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HISTOPATHOLOGY STUDY TO PROVE THE PROTECTIVE EFFECT OF MAJORANA HORTENSIS AGAINST OXIDATIVE STRESS

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ABSTRACT

Majorana hortensis leaves have been proved to have potential antioxidant activity which was further proved by *in vitro* studies using precision cut liver slices. Histopathological examinations of the liver slices were used as to examine the hepatic architecture after the exposure of oxidant, namely H_2O_2. The microscopic lesions produced were usually fatty metamorphosis or centrizonal necroses which were studied. The group which consisted of oxidant and methanolic extract of the leaves showed a protective effect compared to the group which was exposed to the oxidant alone.

KEYWORDS: *Majorana hortensis*, oxidative stress, liver slices, hydrogen peroxide.

INTRODUCTION

Majorana hortensis (*M.hortensis*) is a Mediterranean perinneal herb of Lamiacea family. Commonly it is called sweet majoram and used to add flavor in culinary purpose. It has been proved to have high potential as an antioxidant.[1] An alternate in vitro source such as precision cut goat liver slices were used in the study which was examination histopathologically. It helps to envisage the anatomy and gives the insight into the functioning of tissues.[2] The live slices were chosen as it plays a key role in the metabolism and biochemical transformations of oxidants or pollutants which reflects its integrity by creating lesions and other histopathological alterations of the liver parenchyma.[3] They are used as popular experimental tools for the investigation of liver injury. The liver damage produced by these agents is seen shortly after the exposure to the toxic substances and is dose related.[4] Having analysed the biochemical parameters in order to assess the response of the organs to oxidative stress, a treatment with the leaf extract, the tissues (liver slices) were also examined for histopathological changes like necrosis, edema and lymphocyte infiltration in the presence of the oxidant (H_2O_2) with and without the leaf extract. The procedure of Luna 1968[5] was followed for this study. The hepatic lobule is defined histologically as a hexagonal region of parenchyma which surrounds the central vein at its centre.

MATERIALS AND METHODS

The tissues were placed in 10% formal saline (10% formalin in 0.9% NaCl) for one hour. They were then left overnight in running water after securing the moutns of the vessels with cotton gauze. The tissues were dehydrated in ascending grade of isopropanol by immersing in 80% isopropanol overnight followed by 100% isopropanol for one hour. The dehydrated tissues were cleared in two changes of xylene, one hour each. Then the tissues were impregnated with histology grade paraffin wax at 60ºC. The impregnated tissues were embedded in paraffin blocks using the same grade wax. The paraffin blocks were mounted and cut with a rotary microtome at 3 micron thickness. The sections were floated on a tissue mount and cut with a rotary microtome at 3 micon thickness. The sections were floated on a tissue floatation bath at 40º C and taken on a glass slide smeared with equal parts of egg albumin and glycerol. The sections were then melted in an incubator at 60ºC and after 5 minutes allowed to cool.

Tissue staining: The sections were deparaffinized by immersing in xylene for 10 minutes in a staining jar. The deparaffinized sections were washed in 100% isopropanol and stained in Ehrlich's hematoxylin for 8 minutes. After staining in hematoxylin, the sections were washed in tap water and dipped in a alcohol (8.3% HCl in 70% alcohol) to remove excess stain. The section stained in 1% aqueous solution of eosin for 1 minute. The excess stain was washed in tap water and the sections were allowed to dry. The complete dehydration

of the stained sections were ensured by placing the sections in the incubator at 60ºC for 4 minutes. When the sections were cooled, they were mounted in DPX mountant. The cell architecture in the liver was observed under high power objective in a microscope.

Groups used for the study: Four different groups were studied to determine the protective effect of the methanolic extract of the *M.hortensis* leaves. First was the control which contains only the liver extract. Second contains only the methanolic extract of the leaf (1g/mL). The third treatment group contains the oxidant namely H_2O_2 (10%) and the fourth group is exposed to the oxidant and also the leaf extract to analyze the extent of protection rendered by the leaf in the presence of the oxidant.

RESULTS

Histopathological analysis was carried out to confirm the spectrophotometric analysis. In the control group, the liver tissue showed normal architecture with intact portal triad. In the presence of the oxidant, the liver tissue showed cellular edema. This is presumably due to the presence of stress, which caused the normal cells to swell and exhibit edema thereby blocking the sinusoids or minimizing the sinusoidal spaces. The liver tissue in the presence of the plant extract alone showed a lower extent of cellular edema.

Untreated goat liver slices showed normal hepatic architecture and no fatty changes (Fig.1a). Administration of H_2O_2 resulted in fatty change and lobular ballooning followed by degeneration of hepatocytes. Administration of the methanolic leaf extract of *M. hortensis* exhibited significant improvement (Fig.1b). The hepatocytes showed only mild fatty change The liver showed only mildly distorted architecture. Central vein showed no distinct changes. Portal triad exhibited only mild fibrosis with mild inflammation. Histological profile of the liver slices exposed to the oxidant alone reported to show intense centrilobular necrosis, steatosis, swelling of hepatic cytoplasm, ballooning degeneration, fatty changes and broad infiltration of lymphocytes and Kupffer cells around the central vein (Fig. 1c). When the oxidant was co-administered along with the leaf extract, notable damage was observed in certain areas, but some parts of the liver tissue showed recovered areas with normal architecture. The periportal areas showed some edema but the peripheral areas showed preserved architecture. This indicated that the methanolic extract of the *M. hortensis* leaves is effective in protecting the liver tissue from oxidative damage. The liver showed distorted architecture with nodule formation, distorted central vein and the portal triad showed fibrous portal expansion with moderate fibrosis and moderate inflammation (Fig. 1d). Histological observations basically supported the results obtained from the other biochemical investigations. The mechanism of action of the plant remains to be studied. The above results support the incorporation of *M. hortensis* as a component with protective effect against the action of oxidative stress by hydrogen peroxide.

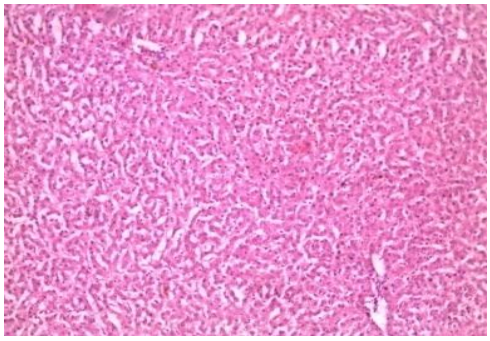

Fig. 1a: Untreated Liver Slice.

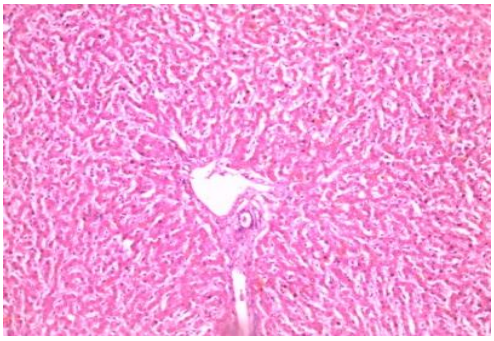

Fig. 1b: Leaf Extract.

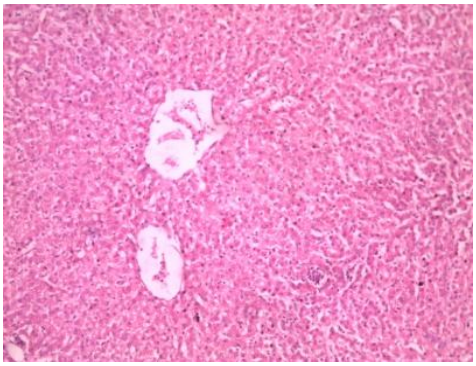

Fig. 1c: Oxidant.

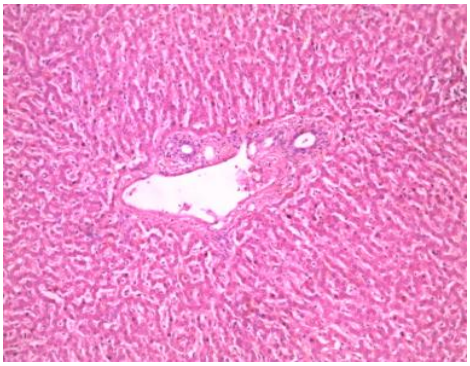

Fig. 1d: Oxidant + Leaf Extract.

Figure 1: Histopathological Architecture of the goat liver slices.

DISCUSSION

Extrapolations from the results obtained in precision cut liver slices to the in vivo condition have been successfully established for metabolic clearance, metabolism, and toxicity of several drugs.[6] The goat liver slices exposed to oxidant in the presence and absence of the leaf extract were analyzed histopatholgically and the results were in agreement to those obtained with the antioxidant status of the slices. The presence of the oxidant caused tissue damage, which was reverted back to a certain extent due to the presence of the leaf extract. There is a rich source of literature that adds credibility to the fact that histopatolgical analysis supports all *in vitro* studies carried out in cell free system. It was suggested[7] that careful histopathologic evaluation and documentation of all manipulations in any model are needed if progress in the field is to occur and he carried into human studies. Tenpe[8] concluded that the *Oroxylim indicum* leaf extract was a good antioxidant and expressed hepatoprotective activity histologically. Ali[9] demonstrated the protective ability of bark of *Khaya sengalensis* on liver injury induced by CCl_4 in albino rats. It was indicated[10] the protective role of methanolic extract of *Digera muricata* on levels of antioxidants in rat thyroid against the toxicity of CCl_4. The protective effects of *Hellinu linteus* on CCl_4 induced rata liver damage showed CCl_4 induced histological changes like increased degeneration, necrosis, hepatitis and portal triaditis.[11] The protective effect of the *Moringa oleifera* leaf extract against CCl_4 induced oxidative stress in precision cut liver slices was confirmed by histopathology analysis.[12]

CONCLUSION

Thus it can be inferred from the results obtained from the histological architecture that the methanolic extract of *M.hortensis* protected the liver from the histological damage by H_2O_2. Also, the histopathology study has shown similar results as observed using other parameters. This indicates that the precision cut liver slices can be used for histopathology study as an alternative for *in vitro* studies which can yield reproducible results.

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