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HISTOPATHOLOGICAL CHANGES IN THE GILL, LIVER AND INTESTINE OF *HETEROPNEUSTES FOSSILIS* (BLOCH) TREATED WITH EXTRACTS OF DIFFERENT PARTS OF THE PLANT *MADHUCA INDICA* (G. F. GMEL)

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ABSTRACT

Histopathological studies of gill, liver and intestine of the experimental fishes *Heteropneustes fossilis* (Bloch) were made after 24 hours exposure to 50% ethyl alcohol extracts of *Madhuca indica* (G. F. Gmel) plant parts. The principal changes in the gills included distorted and disintegrated gill arches, shrunken and oedamatous distensions in the primary lamellae, swollen, shortened and coalesced secondary lamellae, vacuolated and disrupted epithelial and pilaster cells and disorganized gill filaments. The changes in the liver included compactly or loosely arranged hepatic cells, dialated and swollen central vein, blood coagulated portal vein, reduced or swollen hepatic artery and dispersed sinusoids. In the intestine disintegrated serosa, swollen and partially ruptured muscularis, vacuolated submucosa, damaged mucosa, disintegrated and reduced lamina propria and distended and coalesced villi were observed. On the basis of affectivity the most affected organ was the gill followed by liver and intestine. Affectivity of the plant parts on the three organs was in the order seed > bark > leaf for gill, intestine and was seed > leaf > bark for liver.

Key words: Histopathology, Toxicity, Botanicals, Plant parts, *Heteropneustes fossilis*, *Madhuca indica*.

INTRODUCTION

Histopathological effects of fish poisons on different organs of fish lead to know about the impact of poisons on the ecosystem. The tissues of fresh water fishes show various responses when exposed to toxicants (Gardner and Laroche 1973). According to Vijayamadhawan and Iwai (1975) damages of tissue vary with nature of toxicants, medium and duration of exposure. The toxic materials accumulate in the body systems and cause disorder, which ultimately may lead to death of the organisms.

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Histopathological changes in the organs due to administration of toxic agents to the organisms are immense. Besides documented histopathological effects of metal salts, pesticides are known to exert significant pathological effects on different organ systems of fishes (Shivarajah *et al.* 1978, Sastry and Sharma 1979, Dubale and Shah 1979, Mathiessen and Roberts 1982, Sultan and Khan 1983, Kulshrestha and Jauhar 1984, Thakur and Pandey 1989, Dutta *et al*. 1993, Pandey *et al.* 1997, Oronsaye 1997 and Poleksic and Karan 1999).

Some information have been reported on the histopathological effects of plant piscicidal compounds on fish organs (Bhatt and Singh 1985, Bhatt *et al.* 1987, Bhatt 1991a, 1991b, 1992, Fafioye *et al.* 2004, Obomanu *et al.* 2007 and Olaifa *et al*. 2008). In Bangladesh, some research works had been reported on histopathological effect of toxicants in various organs of fishes (Kabir and Begum 1978, Kabir and Ahmed 1979, Latifa *et al.* 2002 and Nasiruddin *et al.* 2005, 2008). The present work was carried out to evaluate the histopathological changes in gill, liver and intestine of the predatory fish *Heteropneustes fossilis* (Bloch) treated with 50% ethyl alcohol extracts of leaf, bark and seed of *Madhuca indica* (G.F. Gmel).

MATERIALS AND METHODS

During the present experiment, the toxicants were obtained from the plant parts i.e. dry leaf, bark and seed of Mahua (*Madhuca indica*) from April 2008-March 2009. In order to extract the toxicants, dry seeds, chopped barks and meshed leaves were pulverized into fine powder separately first in a mortar and then in an electric grinder, and then sieved (mesh size 0.0025^2 cm). The powder was weighted in a sensitive chemical balance. Ten grams of the grinded powder was mixed with 100 ml of 50% ethyl alcohol, stirred for 3-4 hours in a magnetic stirrer, then filtered and the filtrate was obtained as 'Stock solution'.

For each set of experiment a certain calculated volume of stock solution was added to a certain volume of water so that the final volume of each test solution per replicate in all treatments in the aquarium was five liters (APHA 1976). The concentrations prepared in terms of parts per million (ppm) were used directly from the freshly prepared stock solution. In the experiments, fresh, healthy and disease free fishes were collected from the local markets of Chittagong city. The fishes were 10-14 cm in length and 5-13 g in weight. The experiments were performed under the normal laboratory conditions.

The histopathological effects of *M. indica* on the tissues were observed from the fishes that were treated with 500ppm, 750ppm and 500ppm of the

second highest concentration of 50% ethyl alcohol extracts of dry leaf, bark and seed respectively. A controlled set was similarly maintained in tap water free of any kind of extracts. After 24 hours of treatment, tissues of the three organs i.e. gill, liver and intestine were collected from both controlled and experimental fishes. To avoid post-mortem changes, tissues were dissected out and kept in saline water and fixed in Bouin's fluid overnight. After 12-16 hours, tissues were preserved in 70% ethyl alcohol. The tissues were dehydrated in progressively graded alcoholic series [70%, 90% and 100% (1 & 2)] and embedded in melted paraffin wax. The embedded tissues were trimmed and placed into rotary microtome to get transverse sections of the tissues at 3-5µ thickness. Dewaxing, hydration and staining with haematoxylin and eosin (aqueous) of the sections were carried out and finally mounted with DPX.

The prepared tissues were studied with the help of a compound microscope and photomicrographs were taken by using an Axiovert 25 CFL microscope fitted with a SLR Canon camera. The photomicrographs of the desired areas of the sections were taken at x10 & x40 magnifications.

RESULTS AND DISCUSSION

Gill of control H. fossilis (Plate-1 A & B)

Gills of *H. fossilis* were composed of finger like filaments attached to the cartilaginous gill bar. There were four pairs of gills in *H. fossilis* and two rows of primary gill lamellae which were borne by the ceratobranchial and epibranchial segments of gill arch. The interbranchial septum between the two rows of lamellae was short, so that the lamellae of the two rows were free at their distal ends. The gill lamellae were supported by gill rays which were partly bony and partly cartilaginous and were connected with gill arch and with each other by fibrous ligaments. Each gill ray was bifurcated at its proximal end and provides a passage for the efferent branchial vessel. Each primary lamellae bear number of secondary lamellae on both the sides. These flat leaf-like structures are the main seat of gaseous exchange. Each secondary lamella consisted of a central vascular layer surrounded by a thin layer of connective tissue and epithelium. The vascular layer consisted of a net work of capillaries supported by the pilaster cells.

Histopathological changes in the gill of H. fossilis treated with dry leaf extract of M. indica (Plate-1 C & D)

Gill filaments were disorganized and some were distorted. Primary gill lamellae were shrunken or elongated, squeezed at the base and swollen at the tip

of the filament forming oedema. Secondary gill lamellae were also shrunken and atrophied. Some were squeezed and some coalesced at the tip of the lamella. Epithelial cells were more or less vacuolated and disrupted. Pilaster cells were disorganized to some extent. Gill rays were demolished in some lamella but very much swollen at the apex of other lamella. Gill arch slightly disorganized.

Histopathological changes in the gill of H. fossilis treated with dry bark extract of M. indica (Plate-1 E & F)

Gill filaments were very much disorganized and distorted. Some primary gill lamellae were atrophied, some swollen and some were disintegrated. Secondary gill lamellae were swollen, shortened and also coalesced with each other. Epithelial cells were vacuolated and disrupted. Pilaster cells were damaged. Gill rays were disintegrated at the base and swollen at the apex. Gill arch more or less disintegrated.

Histopathological changes in the gill of H. fossilis treated with dry seed extract of M. indica (Plate-1 G & H)

Gill filaments were very much distorted and damaged. Primary gill lamellae were distended, elongated in shape, distorted and partially damaged in some region. Secondary gill lamellae were extremely shrunken and coalesced. Damage of epithelium and pilaster cells in some portion was observed. Gill rays were demolished at the base but swollen at the apex in some lamellae. Gill arches were spread and highly distorted and hollowed.

Histology of liver of control H. fossilis (Plate-2 A & B)

Histologically the liver was mostly composed of numerous polyhedral hepatic cells with prominent nuclei and granular cytoplasm. This structure indicated their secretory nature. But these cells had no definite arrangement. Hepatic cells radiated outwards from the central vein and constituted the parenchyma. Blood sinusoids separated the parenchymatous cells. No hepatic lobules and definite cell cords were found in the liver. Portal veins, hepatic veins, hepatic arteries and bile duct spread throughout the liver. Hepatic duct, ductules and blood capillaries were seen between the spaces of hepatic cells.

Histopathological changes in the liver of H. fossilis treated with dry leaf extract of M. indica (Plate-2 C & D)

Hepatic cells were compact in the central region, but swollen and vacuolated at the periphery. Blood sinusoids were dispersed and extended. Portal vein was enlarged with clotted blood. Hepatic artery was reduced in size. Blood vessels were dialated.

Histopathological changes in the liver of H. fossilis treated with dry bark extract of M. indica (Plate-2 E & F)

Hepatic cells were compact in the centre and swollen, vacuolated and degenerated in the periphery with disrupted cell membrane. Nuclei became highly pyknotic. Blood sinusoids were regularly arranged. Central vein was normal to slightly swollen. Hepatic vein was also dialated with clotted blood. Portal vein was very much dialated with coagulated blood.

Histopathological changes in the liver of H. fossilis treated with dry seed extract of M. indica (Plate-2 G & H)

Hepatic cells were more or less compact at the centre but extensively vacuolated at the periphery. Hepatic cells were extremely swollen, cell membrane damaged and nuclei disintegrated. Sinusoids were distended. Central vein was dialated with coaglulated blood. Portal vein was very much enlarged, deshaped and with clotted blood. Hepatic artery was highly distented with clotted blood.

Histology of intestine of control H. fossilis (Plate-3 A & B)

The transverse section of intestine of control *H. fossilis* consisted of four basic layers- mucosa, submucosa, muscularis and serosa. The serosa was very thin and was made up of single layer of peritoneal cells, and also had blood capillaries. The muscularis mucosa consisted of two layers. The outer one was the thin layer of longitudinal muscle fibres and the inner one was the thick layer of circular muscle fibres. The submucosa consisted of loose connective tissue fibres. Mucosa layer was thrown into prominent folds forming villi and was made up of single layer of simple columnar epithelium, which was formed of absorptive cells and goblet cells. The extension of submucosa in the villi was the lamina propria. Villi consisted of absortive and mucus secreting cells.

Histopathological changes in the intestine of H. fossilis treated with dry leaf extract of M. indica (Plate 3 C & D)

Serosa was partly disintegrated in the treated intestine. Muscularis layer was partially ruptured and disintegrated. Submucosa was reduced. Mucosa layer was intact and well organized. Lamina propria was demolished or reduced. Villi were compact or all distended and coalesced.

Histopathological changes in the intestine of H. fossilis treated with dry bark extract of M. indica (Plate 3 E & F)

Serosa layer was partially damaged. Muscularis layer was slightly swollen. Submucosa was normal and with vacuolation. Mucosa was disorganized

and not so intact. Lamina propria was slightly swollen and reduced. Villi were deshaped, shortened and swollen in some region.

Histopathological changes in the intestine of H. fossilis treated with dry seed extract of M. indica (Plate 3 G & H)

Serosa was swollen. Muscularis layer was highly swollen and circular muscle layer damaged in some areas. Submucosa layer was proliferated and very much disorganized. Mucosa extensively damaged, irregular and highly deshaped. Lamina propria was disintegrated. Villi were very much disorganized and irregular in shape, shortened in some places and shrunked in others.

The histopathological changes in the gill, liver and intestine of *H. fossilis* of the present observation showed similarities with the findings of Latifa *et al*. (2002) and Nasiruddin *et al*. (2005, 2008), when the test fishes were treated with *Diospyros ebenum* bark, *Cassia siamea* and *Datura metel* seeds and *Acacia auriculaeformis* and *Mesua ferrea* seed, leaf and bark extracts.

Gill is one of the most susceptible organs affecting the respiratory and osmoregulatory activities of the fish and gill function disorders affect the physiology severely and may cause death of fish (Smart 1976). Liver is the major organ of detoxification. The histopathological alterations lead to a reduction of functional efficiency of the liver. Liver necrosis in the toxicated fish would ultimately lead to cell death (Stoker *et al.* 1985). Necrosis of the intestinal wall might have interfered with the normal function of digestion and absorption. It has been reported that toxicants produced lesions on intestinal villi, necrosis of epithelial cells, degenerative epithelial and sub-epithelial connective tissue and acting on the intestinal mucus membrane destroyed its absorptive and secretory functions (Schiller 1979).

In the present experiment, a comparative account of the extract of three parts of *M. indica* i.e. dry leaf, bark and seed with respect to histopathology was observed on the gill, liver and intestine of *H. fossilis.* In the present finding the trends of toxicity of the three plant part extracts on the studied organs were observed in the gill maximum by seed > bark > leaf; in case of liver: seed > leaf > bark; and in the intestine: seed > bark > leaf. Thus, it is seen that the seed extract was the most effective of the three parts of the Mahua plant and the least toxic is the leaf extract. Moreover, it was seen that the most affected organ was the gill, then liver and then intestine. The active ingredients of these plant parts definitely caused histopathological necrosis, vacuolation, lesion, oedema, heamosiderasis etc. in the studied organs and influenced the physiological activities of *H. fossilis* to a great extent resulting in death.

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Legend of Plates-1, 2 & 3

PLATE- 1: PHOTOMICROGRAPHS OF THE L.S. OF THE GILLS OF *HETEROPNEUSTES FOSSILIS* IN CONTROL (A & B) AND TREATED WITH DRY LEAF (C & D), DRY BARK (E & F) AND DRY SEED (G & H) EXTRACTS OF *MADHUCA INDICA* (GF-GILL FILAMENTS, PGL-PRIMARY GILL LAMELLAE, SGL-SECONDARY GILL LAMELLAE, EP-EPITHELIAL CELL, PC-PILASTER CELL, EV-EFFERENT BRANCHIAL VESSEL, GR-GILL RAYS AND GA- GILL ARCH) (X 10 & X 40). (Page 128)

PLATE- 2: PHOTOMICROGRAPHS OF THE T.S. OF THE LIVER OF *HETEROPNEUSTES FOSSILIS* IN CONTROL (A & B) AND TREATED WITH DRY LEAF (C & D), DRY BARK (E & F) AND DRY SEED (G & H) OF *MADHUCA INDICA* (HA-HEPATIC ARTERY, HV-HEPATIC VEIN, S- BLOOD SINUSOID, CV- CENTRAL VEIN, PV- PORTAL VEIN, BV-BLOOD VESSEL AND HC- HEPATIC CELL) (X 10 & X 40). (Page 129)

PLATE- 3: PHOTOMICROGRAPHS OF THE T.S. OF THE INTESTINE OF *HETEROPNEUSTES FOSSILIS* IN CONTROL (A & B) AND TREATED WITH DRY LEAF (C & D), DRY BARK (E & F) AND DRY SEED (G & H) OF *MADHUCA INDICA* (S-SEROSA, MUS-MUSCULARIS, SM-SUBMUCOSA, M-MUCOSA, LP- LAMINA PROPRIA AND V-VILLI) (X10 & X40). (Page 130)

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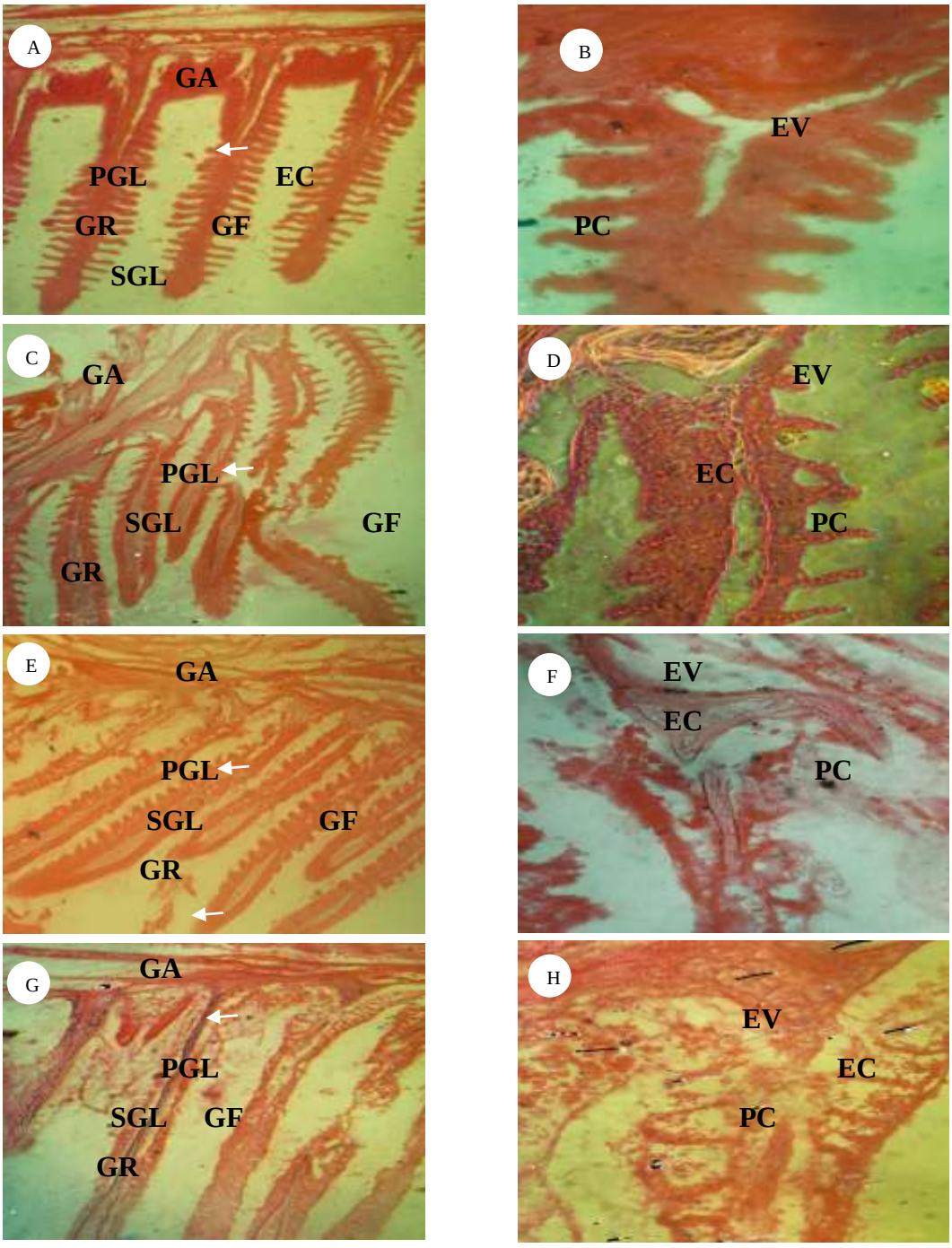
Plate- 1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HISTOPATHOLOGICAL CHANGES IN *H. fossilis*

Plate- 2

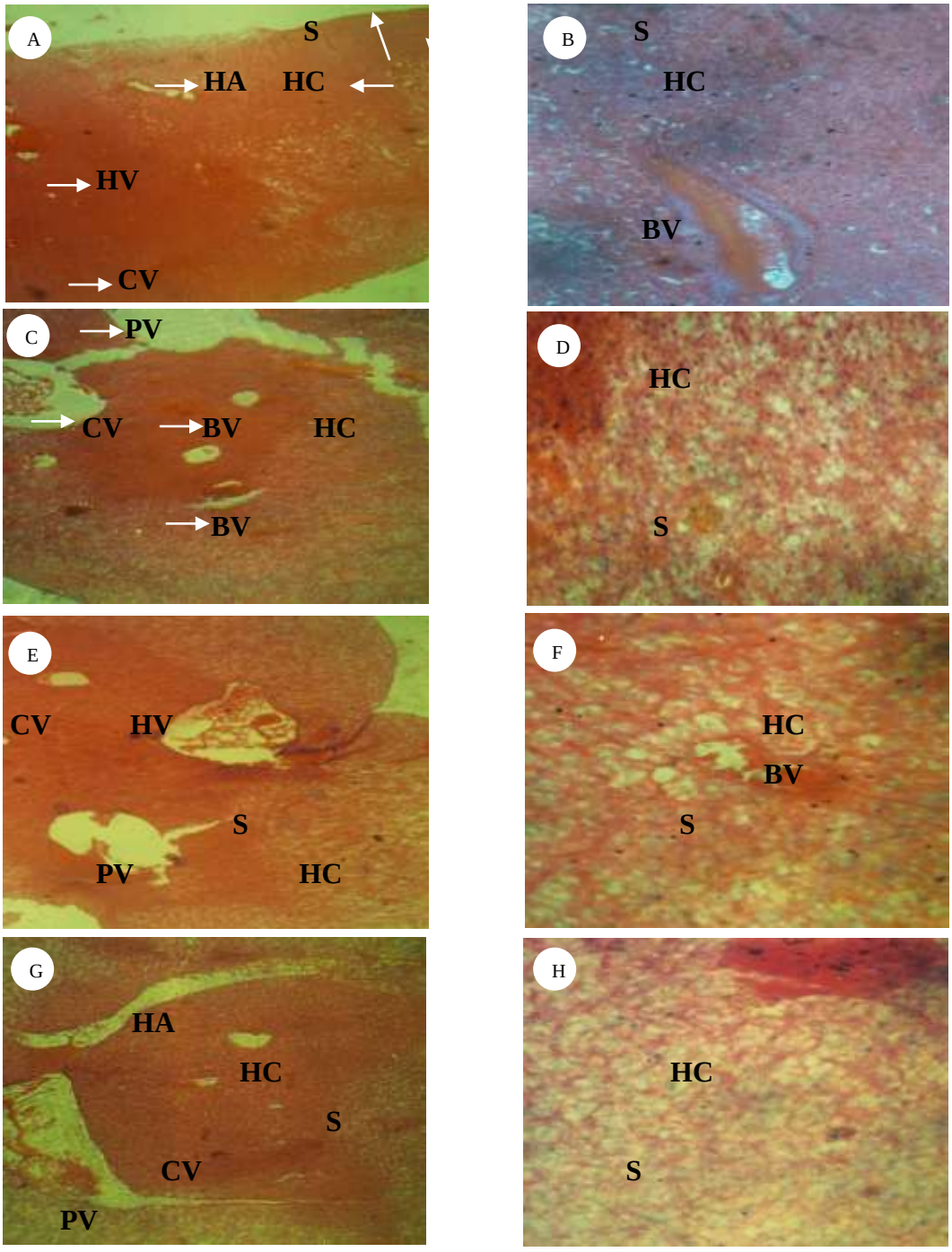
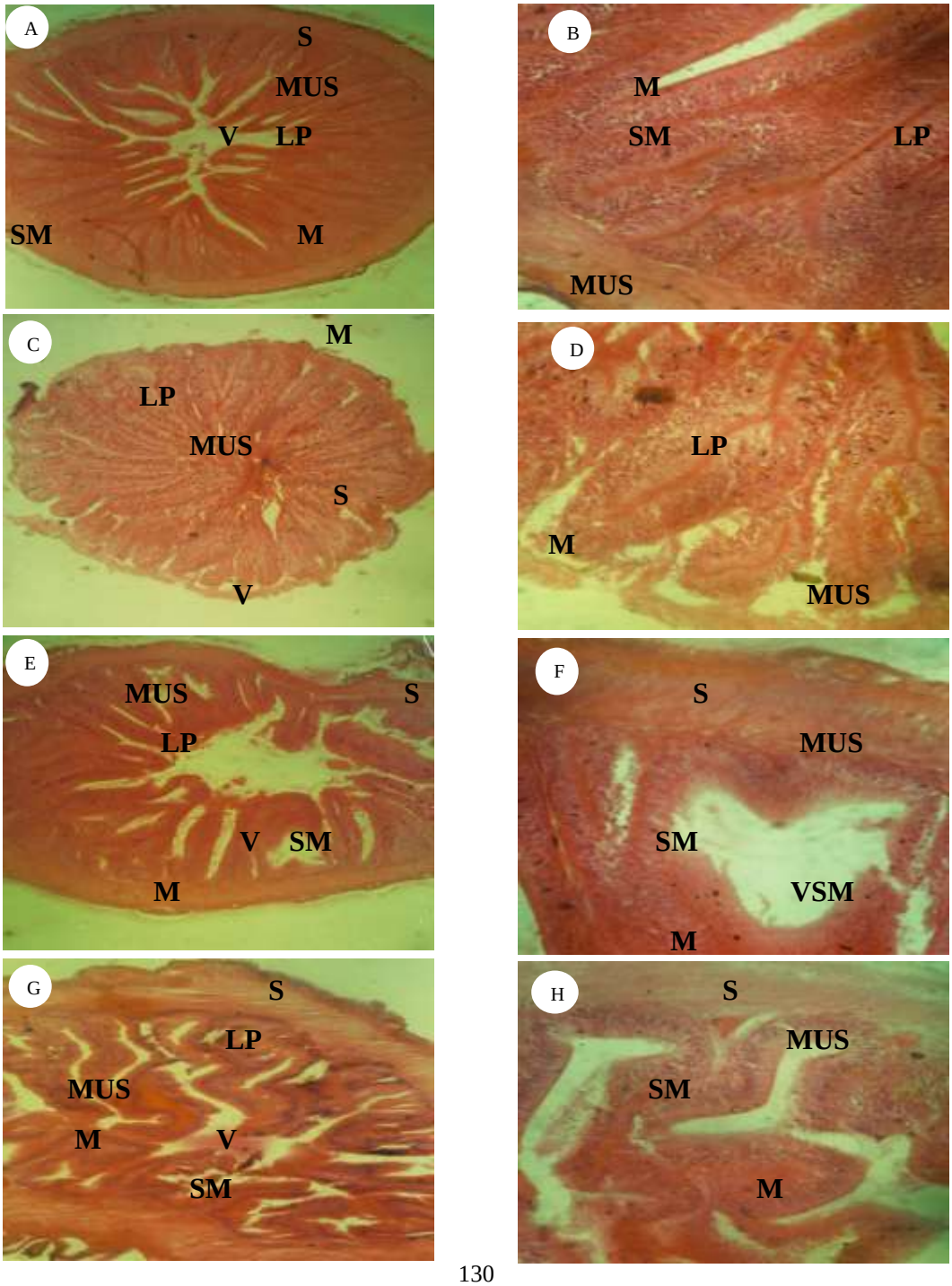


Plate-3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