroon; writing—review and editing, Dr. Quratulane Gillani; supervision, Dr, Quratulane Gillani;. All authors have read and agreed to the published version of the manuscript."

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