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Efficacy of taurine and garlic extract in alleviating the histopathological changes in gills induced by long-term exposure to copper sulphate in *Clarias gariepinus*

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INTRODUCTION

Copper is a micronutrient essential for all living organisms required in small amounts, and important life functions cannot function properly in its absence[1]. Copper also forms an essential part of cytochrome oxidase, is a component of many metalloenzymes and is involved in essential redox reactions within the cell[2]. However, copper becomes toxic to aquatic biota when its concentration exceeds than the biological requirement. The toxic effect of copper is related to its capacity for catalyzing oxidative reactions, leading to the production of reactive oxygen species[3]. In freshwater environments, copper can act as Na analogues and competitors in gill transport systems, and out-compete Na, thereby blocking transport systems[4].

Recent trends in controlling and treating diseases favour natural antioxidant, which could chelate heavy metals into non-ionized and less toxic complex to be excreted in urine or faeces. Taurine (2-aminoethane sulphonic acid, $^{+}NH_3CH_2CH_2SO_3^{-}$) is the major free intra cellular non protein sulphur amino acid[5] found in milimolar concentrations in many animal tissues[6]. Taurine is a conditionally essential amino acid and is either derived from food/feed or biosynthesized in the liver[7]. Taurine is unique in that it is not linked to any protein by a peptide bond and it is not part of any protein[8]. Taurine is present in high concentration in most tissues particularly in lymphocytes[9]. The zwitterionic nature of the taurine gives it high water solubility and low lipophilicity[10]. Taurine is involved in a number of physiological processes including bile acid conjugation, osmoregulation, detoxification of xenobiotics, cell membrane stabilization, modulation of cellular calcium flux, and modulation of neuronal excitability[10].

Garlic and garlic extracts, used for millennia in folk medicine is still a mainstay for various torments. It contains at least 33 sulphur compounds, several enzymes, 17 amino acids, and minerals such as selenium[11]. The consequence of synergism between various compounds is responsible for the antioxidant activity of garlic. One of the most biologically active compounds, allicin (diallyl thiosulfinate or diallyl disulfide) does not exist in garlic until it is crushed or cut. Garlic compounds are having tremendous antioxidant property which exerts actions by scavenging ROS[12]. Garlic extract has been found to suppress the activity of ceruloplasmin accumulation of heavy metals (copper and zinc) in the tissues of fish, *Oreochromis niloticus*[13].

Fishes are ideal organisms to monitor aquatic systems because they occupy positions towards the apex of food pyramids and may, therefore, reflect effects of heavy metals on other organisms including human beings as well as direct stresses on themselves[14]. Histopathological indicators are beneficial in that they show the net effect of biochemical and molecular changes in the organism resulting from exposure to a contaminant. This work was designed to study the efficacy of taurine

Regular Paper

and garlic extract in alleviating the histopathological changes induced by long-term exposure to copper sulphate in *Clarias gariepinus.*

MATERIALS AND METHODS

Test organism

The African catfish, *Clarias gariepinus* of average weight 98.43 ± 24.09 g and length 20.5 ± 2.5 cm was selected as test organism in this study because of its hardy nature and ability to acclimatise quickly in the laboratory conditions. *Clarias gariepinus* is an exotic fish and was brought to India in 1994 from neighbouring country Bangladesh[15]. Alive, healthy and disease free specimens of catfish, *Clarias gariepinus* of either sex belonging to a single population were purchased on order from the local fish market of Sagar (M.P). The specimens were transported in plastic containers filled with oxygenated and cool water to reduce their activity and stress before reaching the fish laboratory of the Department of Zoology, Dr. Harisingh Gour University, Sagar (M.P) India. Before introducing those in the aquariums, fish were treated with 0.01% $KMnO_4$ solution for 15 minutes to obviate any dermal infection. Fish were then kept for a period of fifteen days for acclimatization in laboratory conditions. Faecal remains and food residues were removed by net every other day.

Determination of median lethal copper sulphate concentration (LC_{50})

After acclimatization experimental fish were selected at random and were kept in a static system of water. The feeding was stopped one day prior the exposure to $CuSO_4$ and fish were not fed throughout the test. The acute toxicity tests were performed according to the static non-renewable bioassay procedure[16] as described in the previous study[17] (Jiraungkoorskul *et al*., 2007). The experimental design consisted of a control and six concentrations (24, 31, 38, 44, 50 and 55) of copper sulphate ($CuSO_4.5H_2O$), two replicates per group and with twenty fish in each replicate. The 96h LC_{50} was computed with probit analysis using statistical SPSS 16 package[18].

Experimental design

Experiment was setup in seven groups (viz. I to VII) containing 20 fish (*Clarias gariepinus*) in each group and were kept in fiberglass aquariums (120L) with or without simultaneous treatment of water with copper sulphate, taurine and garlic extract. The fish of group I were kept as control and the fish of this group were maintained in water containing no added copper sulphate. The fish of groups of II, IV and VI were challenged with 4 ppm solution of copper sulphate, where as groups III, V and VII were exposed to 8 ppm $CuSO_4$. Simultaneously, groups II and III were maintained as $CuSO_4$ exposed non antioxidant treated control where as groups IV and V were treated with taurine (5 ppm) and groups VI and VII were treated with garlic extract (5ppm) during the entire experiment period of 90 days. Dose selection and mode of administration of garlic extract and taurine was based on published studies[19]. All the fish were fed with commercially available fish pellet feed (Tokyo®, Japan) throughout the experiment.

Taurine

Taurine was purchased from HiMedia Laboratories, Delhi India.

Preparation of garlic powder

Fresh peeled garlic cloves were weighed and spread on to sterile filter-papers and dried at 40°C temperature in hot air oven. They were allowed to cool and thereafter, powdered in grinder as per the method of[20].

Preparation of ethanolic garlic extract

The ethanolic garlic extract was prepared with slight modification of[19]. Dried garlic powder (100 g) was dissolved in absolute 100 ml ethanol and 50 ml distilled water and left for 24h at room temperature. The mixture was filtered through filter paper and the filtrate was then subjected to evaporation in laminar air flow for the separation of ethanol from garlic extract. Thereafter, 5 ppm of the extract was prepared as and when required for experimentation.

Source of copper and preparation of stock solution

Analytical grade copper sulphate (99%, $CuSO_4.5H_2O$) supplied by WebChem® was used as metal toxicant throughout the experiment. Dilution of copper sulphate ($CuSO_4$) for bioassay test was carried out by preparing a stock solution by dissolving the 50 g of copper sulphate in 1 litre of distilled water. This solution was diluted directly into 40 liters of tap water in 120 liters capacity aquariums in sufficient amounts to provide the 4 and 8 ppm copper sulphate concentrations in water.

Regular Paper

The sublethal treatment (10 and 20% of 96h LC_{50}) was calculated from percentage mortalities of fish.

Histological study

Kidney of (control and treated) fish were removed aseptically and were fixed in aqueous Bouin's fluid. Preserved tissues were washed under tap water and dehydrated; clarified with xylene and embedded in paraffin blocks. They were cut at 4-5 µ thickness by using microtome and stained routinely with haematoxylin and eosin (H & E) for histological examination[21]. Stained histopathological sections were examined under binocular compound microscope (Zeiss, PrimoStar) on different magnifications and photographed.

Semiquantitative scoring

Histopathological alterations was assessed according to[22] by using a scoring ranging from – to +++ depending on the degree and extend of the alterations: (-) none, (+) mild occurrence, (++) moderate occurrence, (+++) severe occurrence. Five slides were observed from each organ and treatment.

RESULTS

Median lethal concentration (LC_{50}) of copper sulphate

There were no registers of death among the fish of the control group (0 mg/l of $CuSO_4$) during the determination of LC_{50}-96h, as expected. Initially, on $CuSO_4$ exposure, fish became agitated, looked for the water surface. Susceptibility of catfish, *Clarias gariepinus* to lethal effect of copper sulphate was duration and concentration dependent as mortality increased with an increase in its concentration. The 96h LC_{50} value based on probit analysis was found to be 40.38 ppm; the lower and upper lethal confidence limits for copper sulphate indicate a wide range of 37.38 to 43.67 ppm (TABLE 1 and Figure 1).

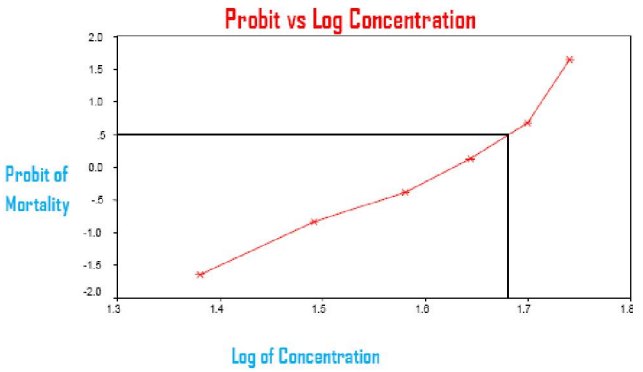
Histopathological study of gills

Group I (control)

The microscopic examination of gills of *Clarias gariepinus* revealed the normal histoarchitecture (Figure 1 & 2) and did not show recognizable alterations in any fish of control group throughout the experiment. Gill arch was composed of primary lamellae with two rows of numerous semicircular secondary lamellae that ran perpendicularly to each filament. These primary lamellae consisted of cartilaginous supporting rod, a vascular system with traces of sinusoidal blood spaces and multilayered epithelium. Secondary gill lamellae were lined by a wavy single layer of respiratory simple squamous epithelium composed of pavement cells which are separated from the lamellar blood sinuses by a basement membrane. The secondary lamellar epithelium is supported by pillar cells, which are contractile and separate the capillary channels. Each lamella was composed of a network of interconnected spaces called lacuna. Chloride cells or ionocytes were identified as large epithelial cells with granular appearance, usually present at the trailing edges of the filament and lamellae and tend to project from gill surface. Mucous goblet cells were abundant on the surface of lamellae, appearing as granular domes or vacuolated cells.

TABLE 1 : Showing the mortality of *Clarias gariepinus* at 96h after treatment of different copper sulphate concentrations.

$CuSO_4$Conc.(ppm)	Mortality	No. of fish exposed
00	00	20
24	01	20
31	04	20
38	07	20
44	11	20
50	15	20
55	19	20



Graph 1: Showing the linear relationship between probit response and log sub-lethal concentration of copper sulphate on *Clarias gariepinus*

Group II (4ppm $CuSO_4$)

The histopathological examination of gills after 15 and 30 days exposure to copper sulphate exhibited distinct histopathologies including proliferation and hypertrophy of chloride cells and mucous cells at the base of

Regular Paper

gill filament. Abnormal increase in size of few secondary lamellae was observed (Figure 3). There were some areas where tips of secondary lamellae were highly swollen due to blood cell congestion in the vessels and breakdown of pillar cell system and disappearance of lacunae (Figure 4). After 60 and 90 days severe histo-

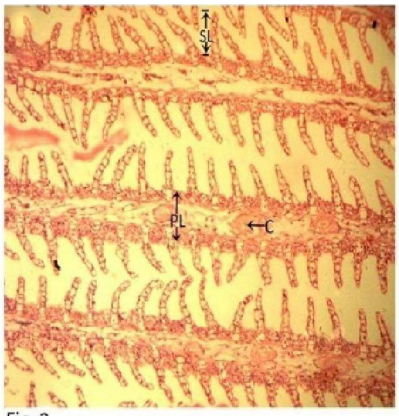


Fig. 2

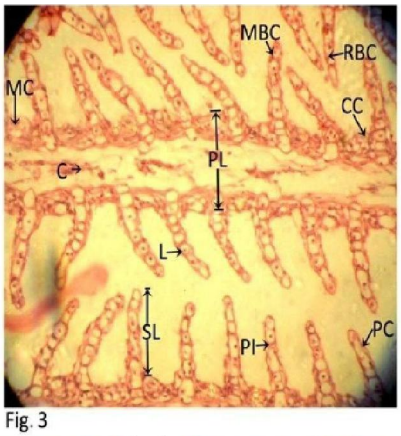


Fig. 3

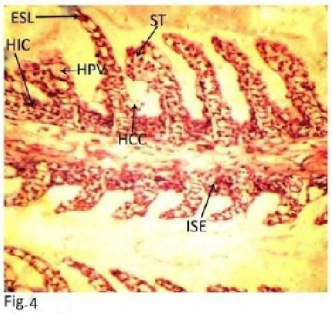


Fig. 4

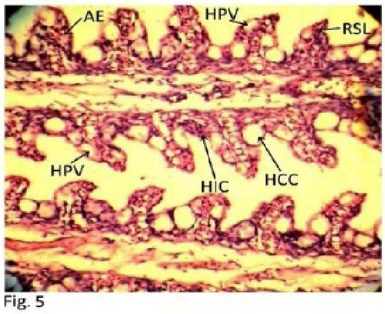


Fig. 5

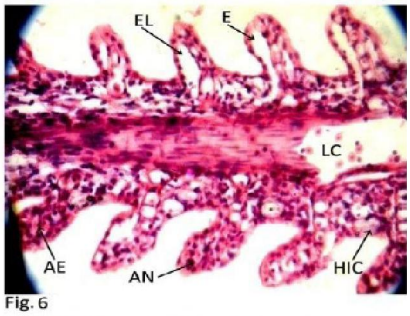
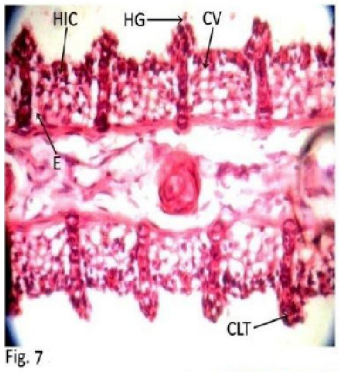


Fig. 6



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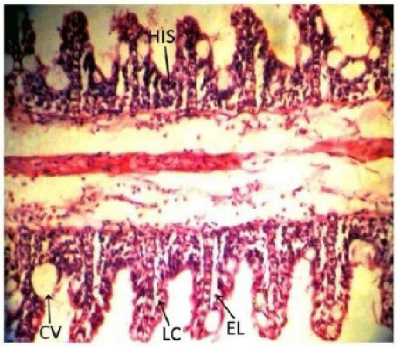

Fig. 8

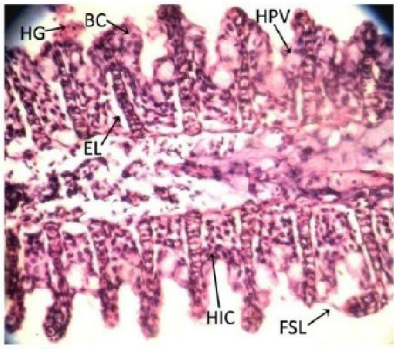

Fig. 9

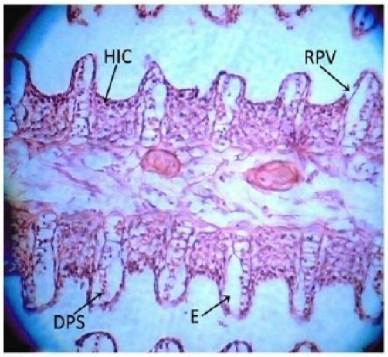

Fig. 10

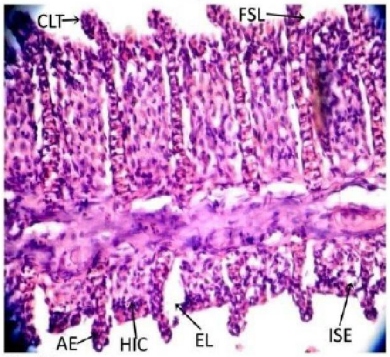

Fig. 11

Plate I- Histology of *C. gariepinus* gill in control (Fig. 2 and 3) and copper sulphate exposed groups II and III (4-11). Fig 2 and 3. Normal gill (primary lamellae (PL), secondary lamellae (SL), X100, X200, respectively. (Fig.4) exposed to 4ppm $CuSO_4$ after 15 days (swollen tips (ST), unusual elongation of secondary lamella (ESL), interstitial edema (ISE) and hypertrophy of chloride cells (HCC). [X280]. (Fig.5) exposed to 4ppm $CuSO_4$ after 30 days (hypertrophy of pavement cells (HPV), hyperplasia of interlamellar cells (HIC) [X280]. (Fig.6) exposed to 4ppm $CuSO_4$ after 60 days (epithelial lifting (EL), lamellar aneurysms (AY) [X280]. (Fig.7) exposed to 4ppm $CuSO_4$ after 90 days (hyperplasia of interlamellar cells (HIC), club-like lamellae tips (CLT) and haemorrhage (RG), [X280]. (Fig.8) exposed to 8ppm $CuSO_4$ after 15 days (cytoplasmic vacuolation (CV) and hyperplasia of interlamellar cells (HIC) [X280]. (Fig.9) exposed to 8ppm $CuSO_4$ after 30 days (haemorrhage (RG), hypertrophy of pavement cells (HPV), fusion of secondary lamellae (FSL) [X280]. (Fig.10) exposed to 8-ppm $CuSO_4$ after 60 days (epithelial lifting (EL), hyperplasia of interlamellar cells (HIC),disintegration of pillar cell system (DPS), [X280]. (Fig.11) exposed to 8ppm $CuSO_4$ after 90 days (extensive hyperplasia of interlamellar cells (HIC), club-like lamellae tips (CLT), fusion of secondary lamellae (FSL), epithelial lifting, (EL), [X280].

pathological alterations were detected in gill filaments and characterized primarily by dilation of the secondary lamellar blood sinuses and vascular congestions (aneurysms) due to disintegration in pillar cell system (Figure 5). Lifting of respiratory epithelia and edema in the filamentar epithelium was quite prominent. Excessive infiltration of leucocytes and erythrocytes was observed in secondary lamellae and central blood sinuses. The tips of secondary lamellae were swollen due to congestion of blood spaces by erythrocytes indicating circulatory anomaly. The filamentary epithelium of gills after 90 days revealed decrease in interlamellar space due to extensive hypertrophy and hyperplasia of the chloride and pavement cells (Figure 6)

Group III (8ppm $CuSO_4$)

After 15 days exposure the most common histopathological gill changes were lifting of the lamellar epithelium and increase in intracellular vacuolation in pavement cells which resulted in edematous changes due to collapse of pillar cells in secondary lamellae (Figure 7). After 60 days gills exhibited severe distinct histopathologies including hyperplasia of interlamellar epithelial cells resulted in complete fusion of secondary lamellae and disappearance of the space between contiguous lamellae. Haemolysis and haemorrhage were also reported due to severe conjestion of blood (Figure 8). After prolonged exposures (60 and 90 days) the gill tissue revealed most frequent alteration in the secondary lamellae with severe edema and rupture of pavement cell layer (Figure 9). The fusion of secondary lamellae along the entire length was observed due to severe hyperplasia and hypertrophy of interlamellar cells. The pillar cell damage led to loss of supportive properties and degeneration of blood channels at the proximal portion with subsequent mild accumulation of blood cells (Figure 10).

Group IV (4 ppm $CuSO_4$ and 5 ppm taurine)

The histoarchitecture of filamentary epithelium of gill exhibited mild histopathological alterations including hypertrophy of chloride cells at base of secondary lamellae with very slight hyperplasia of mucous cells after 15 days (Figure 11). Occasionally, slight lifting of epithelium was also seen at the distal portion of secondary lamellae after 30 days (Figure 12). The histopathological changes observed after 60 days were mild lifting of lamellar epithelium and mild hyperplasia of chloride and mucous cells. At many places rupture of lamellar epithelium was also reported. Occasionally, blood channel of secondary lamellae were dilated and disorganized (Figure 13). Severe infiltration of leucocytes due to hyperemia was observed after 90 days in secondary lamellae with formation of lamellar aneurysms. However, less frequent rupture of lamellar epithelium with mild haemorrhage was also seen (Figure 14).

Group V (8 ppm $CuSO_4$ and 5 ppm taurine)

The histoarchitecture of gill exhibited mild histopathological alterations including slight hyperplasia of interlamellar cells with interstitial edema. Occasionally, the blood sinuses at the proximal portion were dilated with accumulation of few erythrocytes after 15 days (Figure 15). After 30 days gill revealed slight hyperplasia and hypertrophy of epithelial cells with Infiltration of leucocytes was also reported in secondary lamellae (Figure 16). The blood channels of secondary lamellae were dilated with formation of aneurysm after 60 days (Figure 17). Hyperplasia of interlamellar cells was seen after 90 days with partial fusion of adjacent lamellae. Occasionally, edema and epithelial lifting were reported at bases of lamellae (Figure 18).

Group VI (4 ppm $CuSO_4$ and 5 ppm garlic extract)

The histopathological examination of gills after 15 days revealed slight lifting of secondary lamellae with accumulation of few blood cells in the edematous spaces in filamentary epithelium (Figure 19). After 30 days the gill sections revealed mild histopathological changes including hypertrophy and hyperplasia of the mucous and chloride cells at the base of the secondary lamellae (Figure 20). Degeneration of blood channel at the proximal portion and epithelial lifting in secondary lamellae were observed after 60 days. However, less frequent hyperplasia and hypertrophy of chloride and mucous cells were also seen (Figure 21). After 90 days hypertrophy of pavement cell layer and epithelial lifting in secondary lamellae with formation of edematous spaces was reported. Shrinkage of blood channel was observed in proximal portion of few secondary lamellae due to degeneration of pillar cells (Figure 22).

Group VII (8 ppm $CuSO_4$ and 5 ppm garlic extract)

The gill revealed minor histopathological alterations

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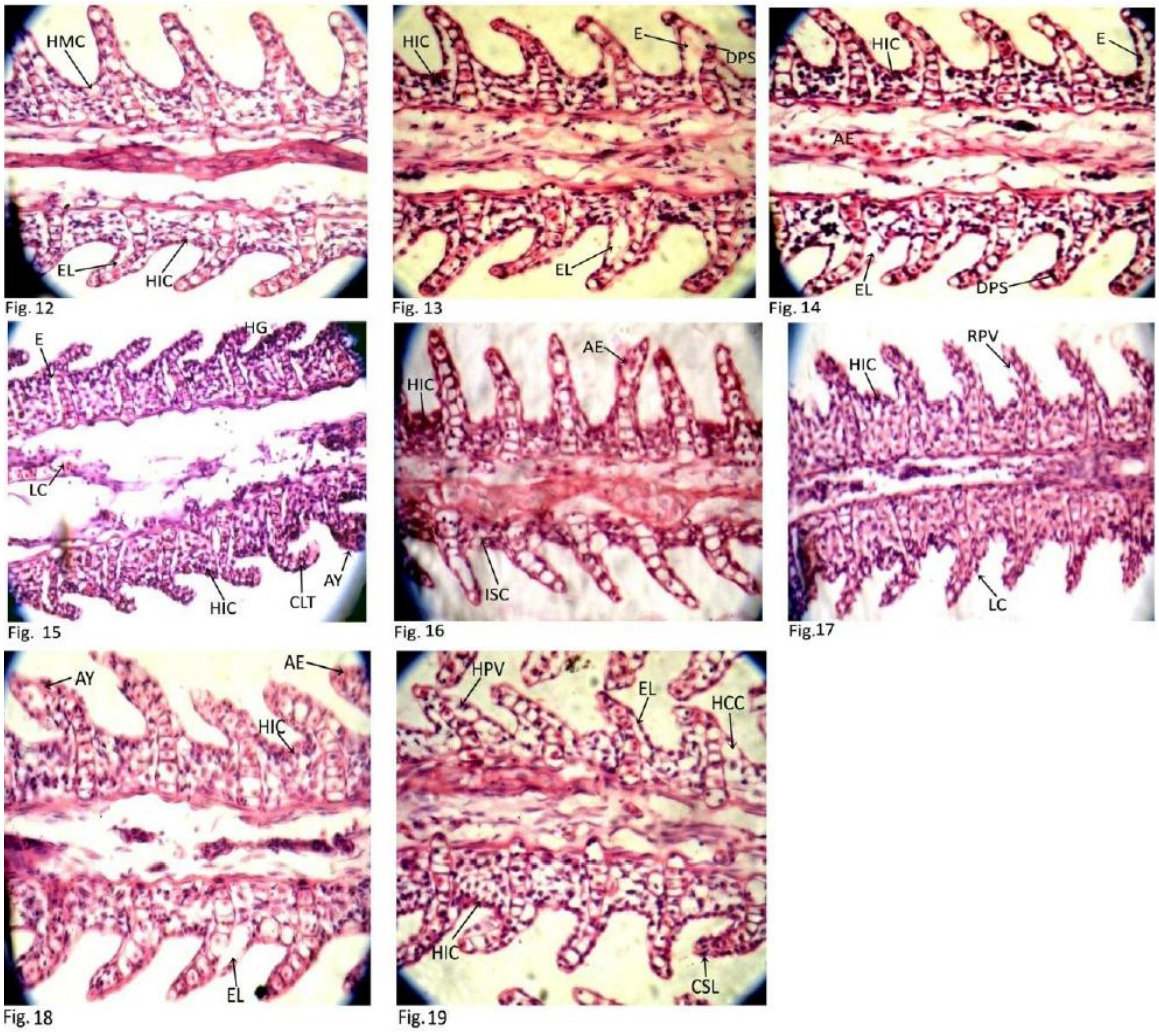

Plate II- Histology of *C. gariepinus* gill on in groups IV and V exposed tocopper sulphate and 5ppm taurine (12-19). (Fig. 12) exposed to 4ppm CuSO4 and 5ppm taurine a 15 days (mild hypertrophy of chloride cells (HCC), mild lamellar epithelial lifting (EL), [X280]. (Fig. 13) exposed to 4ppm CuSO4 and 5ppm taurine exposed to4ppm Cu after 30 days (lamellar epithelial lifting (EL), slight disintegration of pillar cell system (DPS), [X280]. (Fig. 14) exposed to 4ppm CuSO4 and 5ppm taurine after 60 (edema (E), slight disintegration of pillar cell system (DPS) mild hyperplasia of interlamellar cells (HIC), [X280]. (Fig. 15) exposed to 4ppm CuSO4 and 5ppm tau after 90 days (hyperplasia of interlamellar cells (HIC), mild haemorrhage (HG), club-like lamellae tips (CLT), mild lamellar aneurysms (AY), [X280]. (Fig. 16) exposed to 8 CuSO4 and 5ppm taurine after 15 days (accumulation of few erythrocytes (AE), mild hyperplasia of interlamellar cells (HIC) [X280]. (Fig. 17) exposed to 8ppm CuSO4 5ppm taurine after 30 days (infiltration of leucocytes (LC), slight hyperplasia of interlamellar cells (HIC) and mild rupture of pavement cell layer (RPV), [X280]. (Fig exposed to 8ppm CuSO4 and 5ppm taurine after 60 days (mild lamellar aneurysms (AN), hyperplasia of interlamellar cells (HIC), epithelial lifting (EL), [X280]. (Fig. 19) osed to 8ppm CuSO4 and 5ppm taurine after 60 days (curling of secondary lamellae (CSL), hypertrophy of chloride cells (HCC), hypertrophy of pavement cells (HPV), epithelial lifting (EL),[X280].

including hypertrophy of lamellar epithelium at the base of secondary lamellae. Increased infiltration of blood cells was reported in the dilated blood sinuses of secondary lamellae and in central venous sinuses after 15 days (Figure 23). After 30 days curling and mild epithelial lifting of secondary lamellae was observed (Figure 24). Severe lifting of lamellar epithelium with formation edematous spaces was seen after 60 days (Figure 25). Disorganization of blood sinuses and mild haemorrhage from secondary lamellae were found together with necrosis (Figure 26). After 90 days severe hyperemia, lamellar aneurysms (teleangiectasia) and haemorrhages with rupture of lamellar epithelium was seen (Figure 27).

DISCUSSION

Fish are an invaluable test model in environmental toxicology for the determination of lethal and sublethal effects of aquatic pollutants. Monitoring histopathological changes in fish is a sensitive and accurate way to assess the effects of xenobiotics. Although the toxicological effects of copper on fish are well documented, the variability of the reported results are large[23, 24]. This study was also designed to evaluate the efficacy of protective effects of taurine against the copper induced histopathological changes in gill due to its potential antioxidant activity.

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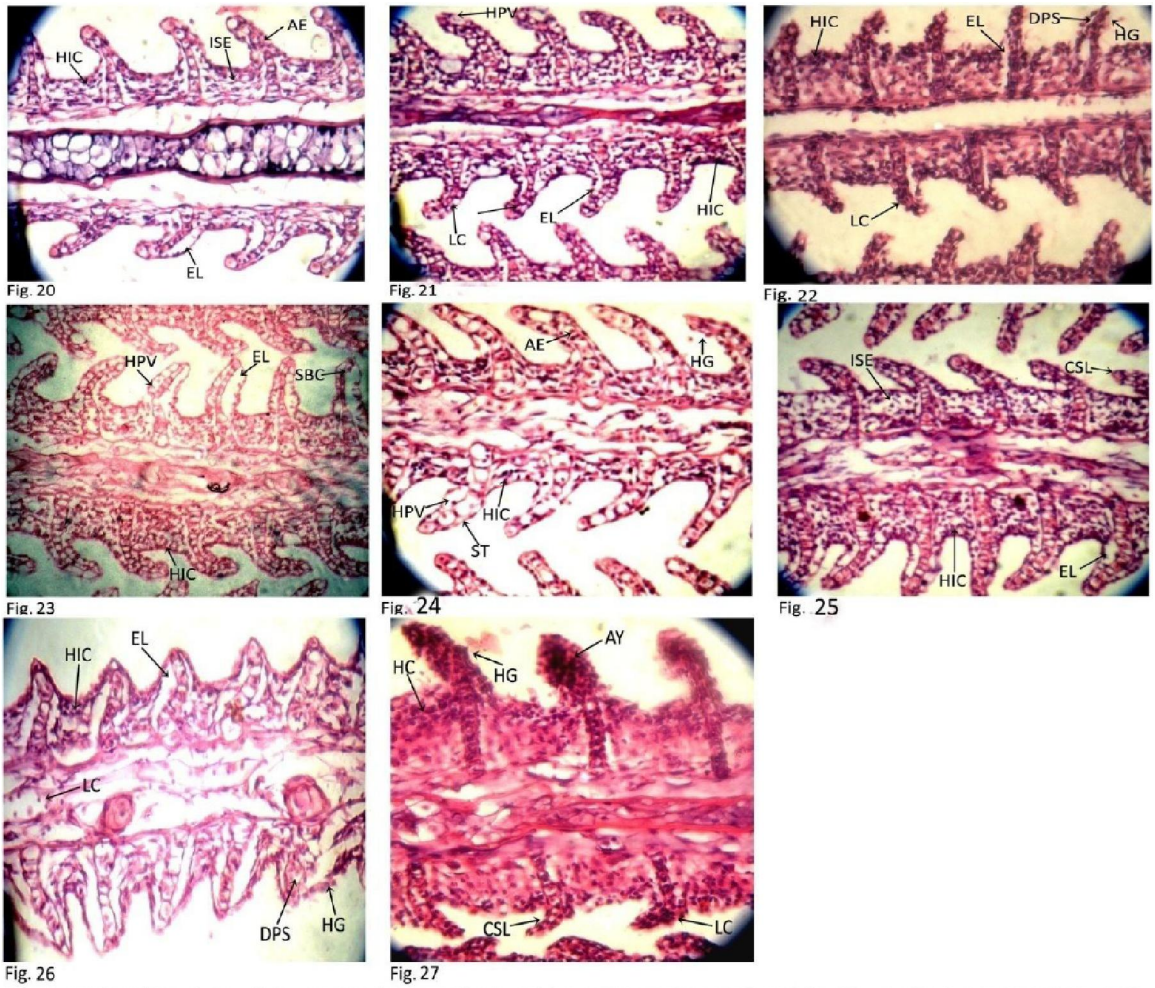

Fig. 20 Fig. 21 Fig. 22 Fig. 23 Fig. 24 Fig. 25 Fig. 26 Fig. 27

Plate III- Histology of *C. gariepinus* gill in groups VI and VII exposed copper sulphate and 5ppm garlic extract (20-27). (Fig. 20) exposed to 4ppm CuSO4 and 5ppm garlic extract after 15 days (mild epithelial lifting (EL) and mild hyperplasia of interlamellar cells (HIC), [X280]. (Fig. 21) exposed to 4ppm CuSO4 and 5ppm garlic extract after 30 days (hypertrophy of pavement cells (HPV), hyperplasia of interlamellar cells (HIC), disintegration of pillar cell system (DPS) [X280]. (Fig. 22) exposed to 4ppm CuSO4 and 5ppm garlic extract after 60 days (hyperplasia of interlamellar cells (HIC), mild haemorrhage (RG), infiltration of leucocytes (LC) [X280]. (Fig. 23) exposed to 4ppm CuSO4 and 5ppm garlic extract after 90 days (severe epithelial lifting (EL), hypertrophy of pavement cells (HPV), shrinkage of blood channe l (SBC) [X280]. (Fig. 24) exposed to 8ppm CuSO4 and 5ppm garlic extract after 15 days (hypertrophy of pavement cells (HPV), mild haemorrhage (RG), swollen secondary lamellae tips (ST), [X280]. (Fig. 25) exposed to 8ppm CuSO4 and 5ppm garlic extract after 30 days (Interstitial edema (ISE), epithelial lifting (EL) curling of secondary lamellae (CSL), [X280]. (Fig. 26) exposed to 8ppm CuSO4 and 5ppm garlic extract after 60 days (showing severe epithelial lifting (EL), disintegration of pillar cell system (DPS), edema (E), [X280].. (Fig. 27) exposed to 8ppm CuSO4 and 5ppm garlic extract after 90 days (laneurysms (AY), hyperplasia of interlamellar cells (HIC), haemorrhage (HG), [X280].

Median lethal concentration (LC_{50}) of copper sulphate

In present study there was corresponding increase in mortality response of the test fish with increased exposure and time. Forgoing results of Finney's probit analysis revealed 40.38 ppm as LC_{50} value of copper sulphate exposed to *Clarias gariepinus* for 96h. The results of this investigation support the observations, in this regard, forwarded by[25] who reported 72h LC_{50} value of $CuSO_4$ as 40.6 mg/l in *Oreochromis niloticus* and[26] who evaluated 17.5 mg/l as the 96h LC_{50} value of copper sulphate on juveniles of *Colossoma macropomum*. Copper was found significantly more toxic to *Oreochromis niloticus* than the catfish[27] and the 96h LC_{50} values for *Oreochromis niloticus* and *Clarias gariepinus* were revealed to be 58.837 and 70.135 mg/l, respectively. The 96h LC_{50} values of copper reported for *Colisa fasciatus* was 4 mg/l[28], for *N. notopterus* as 30 mg/l by[29] and for *Esomus danricus* as 5.5 mg/l by[30]. The 96h median lethal concentration (LC_{50}) value for copper sulphate on *Ictalurus punctatus* was 6.89 mg/l and on *Morone chrysops* was 3.35 mg/l[31]. However, in contrast to our study, much lower LC_{50} values of copper were reported on *Poronotus triacanthus* as 502.95 µg/l[16] and *Danio rerio* as 73.83 µg/l[32] which suggests that these fishes are less tolerant to toxic effect of copper.

Marine teleosts are more less sensitive, with reported 96h LC_{50} values of 800-1000 µg/l range for spiny dog fish, *Squalus acanthias*[33], of 1140 µg/l for sheepshead (*Archosargus probatocephalus*), of 5660 µg/l for Atlantic croacker, *Micropogan undulatus* and

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of 2750 µg/l for pinfish, *Lagadon rhomboides*[34]. Toadfish, *Opsanus beta* are even more resistant with the 96h LC_{50} between 21,600 and 36,100 µg/l[35]. The observed differences in the LC_{50} values of copper might be due to the physicochemical characteristics of the test medium, species and ages of fishes used and their susceptibility rates, which resulted in their subsequent different toxicity values.

HISTOPATHOLOGICAL STUDY

The histological characteristics of specific organs express condition and represent time integrated endogenous and exogenous impacts on the organism stemming from alterations at the lower level of biological organization[36]. The gills have an extensive surface area with minimum diffusion distance, which participate in many important functions in fish, such as respiration, osmoregulation and excretion, remain in close contact with the external environment, and particularly sensitive to changes in the quality of the water, are considered the primary target of the contaminants[37,38].

Histological study of the gills in present investigation showed a typical structural organization of the lamellae in the untreated control group I. However, fish (*Clarias gariepinus*) exposed to 4 ppm and 8 ppm concentrations of copper sulphate in group II and III respectively after 15, 30, 60 and 90 days revealed several histopathological alterations. Moreover, the ameliorative efficacy of antioxidants (5 ppm taurine and 5 ppm garlic extract) was found to minimize the histopathological changes in gills on respective intervals. These gill histopathological alterations has been previously observed by several authors in fish submitted to copper[39-43]. In *Prochilodus scrofa* as a result of copper exposure hyperplasia and thickening in the gill as well as lamellar telangiectasis was reported by[44]. Some pathological changes like thickening of the epithelium as well as telangiectasis was reported in the of gills of fish exposed copper sulphate[45], similarly same lesions in the gill of rainbow trout were also reported after acute exposure to 0.135 mg/l copper sulphate at 48hours[46]. According to[47] such alterations are non-specific and may be induced by different types of contaminants.

The foregoing results of histopathological investigation of gills are in good agreement with[46] who reported several histological alterations on exposure of 0.5, 1.0 and 2.5 mg/l to copper for a period of 21 days in Nile tilapia, *Oreochromis niloticus*. Similarly, histopathological changes in gills of *O. niloticus* which were positively correlated in its effects with the increase of copper concentration and time of exposure[24]. Some studies revealed that interstitial edema is one of the more frequent lesions observed in gill epithelium of fish exposed to heavy metals[47]. The results of this study confirm the occurrence of edema independently of copper levels, as in other fish species[48,49]. The lifting of lamellar epithelium is other histological change observed, probably induced by the incidence of severe edema[50-52]. According to[53] this lesion can induce changes in pillar cell normal structure, with consequent loss of their support function and probably, and was responsible for the emergence of lamellar aneurysms in fish exposed to cadmium.

Edema with lifting of lamellar epithelium could serve as a mechanism of defense, because separation of epithelia of the lamellae increases the distance across which waterborne pollutants must diffuse to reach the bloodstream[50]. As a consequence of the increased distance between water and blood due to epithelial lifting, the oxygen uptake is impaired. However, fishes have the capacity to increase their ventilation rate, to compensate low oxygen uptake[38]. The compensatory changes may become maladaptive if the duration of the stress factor(s) exceeds the biological tolerance limits[54]. According to[55] any discontinuity of epithelial lining of the gill due to massive wear and tear may lead to a negative ion balance and to changes in haematocrit and mean cellular haemoglobin values of blood. The edematous spaces, along with hypertrophied epithelial lining, results in inadequate gas exchange and consequently a reduced diffusion capacity, although they have created an additional barrier for prevention of penetration of waterborne xenobiotics. However, these changes also can be due to the exposition to different kinds of pollutants, such as endosulfan[56], arsenic[57], drugs[58] and other heavy metals, as aluminium[59], lead[60], cadmium[61], and nickel[51]. Thus, this signifies that these alterations are not specifically induced by copper or other heavy metals.

Most part of the gill lesions caused by sublethal exposures affects lamellar epithelium however, some alterations in blood vessels may also occur, when fishes suffer a more severe type of stress. In this case, damaged pillar cells can result in an increased blood flow inside the lamellae, causing dilation of the marginal chan-

nel, blood congestion or even an aneurysm[62,63]. The formation of an aneurysm is related to the loss of adhesion between the epithelial cells and underlying pillar cell system accompanied by a collapse of structural entity of secondary lamella[64] due to a larger quantities of blood flow that push the lamellar epithelium outward[65] or even because of the direct effects of copper[38,66] on these cells. The teleangiectasia may affect blood circulation leading to respiratory impairment. Evaluating the effects of the copper ion on *P. scrofa* juveniles during 96h of exposure[67] found aneurysms in the secondary lamellae of specimens exposed to 20.0, 25.0 and 29.0 µgCu/l, and in some cases could observe rupture of the secondary lamellae and bleeding These results corroborate those of the present study, where copper caused haemolysis and haemorrhage at high concentrations. It is the condition in which blood congests in the gill, due to the presence of metabolites and an overall change. When the tissue increases its activity, there is a well characterized fall in the partial pressure of oxygen and pH, an increase in the partial pressure of carbon dioxide, and a rise in temperature and the concentration of potassium ions. The blood vessels near the injury site dilate, the permeability of the capillary walls increases, which produces an exudation of the fluid which leads to a congestion of the blood cells in these vessels. The blood-derived exudate can enter nearby epithelia[68].

Due to continuation of sublethal copper sulphate exposure in present study, uncontrolled degeneration of gills takes place and pavement cells become haphazardly arranged. Consequently, the space between the neighbouring secondary lamellae was almost entirely filled with polygonal epithelium and gill filaments appeared a solid mass of cells. Accumulation of cellular debris on the gill lamella was one of the histopathologic findings. Contact of fish gill with copper sulphate can cause access mucus secretion and because of substantial net negative charge of gill surface, gill have a high affinity for cationic metals. Therefore, the accumulation of superficial debris may be a result of precipitation of copper ions in mucus secretions[69]. Hypertrophy and proliferation of mucous cells was also reported in present investigation in the fused surface of the secondary lamellae. This may be considered as a protective response to carry out the transport of toxins. Cell proliferation with thickening of gill filament epithelium is one histological change found in fish exposed to copper by several authors[50,70,71].

During the present investigation, the number of the chloride cells in the epithelial linings of *C. gariepinus* increased significantly following exposure to copper sulphate solution. Proliferation of chloride cells are thought to be compensatory response to ion loss and was observed following exposure to water-borne copper in *H. fossilis* by[69] in *Ctenopharyngodon idella*, and therefore chloride cell hyperplasia may therefore be good biomarker of adaptation[72]. The chloride cell hyperplasia in the lamellae following exposure to heavy metal salts have also been observed by[22, 73]. Following $ZnCl_2$ exposure, the number of such cells present in the respiratory epithelia of the secondary lamellae of the gills also increases at several stages of exposure[74]. Also, the pathological changes in the chloride cells on exposure to heavy metals may indicate osmoregulatory dysfunction, which is the main function of the chloride cells[75]. Chloride cells proliferation may be due to an added function of oxygen transport due to injury to gill tissue proper. Leukocytes infilteration caused their accumulation in the subepithelial spaces of secondary lamellae and necrotic gill tissues. This may be an inflammatory reaction response to copper[76] or to phagocyte the copper particles and tissue debrises[77].

Taurine supplementation through water at 5 ppm in groups IV and V seems to be beneficial upto some extent to alleviate copper sulphate induced toxicity in tissue damage in gill as observed in microscopic changes in the present study. The lesions were less severe in gills when compared to those observed in the fish from group II and V. The beneficial effects could be attributed its zwitterionic nature and concur to those of[10]. However, fish do contain high concentration of taurine[07] and the extra-supplementation might have protected the gills to alleviate Cu toxicity. The pretreatment with taurine after cyclophosphamide injection produced a significant decrease in urinary bladder weight (edema) and a marked decrease in vascular congestion and haemorrhage, as well as a profound improvement in histological structure[78].

Mild degeneration of chloride cells found in taurine treated fishes might be due an antioxidant property of taurine to maintain membrane organization and thus prevents ion leakage and water influx, and subsequently, avoid cell swelling or hypertrophy[79,80]. The stabilizing effect of taurine on cellular membrane has been suggested to be associated with the interaction between taurine and polyunsaturated fatty acids in the membrane[81].

Regular Paper

This property of taurine may also partly account for its protection against copper induced gill necrosis.

Addition of garlic extract at 5 ppm to group VI and VII found to be effective to reduce copper sulphate induced histopathological changes in gills. Occurrence of mild hyperplasia of interlamellar cells and less frequent hypertrophy of chloride and mucous cells explicates that garlic extract might be involved in the alleviation of copper toxicity due to its antidotal and immunomodulatory activities[82,83] and presence of selenium[84,85]. This finding concur to those of [86] who noted that cadmium induced histopathological alterations in rats were significantly alleviated by garlic administration and attributed this protective role of garlic to its potential to chelate metal and enhance the antioxidant defense system. Similarly[87] used aqueous garlic extract to ameliorate the histopathological changes induced by acetic acid in colon of rats.

It could be concluded that the intoxication by copper resulted severe histopathological changes in the gills of *Clarias gariepinus*. Garlic and taurine supplementation counteracted these toxic effects partly and bringing structural improvement in gills. This could be due to their cytoprotective properties and antioxidant nature, which combines free radical scavenging with metal chelating properties.

ABBREVIATIONS

AE = Accumulation of erythrocytes
AY = Aneurysm
BC = Blood congestion
C = Chondrocytes
CC = Chloride cells
CLT = Club-like lamellae tips
CSL = Curling of secondary lamella
DG = Dilated glomerulus
DPS = Disintegration of pillar cell system
DSE = Desquamation of epithelial layer
E = Edema
EL = Epithelial lifting
ESL = Elongation of secondary lamella
FSL = Fusion of secondary lamellae
HCC = Hypertrophy of chloride cells
HG = Haemorrhage
HIC = Hyperplasia of interlamellar cells
HL = Haemolysis
HMC = Hypertrophy of mucous cells
HPC = Hypertrophy of chloride cells
HPV = Hypertrophy of pavement cells
ISE = Interstitial edema
LC = Infiltration of leucocytes
MBC = Marginal Blood channel
MC = Mucous cells
PC = Pavement cells
PI = Pillar cells
PL = Primary lamella
PN = Pycnotic nuclei
PPM = Parts per million
RBC = Red blood cells
RPV = Rupture of pavement cell layer
RSL = Reduction of secondary lamella
SBC = Shrinkage of blood channel
SL = Secondary lamella
ST = Swollen tips
PI = Pillar cells
PL = Primary lamella
PN = Pycnotic nuclei
PPM = Parts per million
RBC = Red blood cells
RPV = Rupture of pavement cell layer
RSL = Reduction of secondary lamella
SBC = Shrinkage of blood channel
SL = Secondary lamella
ST = Swollen tips

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