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Full Length Research Paper

Evaluation of Hepatic activity of various morphological parts of *Musa paradisiaca* L.

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The objective of present study was to investigate the hepatic activity of methanolic extract of various morphological parts (bract, flower, trachea and tracheal fluid) of *Musa paradisiaca* L. for their effect on liver of experimental mice. The methanolic extract of morphological parts of *Musa paradisiaca* (bract, flower, trachea and tracheal fluid) at the dose of (100, 250 and 500 mg/kg b.w) and silymarin (25 mg/kg) was orally administered once daily for 28 days and toxicity evaluation studies were carried out. Liver damage was assessed by biochemical parameters such as total bilirubin, direct bilirubin, indirect bilirubin, alkaline phosphatase (ALP), alanine transaminase (ALT), aspartate aminotransferase (AST), serum protein, serum albumin, serum globulin and A/G ratio and by histopathology of carbon tetrachloride (CCl_4) induced hepatic injury in mice. Results of the experiment showed that there is significant ($P< 0.05$) diversity between the groups that were treated with CCl_4, silymarin and different doses of different morphological parts of plant as compared to control group. Histopathological studies also supported the biochemical parameters. Different morphological parts of *M. paradisiaca* such as bracts, flower, trachea and tracheal fluid have potential to cause the hepatotoxicity that depends on the dose and the time duration in experimental mice.

Key words: *Musa paradisiaca* L., hepatic activity, histopathology, biochemical parameters.

INTRODUCTION

The liver, a major body organ plays an important role in the metabolism of the lipids, protein and carbohydrates, metabolic homeostasis, detoxification, biotransformation and excretion of many endogenous, environmental and pharmaceutical chemicals storage of glycogen, biochemical's necessary for digestion (bile), production of several coagulation factors, hormones (angiotensinogen), growth factors, vitamin A, D and B12 and protects the body from toxic by-products of metabolisms and potentially injurious substances namely endotoxins that are absorbed from the intestinal tract (Zhang et al., 2014; Madhu et al., 2012; Ajith et al., 2007). In last few decades liver injury and dysfunction is mainly caused due to exposure to toxic chemicals, certain drugs such as

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chemotherapeutic agents, thio-acetamide, carbon tetrachloride, xenobiotics, chronic alcohol consumption, microbes, environmental pollutants, viruses, and auto-immune diseases (Zhang et al., 2014; Showkat et al.,2013; Mohanraj et al., 2013).

Carbon tetrachloride is a potent hepatotoxin that metabolized to tri-chloromethyl radicals by cytochrome P_{450} which leads to increase in hepatic lipid peroxidation and oxidative stress that why widely used to produce hepatotoxicity to evaluate hepatoprotective effects of natural products (Ottu et al., 2013). There are many reports which show similarities between CCl_4 induced liver damage and human liver cirrhosis (Halim et al., 1997). That is why CCl_4 induced liver damage is generally used as experimental model for screening of hepatoprotective and hepatocurative drugs.

The intensity of hepatic damage is generally accessed by measuring the activities of hepatic cytoplasmic enzymes [serum glutamate pyruvate transaminase (SGPT), serum glutamate oxalacetate transaminase (SGOT), serum alkaline phosphatase (ALP)], serum bilirubin concentration and histological studies (Stierum, 2005). The extent of oxidative stress may be predicted by estimating the serum glutathione level (Sallie et al., 1991).

In recent years natural products and their active principles are the sources for new drug discovery and treatment of diseases (Ajith et al., 2007). Lack of toxicity and claims of therapeutic efficiency of many plants in recent years have been proved scientifically. In view of the potential use of medicinal plants as a source of alternative medicine in many diseases and claims made by the people in different countries, many species of plants kingdom containing chemical constituent of medicinal value which have to be discovered yet. Large numbers of plants are needs to be examined thoroughly for their possible pharmacological value (Ethadi et al., 2013; Tauqeer et al., 2014).

Musa paradisiaca L. (Musaceae) is evergreen tropical monoherbacious plant,commonly known as kela (Urdu) Kadali, Bali Hannu (Hindi) and Plantain (English). It is major food crops in the humid and subhumid parts of Pakistan, Africa, India, Burma, Bangladesh, America, and Australia (Paul et al., 2013; Sanjeev et al., 2012; Sunil et al., 2012; Shodehinde et al., 2012). *M. paradisiaca* root is used as tonic for congestion of the liver and to prevent scurvy, anaemia, veneral disease.

The leaves are used in inflammation of eye, healing wounds and ulcers. The flowers check excessive bleeding during menstruation and are used in the case of diabetes. The fruits are used in diarrhea, indigestion and flatulence. The stems are used for ulcer, jaundice, nervous disorder, hysteria, diarrhea, dysentery, antidote for opium poisoning, asthma, hair loss, treatment of piles (Sanjeev et al., 2012; Enye et al., 2013).

Objective of our study was to evaluate the various morphological parts (bract, flower, trachea and tracheal fluid) of *M. paradisiaca* for its effect on liver.

MATERIALS AND METHODS

Plant material

M. paradisiac plant was collected from District Muzaffar-Garh, (Punjab) Pakistan during November 2013 and authenticated by Dr. Mansoor Hameed, Associate Professor, Taxonomic Laboratory, Department of Botany, University of Agriculture Faisalabad with voucher number 131-2014. Each part of plant was also deposited in the herbal museum Department of Pharmacognosy, Faculty of Pharmacy, University of Karachi. The parts of plant [bracts, flower and Flowering stalk (trachea)] were separated and dried in shade at room temperature for about one month.

Preparation of extract

2.5 kg bracts, 2.0 kg flowers and 2.0 kg trachea (flowering stalk) were coarsely powdered by mortar and pestle separately and then passed through sieve number 40 and stored in air tight container. Extraction was carried out separately for each part in 7.5, 5.0 and 4.0 L methanol (Merck, Germany) respectively in glass aspirator by maceration for seven days with occasional shaking at room temperature then filtration was carried out by using Whatman filter No 1. The filtrate were evaporated to dryness in rotary vacuum evaporator (Rotavapor R-200, Buchi) at temperature 45°C with rotation 3.0 rpm and pressure 0.07 MPA or 20 in Hg. The dried material were weighed, labeled and stored in refrigerator. 1.0 L tracheal fluid obtained from floral stalk after cutting the bunch of fruit and this is then, lyophilized at -65 to -60°C with vacuum of 30 to 40 milibar in alpha 1-4LSC Christ Germany lyophilizer. The dried material were weighed, labeled and stored in refrigerator (Sanyo biomedical freezer, MDF-U333, Japan).

Drugs and standards

Standard drug silymarin was obtained from A and K Pharmaceuticals. Carbon tetrachloride was purchased from Riedel De Haen. Alanine aminotransferase (ALT), Aspartate aminotransferase (AST), total bilirubin, direct bilirubin, Alkaline phosphatase (ALP), Total protein, Albumin and Globulin reagents for estimation were purchased from Roche Pakistan Ltd. Animal feed pellets were purchased from local market. All other chemicals and solvents used in the study were of analytical grade (Merck, Germany).

Animal

Wistar mice of either sex weighing between 22 and 30 g were taken for the study. Animals were housed in colony cages under standardized conditions at a temperature of 24±2°C, humidity of 50% under 12 h light / dark cycle and they were fed with *ad libitum* with standard pellet diet with free access to food and water. They were allowed to acclimatize for a week before the experiments were started and protocol was approved by the Institutional animal ethics committee for the purpose of control and supervision on animals. The experimental procedures were carried out in strict compliance with the Institutional Animal Ethics committee (Ref. No. Pharm/14/1928).

Carbon tetrachloride-induced hepatotoxicity in mice

Effect of various morphological part of *M. paradisiaca* was tested using CCl_4 model as described by Kavishankara et al. (2014) with slight modification. The animals were randomly divided into fifteen

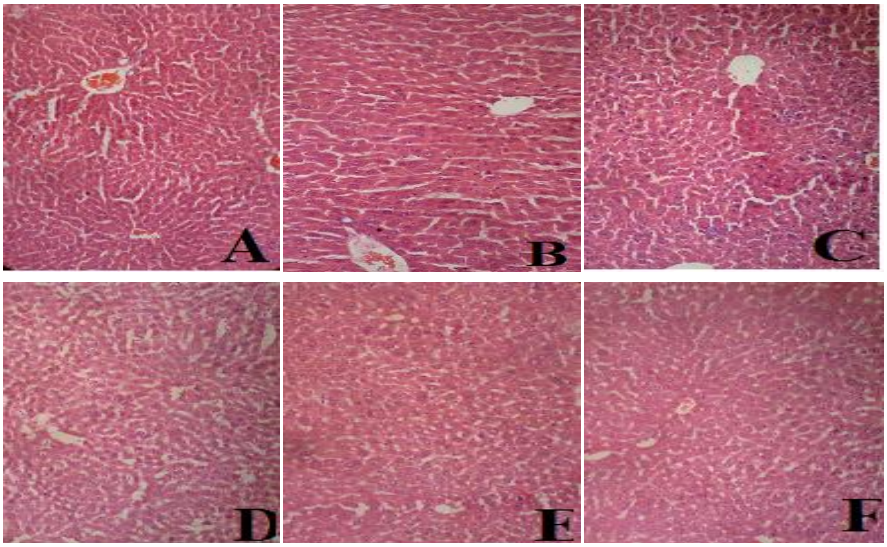

Figure 1. Photomicrographs showing the effect of silymarin, CCL_4, control group and different doses of bract extract of *M. paradisiaca* on mice liver. **A**: liver section of standard drug silymarin (25 mg/kg) treated group; **B:** liver section of CCL_4 (2.0 g/kg) treated group;**C**: normal control group receiving normal saline (2 ml/kg); **D**: methanolic extract of bract (100 mg/kg) treated group,**E**: methanolic extract of bract (250 mg/kg) treated group **F**: methanolic extract of bract (500 mg/kg) treated group (magnification ×10).

groups, consisting of six animals each. Group-I (negative control) received normal saline solution orally (0.2 ml/100 g); Group-II (toxic group) was given a single intraperitoneal dose of CCl_4 (2.0 g/kg b.w); Group-III (standard group) received orally 25 mg/kg b.w of silymarin; Group IV to VI (test groups), received a dose of (100, 250 and 500 mg/kg b.w., p.o.) of methanolic extract of bract of *M. paradisiaca* and CCl_4 (2.0 g/kg b.w., i.p.), respectively; Group VII to IX (test groups), received a dose of 100, 250 and 500 mg/kg b.w., p.o. of methanolic extract of flower of *M. paradisiaca* and CCl_4 (2.0 g/kg b.w., i.p.), respectively; Group X to XII (test groups), received a dose of 100, 250 and 500 mg/kg b.w., p.o. of methanolic extract of trachea of *M. paradisiaca* and CCl_4 (2.0 g/kg b.w., i.p.), respectively; Group XII to XV (test groups), received a dose of 100, 250 and 500 mg/kg b.w., p.o. of methanolic extract of tracheal fluid of *M. paradisiaca* and CCl_4 (2.0 g/kg b.w., i.p.), respectively. All the groups were treated for consecutive 28 days. On completion of experimental period, animals were sacrificed under ether anesthesia. Blood samples were collected and centrifuge. The obtained serums were analyzed for liver function markers. The liver was excised from the animal and immediately processed for histopathological studies (Kavishankara et al., 2014).

Histopathological studies

Mice were sacrificed, livers excised and tissues were washed in normal saline and fixed in10% formalin solution, dehydrated in graded (50 to 100%) alcohol and embedded in paraffin. 5 µm thin microtome sections were made, processed with alcohol-xylene series and stained with haematoxylin. It was then studied under light microscope for any histological protection or damage (Luna, 1968).

Biochemical measurement

Biochemical parameter like ALP, SGOT and SGPT, total bilirubin, direct bilirubin, total protein, albumin and globulin were determined by using automatic chemistry analyzer machine Cobas c311 by Roche.

Statistical analysis

The experimental data are expressed as mean ± SD. Data were subjected to statistical analysis through one way analysis of variance (ANOVA) followed by Tukey's test. The values of $P< 0.05$ is been considered as significant.

RESULTS

Histopathology

In histopathological study of CCl_4 induced hepatotoxicity model liver section of normal liver showed all cells with cellular organelles are normal; CCl_4 intoxicated group rat liver section showed degenerative changes in hepatocyte with vacuolation of cytoplasm and swelling of nucleus at some areas. Nuclear pyknosis was also observed. Cytoplasmolysis and necrotic changes were also present in hepatocytes that are the indication of cell damage; Sylimarin treated group rat liver section showed normal integrity of nucleus and cells.

The extract of bract of *M. paradisiaca* with different doses (100, 250 and 500 mg/kg) showed the moderate degenerative changes and dispersion of cytoplasm. Hepatic cords were shrinked. Necrotic changes in hepatocytes with pyknosis of nucleus at some places, vacuolization in cytoplasm and milder degrees of fatty changes (Figures 1 and 2). Flower extract at different

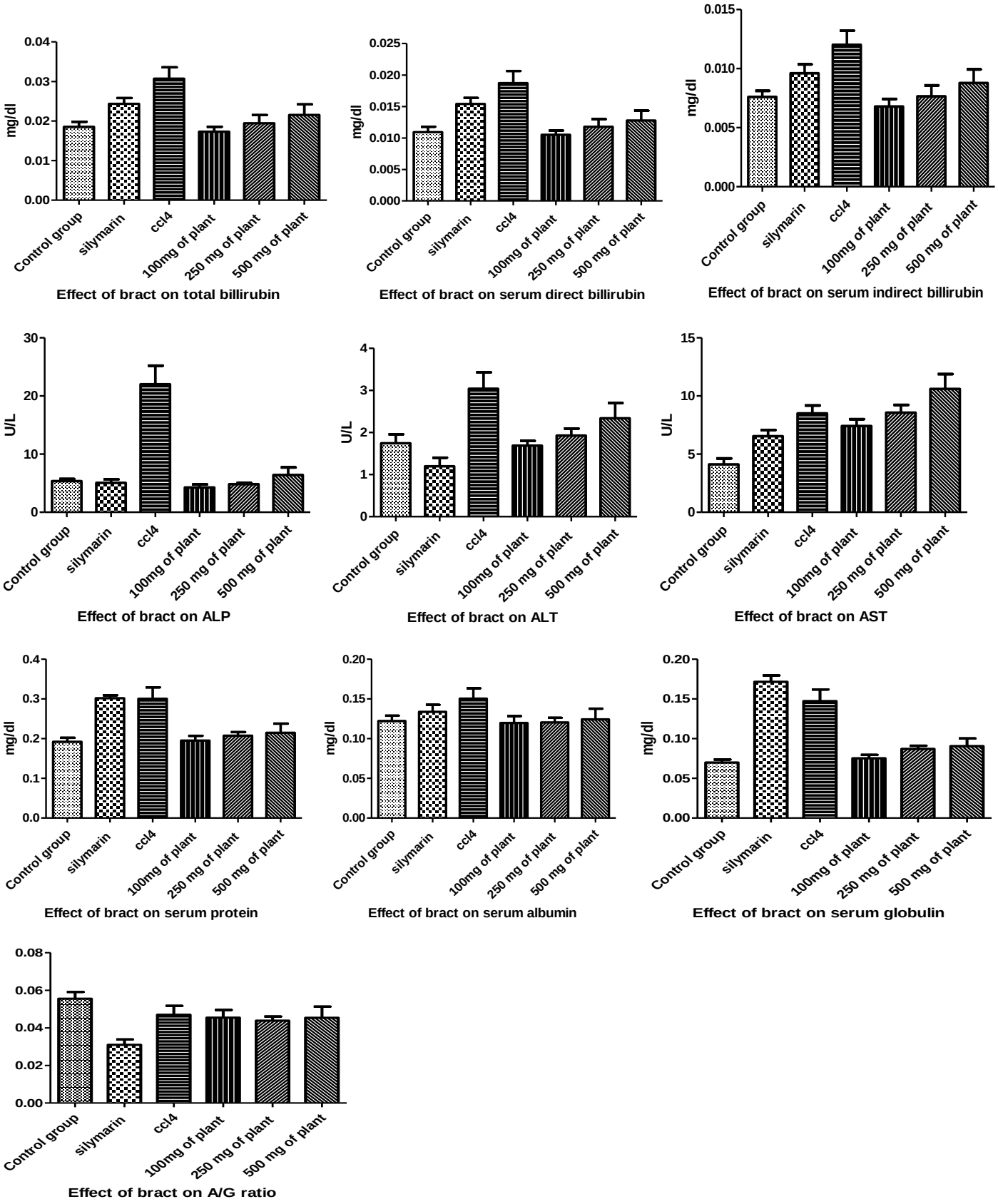

Figure 2. Effect of bract of *Musa paradisiaca* on liver parameters **(**total bilirubin, direct bilirubin, indirect bilirubin, ALP, ALT, AST, serum protein, serum albumin, serum globulin and A/G ratio).

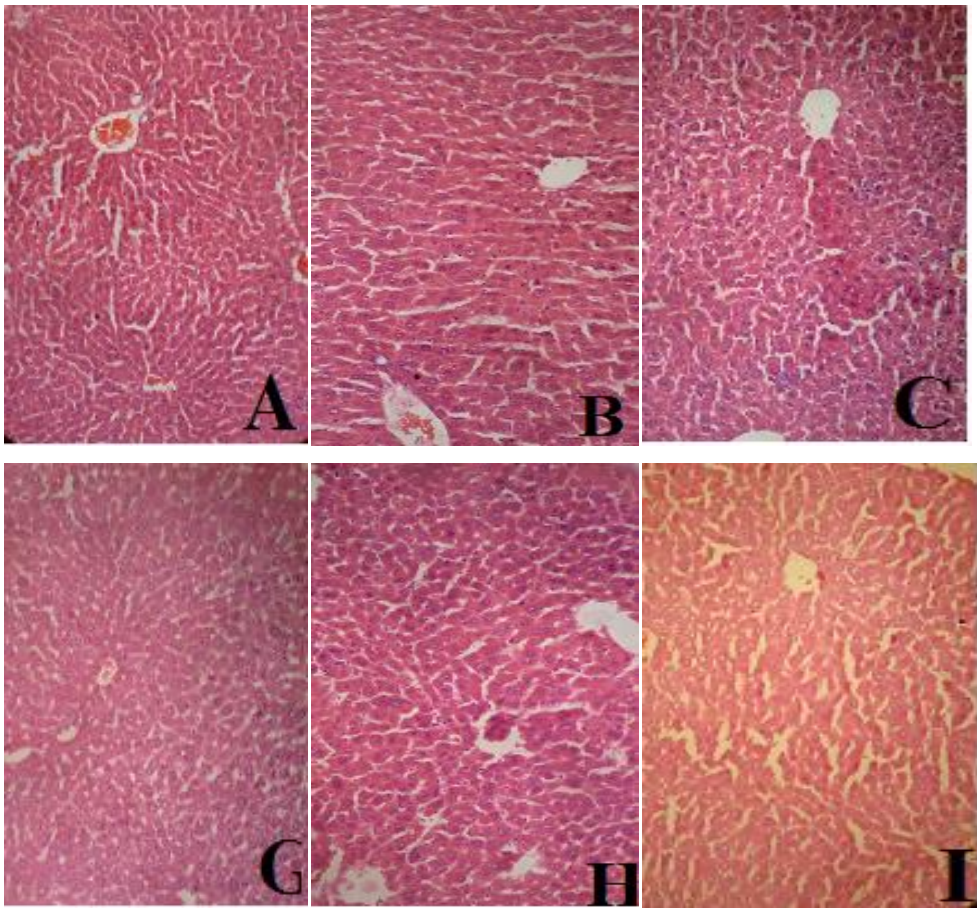

Figure 3. Photomicrographs showing the effect of silymarin, CCL_4, control group and different doses of flower extract of *M. paradisiaca* on mice liver. **A**: liver section of standard drug silymarin (25 mg/kg) treated group; **B**: liver section of CCL_4 (2.0 g/kg) treated group **C**: normal control group receiving normal saline (2 ml/kg); **G**: methanolic extract of flower (100 mg/kg) treated group **H**: methanolic extract of flower (250 mg/kg) treated group **I**: methanolic extract of flower (500 mg/kg) treated group (magnification ×10).

doses (100, 250, 500 mg/kg) shows that necrotic and degenerative changes were observed in group of cells with swollen nucleus at some places while pyknotic nucleus with more a sinophilic cytoplasm (Figure 3). *M. paradisiaca* tracheal extract at different doses (100, 250, and 500 mg/kg) shows slight dilation of sinusoidal spaces in liver cells and mild degree of fatty changes and vacuolization in cytoplasm of hepatocytes (Figure 4). While tracheal fluid of *M. paradisiaca* showed severe vacuolization, degenerative changes and a sinophilic cytoplasm with hyper-chromatic nucleus that has larger size (Figure 5 and 6).

Effect of extracts of *M. paradisiaca* on liver marker

In CCl_4 induced hepatotoxicity animal model pretreatment with silymarin (25 mg/kg) and methanolic extract of bract and trachea of *M. paradisiaca* at the dose of 100, 250 and 500 mg/kg reduced total bilirubin, direct bilirubin, indirect bilirubin, ALP, ALT, AST, serum protein, serum albumin, serum globulin and A/G ratio significantly ($P<0.05$), as compared with CCl_4 intoxicated (Figures 7). Less pronounced effect on liver marker was obtained from the flower and tracheal fluid extract of plant (Figures 4 and 8).

DISCUSSION

Hepatoprotective studies are conducted to investigate the protective effects of the plant extracts against liver damage. Major organ of the body is liver that can be injured by many drugs and chemicals (Hogade et al., 2010; Peng et al., 2009).

The hepatoprotective effects of methanolic extracts of *M. paradisiaca* (bract, flower, trachea and tracheal fluid) were studied in rats by using CCl_4 induced hepatotoxicity at the doses of 100, 250 and 500 mg/kg bw. Liver damage was assessed by biochemical studies

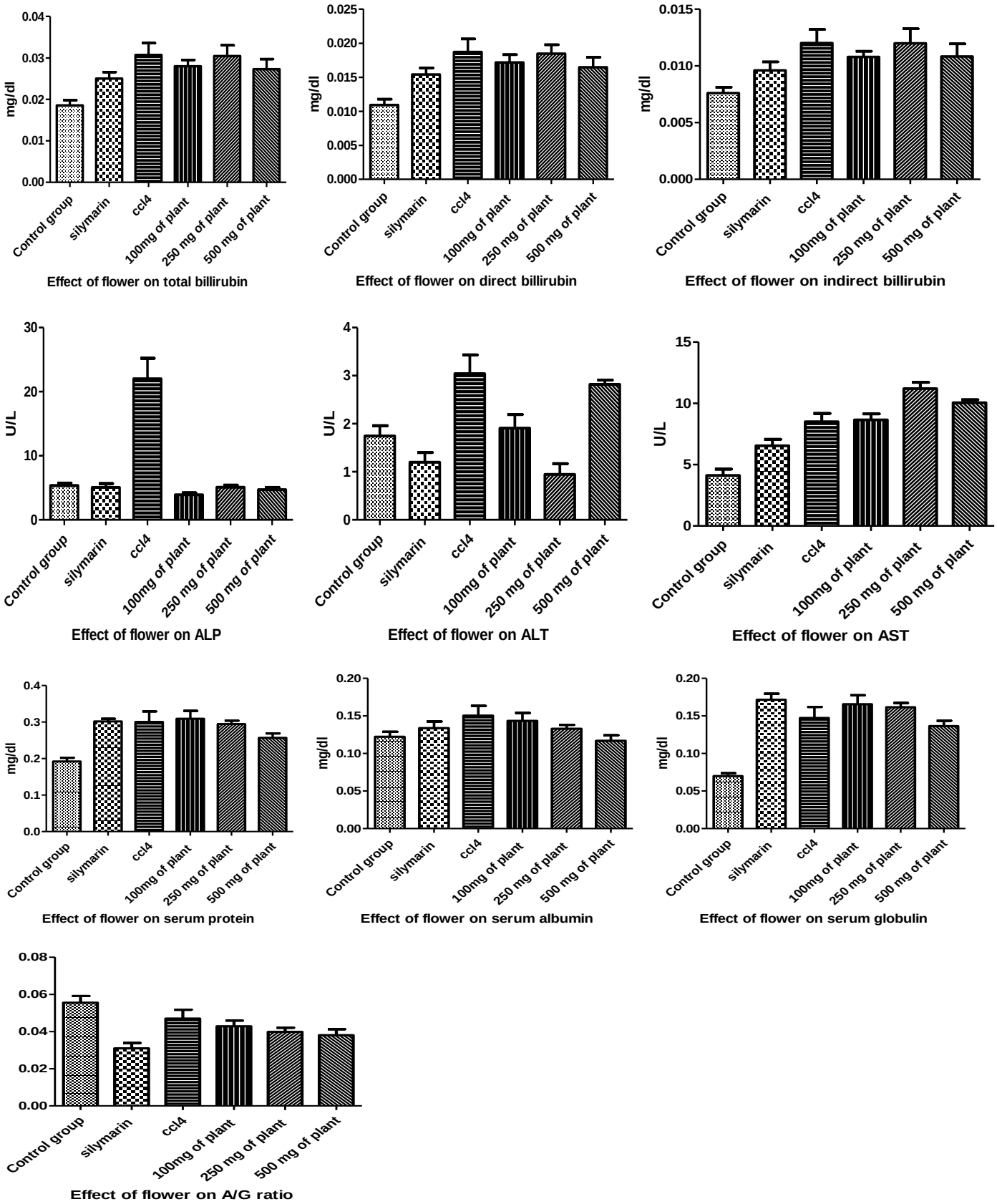

Figure 4. Effect of flower of *Musa paradisiaca* on liver parameters **(**total bilirubin, direct bilirubin, indirect bilirubin, ALP, ALT, AST, serum protein, serum albumin, serum globulin and A/G ratio).

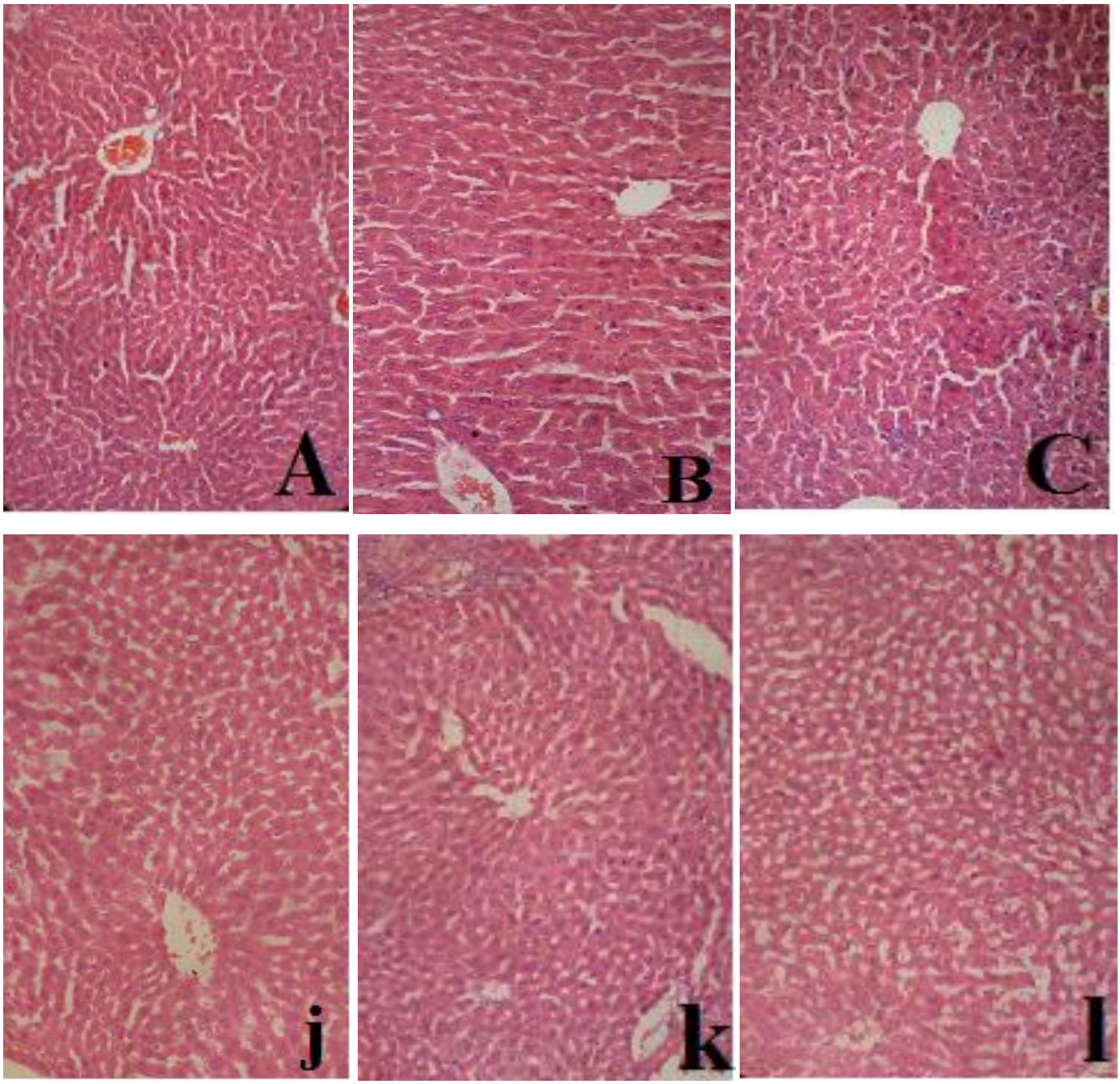

Figure 5. Photomicrographs showing the effect of silymarin, CCL_4, control group and different doses of trachea extract of *M. paradisiaca* on mice liver. **A**: liver section of standard drug silymarin (25 mg/kg) treated group; **B**: liver section of CCL_4 (2.0 g/kg) treated group. **C**: normal control group receiving normal saline (2 ml/kg); **J**: methanolic extract of trachea (100 mg/kg) treated group **K**: methanolic extract of trachea (250 mg/kg) treated group **L**: methanolic extract of trachea (500 mg/kg) treated group (magnification ×10).

(total bilirubin, direct bilirubin, indirect bilirubin, ALP, ALT, AST, serum protein, serum albumin, serum globulin and A/G ratio) and histopathological examinations.

In CCl_4-induced hepatotoxicity model, upon administration of CCl_4 to animals, it undergoes enzymatic activation, majorly by CYP2E1, into the trichloromethyl free radical (CCl_3) within the membrane of the endoplasmic reticulum. This is followed by chloromethylation, saturation, peroxidation and progressive destruction of the unsaturated fatty acid of the endoplasmic reticulum membrane phospholipids. These processes are known as lipid peroxidation, leading

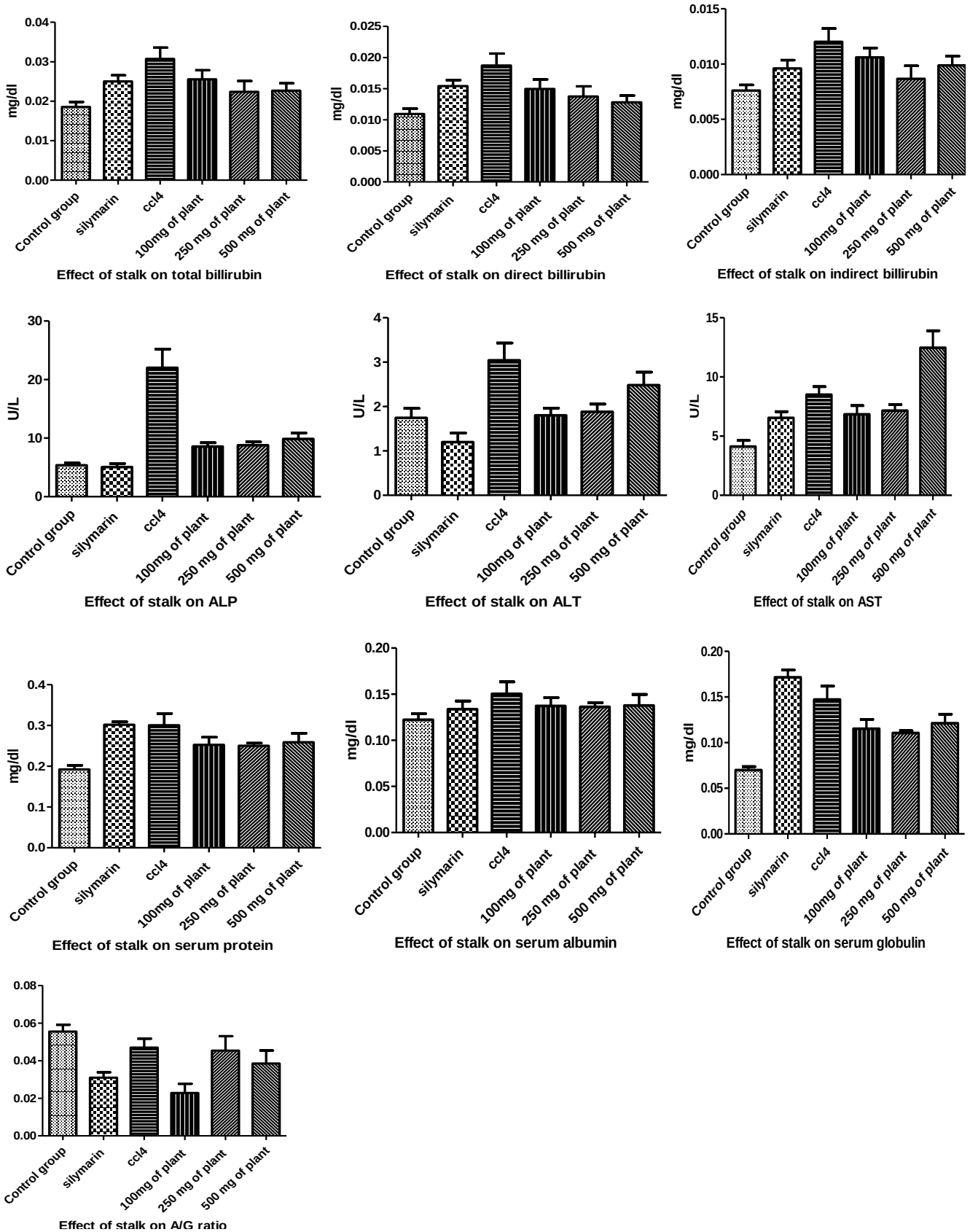

Figure 6. Effect of stalk of *Musa paradisiaca* on liver parameters **(**total bilirubin, direct bilirubin, indirect bilirubin, ALP, ALT, AST, serum protein, serum albumin, serum globulin and A/G ratio).

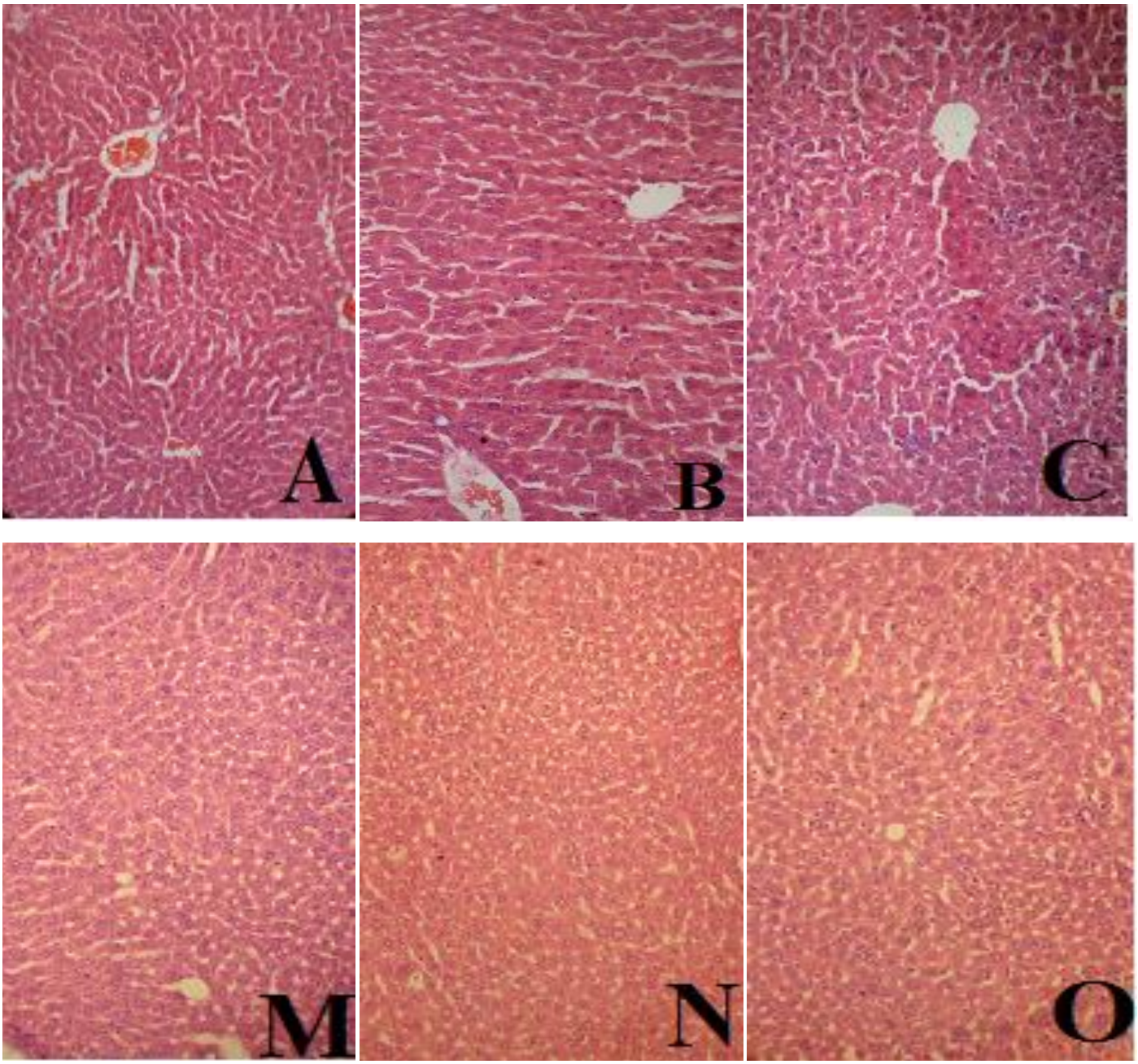

Figure 7. Photomicrographs showing the effect of silymarin, CCL4, control group and different doses of tracheal fluid of *Musa paradisiaca L* on liver histopathology of mice. **A**: liver section of standard drug silymarin (25mg/kg) treated group; **B**: liver section of CCL4 (2.0 g/kg) treated group. **C**: normal control group receiving normal saline (2 ml/kg); **M**: methanolic extract of tracheal fluid (100 mg/kg) treated group **N**: methanolic extract of tracheal fluid (250 mg/kg) treated group **O**: methanolic extract of tracheal fluid (500mg/kg) treated group (magnification 10×).

to functional and structural disruption of hepatocytes. During hepatic damage, cellular enzymes like SGPT, SGOT, ALP, bilirubin (direct and total) leak into the serum resulting in elevation of their serum concentrations (Shenoy et al., 2001).

Measurement of hepatic function markers (SGOT, SGPT, ALP, total bilirubin, direct bilirubin, indirect bilirubin, total protein, serum albumin, serum globulin and A/G ratio) have a clinical and toxicological significance as variation in their values are indications of tissue damage in pathological condition or hepatic dysfunction. Greater amount of release of enzymes from cells are indicative of loss of functional integrity of the cell membrane and cellular leakage and this may be due to abnormal membrane permeability and hepatocyte necrosis (Drotman and Lawhorn, 1978).

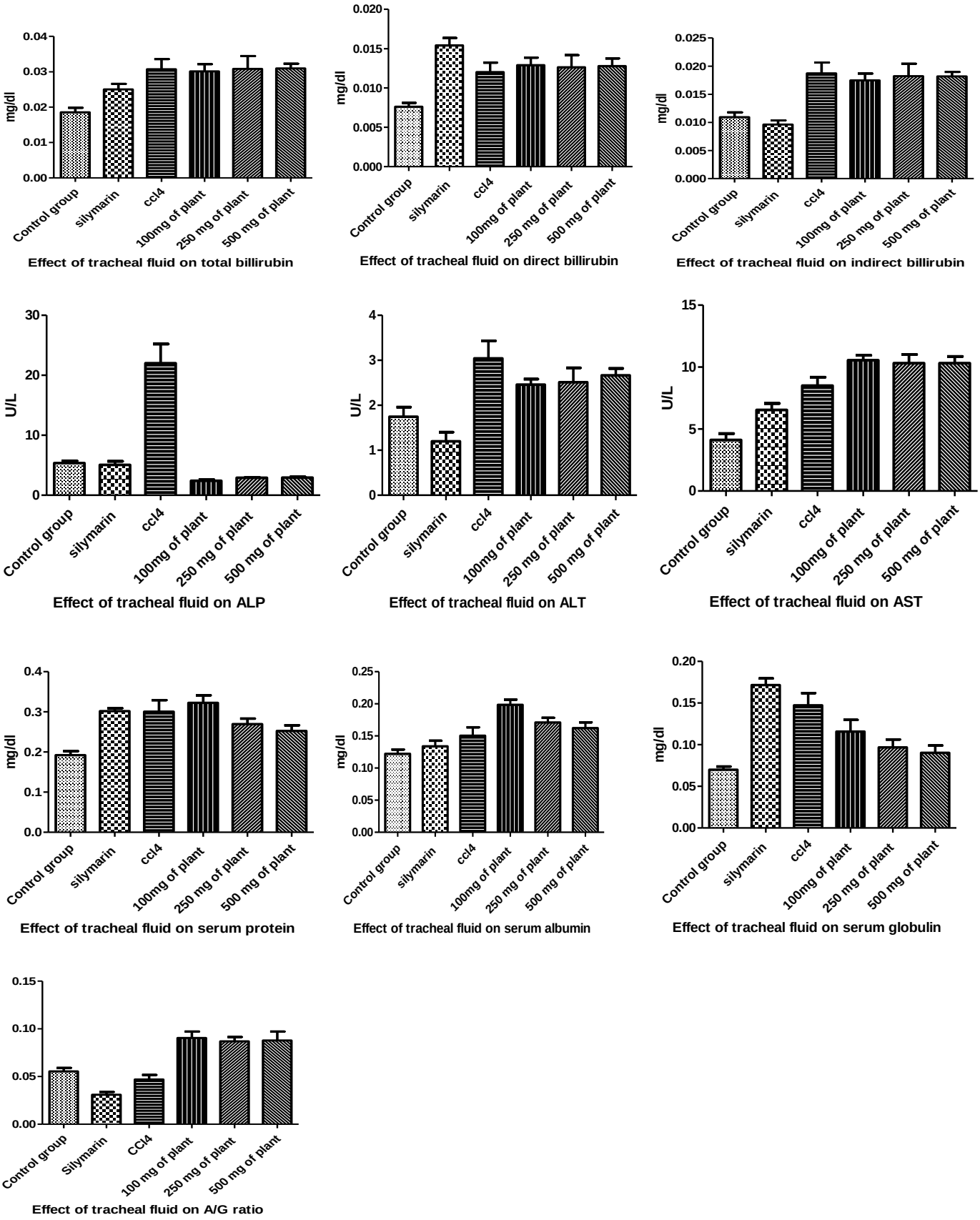

Figure 8. Effect of tracheal fluid of *M. paradisiaca* on liver parameters **(**total bilirubin, direct bilirubin, indirect bilirubin, ALP, ALT, AST, serum protein, serum albumin, serum globulin and A/G ratio).

Conclusion

The result of the present investigation indicates that different morphological parts of *M. paradisiaca* L. such asbracts, flower, trachea and tracheal fluid have potential to cause the hepatotoxicity that depend on the dose and the time duration in experimental mice.

Conflict of Interests

The authors have not declared any conflict of interests.

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