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EFFICACY OF METHANOLIC EXTRACT OF *CYCLEA PELTATA* AS A POTENT ANTICANCER EQUIVALENT

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ABSTRACT

Phytochemical equivalents from Ayurveda are soon replacing chemical drugs used in chemotherapy. The present study was undertaken investigate the anticancer property of extracts obtained from *Cyclea peltata* on breast cancer cell lines. Although the plant has been a widely used folklore medicine with reportedly high diuretic and anti-inflammatory properties, the anticancer potential of the same remains still uncharacterized. The Adult Swiss albino mice selected for this study were grouped separately for screening of biochemical and histopathological parameters and analysis of their comparative effects with a control. The results obtained on comparison of treated samples with control as well as a standard drug revealed the plant extract of *C. peltata* exhibited significant inhibition property against DAL cell lines.

Keywords: *Cyclea peltata*, Ayurveda, anticancer, DAL cell lines, *in vivo*.

INTRODUCTION

Breast cancer is the third leading cancer in women worldwide after cervical cancer and lung cancer with an estimated age standardized incidence rate of 20.7 per 100,000 female [1]. In India breast cancer is the most common cancer and accounts for 25% to 31% of all cancers in wome. The average age of developing breast cancer has shifted from 50 - 70 years to 30 - 50 years and cancers in the young tend to be more aggressive. According to WHO, for the year 2012, an estimated 70218 women died in India due to breast cancer, more than any other country in the world. Treatment in cancer usually requires a multimodality approach including medical, surgical and radiological treatments to reduce mortality and improve palliation in women where cure is not a possibility [2]. Current medical treatments in breast cancer are composed of chemotherapy, hormonal treatment and targeted therapy which, however are sophisticated, expensive and not widely available. Therefore, a search for novel anti-cancer agents from natural products may provide an alternative and cost-effective treatment modality [3].

Cyclea peltata commonly called "Paatakizhangu" or "Rajpatha" is described in the ancient science of Ayurveda for its medicinal value. Due to high medicinal value of this plant, National Medical Plant board of India identified this plant as "medicinal plant species in high trade sourced from tropical forests" [4]. With many traditional claims as diuretic, anti-inflammatory, antipyretic and leprotic, *Cyclea peltata* remains as a plant of great medicinal value and claimed to be useful in cough, snake bites and helmenthiasis [5].

Approximately five decades of systemic drug discovery and development have resulted in the establishment of a large collection of useful chemotherapeutic agents. However, chemotherapeutic treatments are not devoid of their own intrinsic problems. Various kinds of toxicities may occur as a result of chemotherapeutic treatments. Researchers are focusing towards complementary and alternative medicine like

phyto-medicine to manage or arrest the carcinogenic process and provide an alternative to the use of conventional allopathic medicine for treatment of the disease. Many herbs have been evaluated in clinical studies and are currently being investigated to understand their tumouricidal properties against various cancers. In the present study was attempted to confirm the anticancer drug like property from a common plant source botanically named as *Cyclea peltata* (Lam.) Hook.f & Thoms, belonging to the family Menispermaceae.

MATERIALS AND METHODS

Plant material:

The plant *Cyclea peltata* used for the present study was collected from Alappuzha district of Kerala, India. The plant was authenticated by the Department of Botany, Sanathana Dharma College, Alappuzha .A voucher specimen (10002) is prepared as herbarium and submitted to the Department of Zoology, Avinashilingam Institute for Home Science and Higher Education for Women, Coimbatore. The whole plant *Cyclea peltata* was washed in running tap water to remove soil and adhered debris. The plant was then washed using sterile distilled water and shade dried at room temperature. The dried plant material were ground into a fine powder in an electric grinder and subsequently sieved for obtaining fine powder. The sieved powder was collected in two clean air tight containers and used for the further analysis.

Maintenance of mice:

Adult Swiss albino mice (20 ± 5 gm) were purchased from Animal Breeding station, Veterinary College, Mannuthy, Kerala, India. The animals were maintained under standard environmental conditions in polypropylene boxes and fed with standard pellet feed and water ad libitum. The experiments were performed after the approval from the Institutional Animal Ethical Committee and in accordance with the recommendation for the proper care and use of the laboratory animals. DAL cells were procured from Amala Cancer Institute, Thrissur, Kerala, India

Analysis of Biochemical parameters

Experimental mice were divided into 5 groups consisting of 6 animals. The designation of the animal groups and treatment details were as follows:Group I: Normal control; Group II: DAL control; Group III: DAL +5-FU (10 mg/kg); Group IV: DAL + MECP 100mg /kg; Group V: DAL+MECP 200mg/kg. All the animals in each group except Group I received DAL cells (1x106 cells/ mouse i.p). After 24 hrs of DAL inoculation Group IV and Group V received methanolic extract of *Cyclea peltata* at a dose of 100 and 200mg/kg/day. Group III received reference drug 5 fluorouracil (10 mg/kg oral).

After 14 days treatment the animals were sacrificed by cervical dislocation and blood was collected. Serum was separated from blood cells by centrifugation and is used for the estimation of various biochemical parameters.

Acute toxicity and Histopathological analysis

Acute oral toxicity of MECP was performed in Swiss albino mice as per OECD 423 guidelines. The animals were divided into 3 groups. Group 1 and 2 received 3000mg/kg body weight of MECP. Group 3 served as control. After oral administration of these extracts, the animals were continuously observed for behavioral changes for the first 2 and 4 h and then observed for mortality if any, after 24 hours [6].

The animals were anesthetized with diethylether and sacrificed by cervical dislocation. Tissues like liver and kidney were removed and tissues were transferred to ice cooled containers; wiped thoroughly using blotting paper to remove blood and other body fluids and washed in normal saline. The tissues were kept in formalin (10%), fixed and soaked in paraffin, cut at 2-3µm thin, and the slices were stained using hematoxylin and eosin. Tissue slices were photographed using optical microscopy under polarized light.

Statistical analysis

All the grouped data obtained on biochemical assessment were statistically evaluated with one way analysis of variance (ANOVA) followed by Dunnett's test. P values of less than 0.05 were considered to indicate statistical significance. All the results were expressed as mean±SD.

RESULTS

Serum Biochemical parametric assays (Fig 1)

The serum alkaline phosphate level was analyzed in all the experimental groups of animals. In the control group, ALP level was found to be 90.47 ± 2.48 IU/L. In the case of DAL bearing mice ALP level was found to be maximum of 106.25 ± 5.98 IU/L, which was significantly ($p < 0.01$) reduced in MECP treated mice (93.96 ± 1.01and 97.61 ± 1.46) respectively. SGOT level was found to be decreased significantly in all the treatment groups when compared with DAL control group (70.95 ± 0.35). The lower dose (MECP 100 mg/kg) was found to be more significant when compared to that of the higher dose (MECP 200 mg/kg). SGPT level indicated an elevated level of enzyme in DAL control (56.50 ± 0.49) when compared to the normal animals (26.17 ± 0.99). MECP treatment groups differ significantly from DAL treated control (25.90 ± 0.77 & 22.90 ± 1.29).

With regard to total protein content all the treatment groups were found to be significantly reduced when compared with DAL control group (12.94 ± 0.91). MECP treatment with higher dose (200 mg) showed near normal values (6.10 ± 0.64). When compared with the DAL control animals the level of urea and uric acid were found to be significantly reduced in both the MECP

treated groups. Data pertaining to creatinine revealed that treatment with MECP (100 and 200 mg) did not differ significantly from DAL treated control.

In DAL bearing mice there was an increase in cholesterol level (178.90 ± 7.16) when compared with normal control (142.78 ± 2.92). MECP 200 (167.92 ± 9.47) was found to be significant ($P<0.01$) when compared to that of DAL control animals. With administration of MECP (100 & 200 mg) to the DAL induced mice the level of HDL was found to be significantly ($P < 0.01$) increased (46.88 ± 0.83 & 41.28 ± 0.64) compared to that of the DAL control mice (25.26 ± 1.32). The administration of MECP significantly reduced the triglyceride level in 100 & 200 mg treated groups (146.53± 1.42 and 162.14 ± 0.73) when compared with DAL control animals (186.89 ± 7.71).The lower dose of MECP was found to be more significant.

Acute toxicity and Histopathological analysis

C. peltata were found to be safe orally at the dose of 3000 mg/kg body weight. No mortality was seen at this dose in any of the treated animals after 24 hours. So, the effective dose was chosen as 100 and 200 mg/kg body weight. Fig.2 shows the histopathological observation of liver section of control and experimental animals. Control animals showed normal histological appearance with central vein and unremarkable sinusoids and portal tracts whereas DAL induced mice showed portal tract and periportal inflammation. However the groups treated with the standard drug showed lobular architecture was maintained. MECP treated sections of liver tissue showed normal lobular architecture with mild portal tract inflammation. There is no fibrosis, submassive necrosis and carcinoma.

The kidney of normal animals showed normal zonal variation extending from centre to medulla with normal glomeruli and tubules and medulla shows collecting duct. Some variations in DAL induced mice was observed with alterations in glomerular region and suppuration with collection of foamy macrophages. The animals treated with standard drug, MECP 100 and 200 mg exhibited regeneration of glomerular region with slightly dilated tubules. No acute tubular necrosis, malignancy or kidney infarct seen. Renal medulla was found to be normal (Fig.3).

Figure 1. Effect of MECP on Serum biochemical parameters

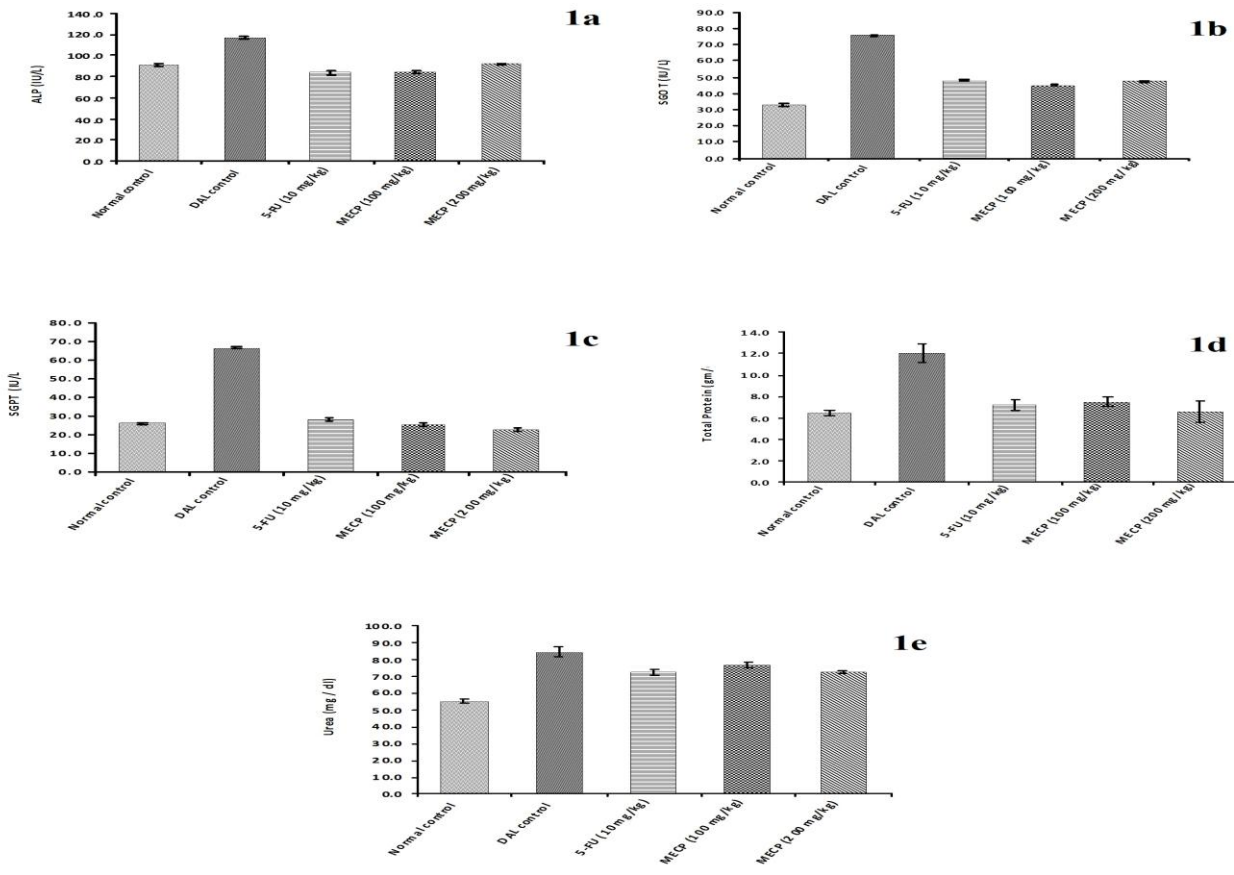


Figure 1 (contd). Effect of MECP on Serum biochemical parameters

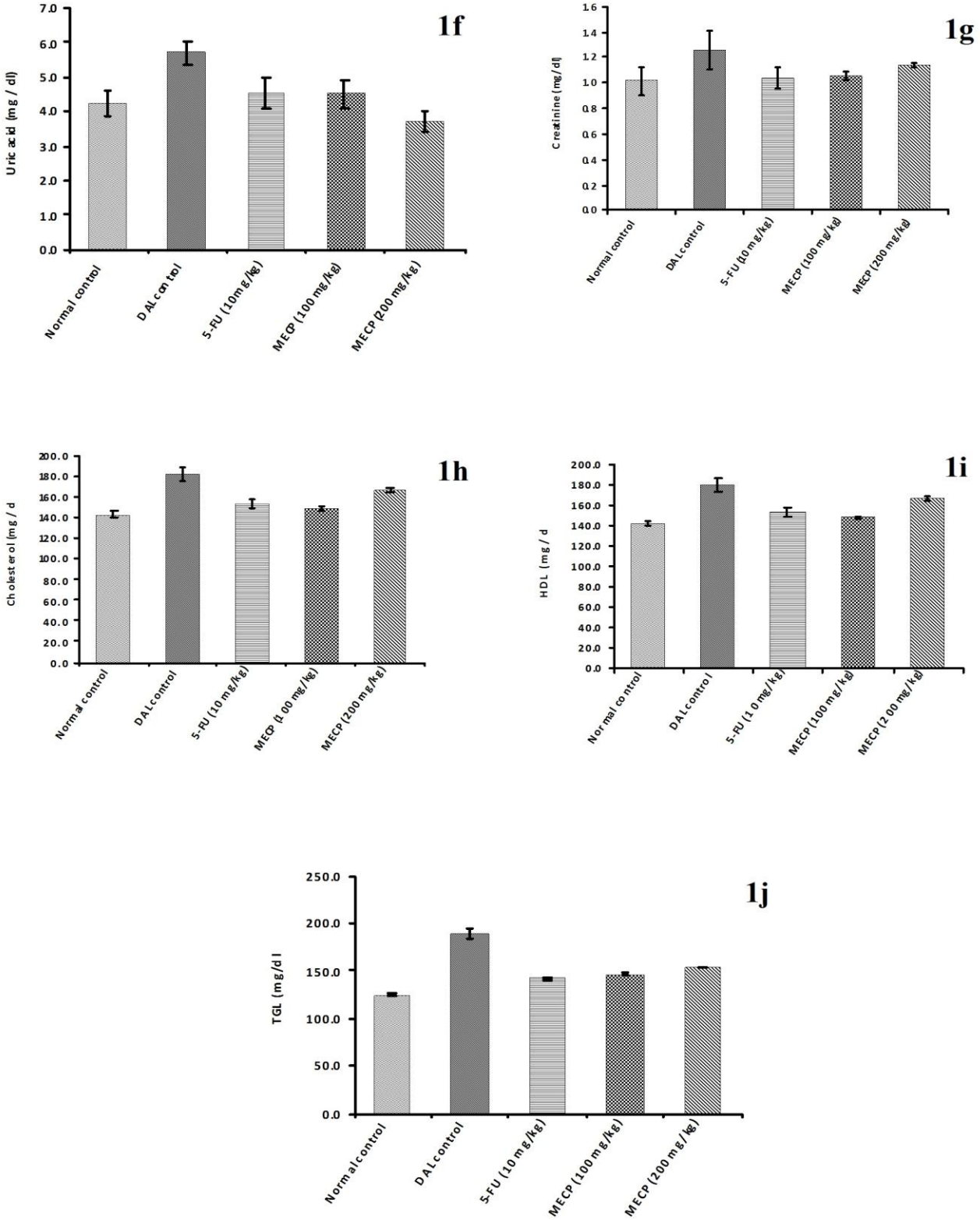
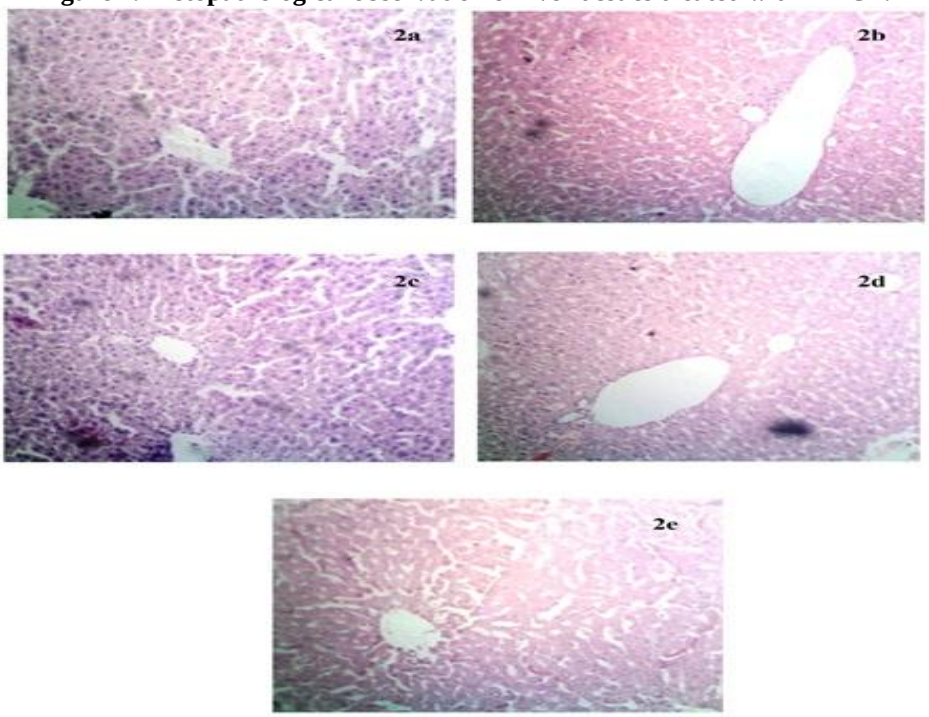
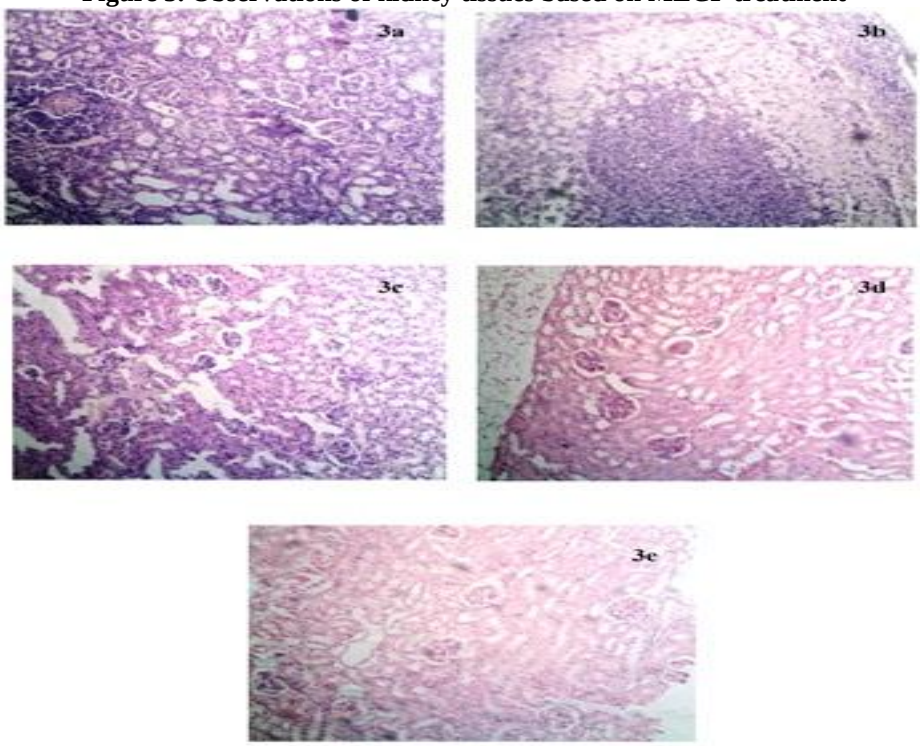


Figure 2. Histopathological observation of liver tissues treated with MECP.



(a): Section of liver of normal control mice showing hepatic cells with well defined nuclei and cytoplasm (b): Section of DAL treated mice liver showing portal tract inflammation (c): Section of MECP+5-FU (10 mg/kg) treated mice liver showing normalcy of hepatic cells (d & e): Section of MECP 100 & 200 mg/kg treated mice liver showing marked improvement over DAL control group.

Figure 3. Observations of kidney tissues based on MECP treatment



(a) Control mice kidney architecture, (b) DAL control kidney architecture, it shows some alteration in glomeruli region. Regeneration of glomeruli region after *Cyclea peltata* treatments (d & e) and 5-fu (c) administered.

DISCUSSION

The persistent search for new anticancer compounds in plant medicines and traditional foods is a realistic and promising strategy for its prevention [7]. These compounds are present in a number of food items and hold great potential as drug candidates due to their safety, low toxicity and wide acceptance among public. Plant derived drugs like vinblastine, vincristine, taxol and campothecin where reported to improvise the chemotherapy of some cancers [8]. So plants contain almost unlimited capacity to generate compounds that fascinates researches in the quest for new and novel chemotherapeutics [9].

Serum estimation of SGPT which is fairly specific for liver tissue is of greater value in liver cell injury, whereas SGOT level may raise in acute necrosis or ischemia of other organs such as the myocardium, besides liver cell injury. Both the enzyme levels are increased on damage to the tissues producing them. Similar case was found with that of DAL control animals.

Serum alkaline phosphatase is produced by many tissues especially bone, liver, intestine, placenta and is excreted in the bile. Serum alkaline phosphatase increases to some extent in most types of liver injury. Bile acids induce alkaline phosphatase synthesis and exert a deterrent effect on the canalicular membrane, allowing leakage into serum [10]. Treatment with MECP reverted back the elevated levels of serum hepatic marker enzymes to near normal level. Similarly lipid profile and protein profile were also normalized by treatment with this plant extract. The data of the results were compared with that of the standard drug 5- fluorouracil (10mg/kg).

The liver is responsible for metabolism and detoxification of most of the components that enter in to our body. The exposure to drugs, food additives, dangerous chemicals etc. and their metabolic products makes the liver unstable such as acute or chronic inflammations like hepatitis, cirrhosis etc. Once the liver became injured, its efficient treatment with chemical drugs is limited. In the present study the histological examination of the liver of DAL control animals showed marked changes (portal and periportal inflammation) indicating the toxic effects of the tumor. After the administration of MECP at different doses (100 & 200 mg /kg) showed only mild portal tract inflammation suggesting less hepatotoxicity compared to DAL control group.

Similar results were reported indicating loss of liver hepatocytes and kidney architecture in DAL bearing mice [11]. However, mice treatment with *L. inermis* extract improves the liver and kidney function and rearranges more or less normal architecture. Hepatoprotective activity of Abutilon indicum on experimental liver damage in rats was studied [12]. In this study the plant extract may interfere with free radical formation which may conclude in hepatoprotective action. Kidney was also considered vital organ in digestion of food since it serves as a filtering machine of absorbed nutrients and elements and blood. Present study revealed that kidney also has marked inflammatory changes to the tissues and cellular damage compared to the control group.

Regeneration of the regions after the administration of MECP could be attributed to the synergistic activity of the phytochemicals in the plant extract especially berberine a natural alkaloid present in *Cyclea peltata*, which has a long history of medicinal use in both Ayurvedic and old Chinese medicine. This could be substantiated by the reports in which berberine reduces hepatic fat content in the rats with nonalcoholic fatty liver disease [13]. Berberine also prevents proliferation of hepatic stellate cells (HSCs), which are central for the development of fibrosis during liver injury [14].

CONCLUSION

The methanol extract of *C. peltata* was shown here to be effective in inhibiting cancer growth and reversing many pathologic states associated with growth of this cancer in mice. Based on the above findings, it may be suggested that *C.peltata* could be used as a natural agent in treatment for cancer therapy.

AUTHORS' CONTRIBUTIONS

Both authors have contributed equally to the manuscript and work.

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