

Impact of abhrak bhasma and SiO₂ on histological architecture of liver and kidney in single dose of carbon tetrachloride intoxicated male albino rat

¹Parashuram Teli, ²Jaywant Jadhav, ³Meghnad Joshi and ⁴Aruna Kanase*

^{1,2,4}Cell Biology Section, Dept. of Zoology, Shivaji University,
Kolhapur-416 004, MH, India.

³Stem Plus Biotech, Sangli - 416416, MH, India
arunakanase@gmail.com
Mob. +91 9767004359

Abstract

Abhrak bhasma is a widely used Ayurvedic drug in various diseases including hepatotoxicity. In the present study, protective effect of graded doses (10, 20, 30 and 40mg/kg body wt) of abhrak bhasma and its silica control (SiO₂) was studied against CCl₄ induced liver and kidney damage in male albino rats during single dose experimental schedule for 24 hrs. Administration of CCl₄ (3.0ml/kg body wt) induced fatty necrosis in hepatic cells without affecting kidney histology. Treatment of abhrak bhasma showed dose dependent protection against CCl₄ induced liver damage by free radical scavenging activity. Silica in SiO₂ form also positively influences liver fatty degeneration induced by single dose of CCl₄ but is associated with some hepatocyte's hypertrophy.

Keywords: Abhrak bhasma, SiO₂, Hepatotoxin, Histological, Centrolobular necrosis, Hypertrophy.

INTRODUCTION

Abhrak bhasma is a widely used Ayurvedic drug in various diseases including hepatotoxicity [1]. It is derived from an ore of silica i.e. mica. Since repetitive heating is required during abhrak bhasma preparation, it is considered as an oxides of particular ore. Our earlier work revealed its hepatoprotective and curative action by evaluating biochemical parameters including serum, liver, kidney function during CCl₄ acute toxicity experimental schedule of 7 days [2,3]. Abhrak bhasma showed no toxicity to liver and kidney histology given as single dose experimental schedule of 24 hrs. Its silica control, SiO₂ used showed some histological alterations in liver and kidney at high doses [4].

Carbon tetrachloride (CCl₄) is a potent hepatotoxin which causes different ailments in liver and kidney [5-7]. The free radicals (CCl₃·) generated by CCl₄ induce peroxidative degradation of membrane lipids of endoplasmic reticulum rich in polyunsaturated fatty acids [8]. This lipid peroxidative degradation of biomembranes is one of the principle causes of hepatotoxicity of CCl₄ [9], which is referred as fatty degeneration. It is associated with centrolobular fatty necrosis [10-12] and is detectable with histological architecture. For this reason, present work was designed to study the effects of abhrak bhasma and its silica control, SiO₂ on liver and kidney histology against single dose of CCl₄ induced toxicity. This is an extended work of our earlier research of abhrak bhasma and SiO₂ toxicity [4].

MATERIAL AND METHODS

Animal:

Male albino rats, *Rattus norvegicus* originally derived from National Institute of Virology Pune, were bred and maintained in the Departmental Animal House (Reg. No. 233/CPCSEA). All rats were fed with standard pellet diet (prepared by Amrit feeds, Sangli, MS, India). Food and water were provided *ad libitum*. The rats, weighing about 130-140g each were used for experiment.

Preparation of abhrak bhasma and Silicon dioxide:

Abhrak bhasma was prepared in the laboratory as described in Rasa Ratna Samucchaya [13]. SiO₂ treatment was given as positive control. To study the detailed dose dependent effects of abhrak bhasma and SiO₂ on histological architecture of liver and kidney during toxicity induced by 3.0ml CCl₄/kg body wt, graded doses of abhrak bhasma and SiO₂ viz. 10, 20, 30 and 40 mg/kg body wt were given orally with honey. Honey control rats were used which showed normal histology of liver and kidney with all doses. Therefore, honey control data is not presented.

Experimental schedule:

The male albino rats were assigned into following groups, each containing 6 animals and the various treatments were given as follows.

Group 1 - The rats were maintained as normal without any treatment.

Group 2 - Hepatotoxicity induced by single dose of 3.0 ml CCl₄/kg body wt for 24 hrs sc.

Group 3- CCl₄ (3.0 ml/kg body wt) sc + 10mg abhrak bhasma/kg body wt po for 24 hrs.

Group 4- CCl₄ (3.0 ml/kg body wt) sc + 20mg abhrak bhasma/kg body wt po for 24 hrs.

Group 5- CCl₄ (3.0 ml/kg body wt) sc + 30mg abhrak bhasma/kg body wt po for 24 hrs.

Group 6- CCl₄ (3.0 ml/kg body wt) sc + 40mg abhrak bhasma/kg body wt po for 24 hrs.

Group 7- CCl₄ (3.0 ml/kg body wt) sc + 10mg SiO₂/ kg body wt po for 24 hrs.

Group 8- CCl₄ (3.0 ml/kg body wt) sc + 20mg SiO₂/kg body wt po for 24 hrs.

Group 9- CCl₄ (3.0 ml/kg body wt) sc + 30mg SiO₂/kg body wt po for 24 hrs.

Group 10- CCl₄ (3.0 ml/kg body wt) sc + 40mg SiO₂/kg body wt po for 24 hrs.

The rats were killed after 24 hrs by giving deep ether anesthesia and liver and kidney tissues were separated from animals and processed for histological preparations.

Histological preparation:

The histological preparations were performed as described in our earlier work⁴. Liver and kidney tissues were removed immediately after killing of rat and cut into small pieces of suitable size for histology. Tissues were fixed in Bouin's fixative, 10% buffered formaldehyde, 4% paraformaldehyde and 4% glutaraldehyde. The tissues were fixed in different fixatives for 18 hrs and were processed for paraffin sectioning as per routine microtechnique [14,15]. The wax sections were cut of 0.5 μ thickness and were further processed and stained with haematoxylin-eosin. The preparations were evaluated under light microscope. The data of various fixatives confirmed the histological alterations.

RESULTS AND DISCUSSION

In the present study, protective effect of abhrak bhasma in graded concentrations (10, 20, 30 and 40 mg/ kg body wt) was studied against CCl₄ induced liver and kidney damage in male albino rats during single dose experimental schedule of 24 hrs. Similar doses of SiO₂ were used as silica control.

Effect of abhrak bhasma and SiO₂ on liver histology against single dose of CCl₄ induced hepatotoxicity:

Liver of normal group of rats showed normal histological architecture of centrilobular and periarterial region. The hepatic cells were radially arranged in hepatic cords. Sinusoids were normal and sinusoidal and Kupffer cells were distributed through the lobule, bile canaliculi were clear, hepatocytes were distinct due to their large shape and staining. Nuclei were basophilic and placed in central region of cells. Cytoplasm was eosinophilic (**Figure 1A & B**).

Alterations occurred in histological architecture of liver of CCl₄ treated rat in comparison with the histological architecture of liver of normal rat were presented in **Fig. 1C & D**. The normal architecture of liver was altered by CCl₄ treatment specifically in centrilobular region. Periarterial region remained normal. Administration of single dose of CCl₄ to the normal rat for 24 hrs induced fatty necrosis in hepatic cells and hence distorted the hepatic cords, blocked the bile canaliculi. The cells showed vacuolated cytoplasm, vacuolar size showed variations from small peripheral to large droplet like structures. In most of the necrotic cells, the centrally placed nuclei were suspended in small amount of cytoplasm, which remained continuous by cytoplasmic strands that traversed through the vacuoles connecting the peripheral rim of cytoplasm. Many of the cells were necrotic. Kupffer cells and sinusoidal cells were arrested in necrotic zone. It was studied earlier and described as hydropic degeneration/fatty degeneration [12] and reviewed by Mehendale [16]. Single dose of CCl₄ lead this zone to restrict to small area surrounding central vein as depicted in **Fig. 1C & D**.

Treatment of various doses of abhrak bhasma showed dose dependent protection against CCl₄ induced liver damage. 10mg abhrak bhasma given orally simultaneous with CCl₄ protected the liver partially. The centrilobular region showed necrosis, but the extent of the area of necrotic region was reduced significantly. Numbers of necrotic cells located in this region were considerably reduced and were retained in immediate vicinity of the vein. Most of the cells on the boundary of the necrotic region showed small vacuoles indicating preliminary stage of necrosis. The area of healthy cells and necrotic cells were located in the same lobular zone. Necrotic region showed the pathological architecture as described above and in region of healthy cells normal histological structure was evident (**Fig. 2A**). Periarterial region of liver was clear showing distinct hepatic cords. Sinusoidal cells and Kupffer cells were distinct and normally distributed (**Fig. 2E**). In centrilobular region of CCl₄+20mg abhrak bhasma treated rats, the region was normal without any necrotic cells or the cells that showed any type of stress. Clear bile canaliculi were noted. Distribution of Kupffer cells and sinusoidal cells

were normal (**Fig. 2B**). Periarterial region showed normal architecture (**Fig. 2F**). Higher doses of abhrak bhasma (30 & 40mg/kg body wt) also resulted in total protection. All the regions of liver were with normal histological architecture (**Fig. 2C & D and 2G & H**). These histological observations have clearly indicated that 20mg and above doses of abhrak bhasma protected CCl₄ induced changes in hepatic architecture like necrotic, fatty and degenerative changes. This protective effect of abhrak bhasma might be because of free radical scavenging activity of abhrak bhasma which is processed mica.

Administration of various doses of SiO₂ simultaneously with CCl₄ showed dose dependent reduction in centrilobular necrotic region with 10mg and 20mg doses with less potency as compared to abhrak bhasma retaining fatty degenerative areas in centrilobular region. With 30mg and 40mg doses of SiO₂ the protection of liver fatty degeneration was shown with abhrak bhasma but with increased hypertrophied cells in centrilobular region and some portion showed alterations like shrunken nuclei, obliterated bile canaliculi. Sinusoids in some region were clear, in some region they were collapsed. Unlike to abhrak bhasma, SiO₂ treated liver showed few alterations in periarterial zone also.

Effect of abhrak bhasma and SiO₂ on kidney histology against single dose of CCl₄ induced hepatotoxicity:

Histological observations showed that single dose of CCl₄ did not influence the normal architecture of both the cortex and medulla region of kidney. It showed normal glomeruli and Bowman's capsule. The proximal and distal tubules were observed normal with clear lumina. Eosinophilic brush border can be identified in proximal tubules. Moderately stained nuclei were observed. The stromal tissue and interstitial tissue can be identified (**Fig. 4**).

Treatment of CCl₄+10/20/30/40mg abhrak bhasma showed normal kidney histology (**Fig. 5**). CCl₄+10/20/30/40mg SiO₂ did not affect kidney structure (**Fig. 6**). Hence the present study revealed that abhrak bhasma protects the histological architecture against CCl₄ induced fatty degeneration in liver. Similarly doses of SiO₂ also protected liver; however the changes are associated with hypertrophy of hepatocytes. The response on sinusoids and bile canaliculi was not homogenous but scattered. Single dose of CCl₄ did not affect kidney structure independently or with any doses of abhrak bhasma/SiO₂.

These results indicate silica in SiO₂ form positively influences liver fatty degeneration induced by single dose of CCl₄ but is associated with some hepatocytes hypertrophy. But abhrak bhasma, which is derived from mica ore protects liver without any alterations in hepatic architecture or adaptive alterations like hypertrophy. Thus, these primary protective responses of abhrak bhasma and SiO₂ are common and indicate that silica plays effective protective role in single dose of CCl₄ toxicity; and in this schedule fail to influence kidney structure independently [4] or with CCl₄ as single dose. These results do not show significance of mica ore processing to form abhrak bhasma except that bile canaliculi and sinusoids responding in scattered fashion.

Since these are primary drug responses against single dose toxicity, to reveal other details of deflections in metabolisms if any, there is need to study these drug effects against acute CCl₄ toxicity and similarly exploiting other metabolisms of liver and kidney to throw light on significance of mica processing described in Ayurveda.

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Figure legends

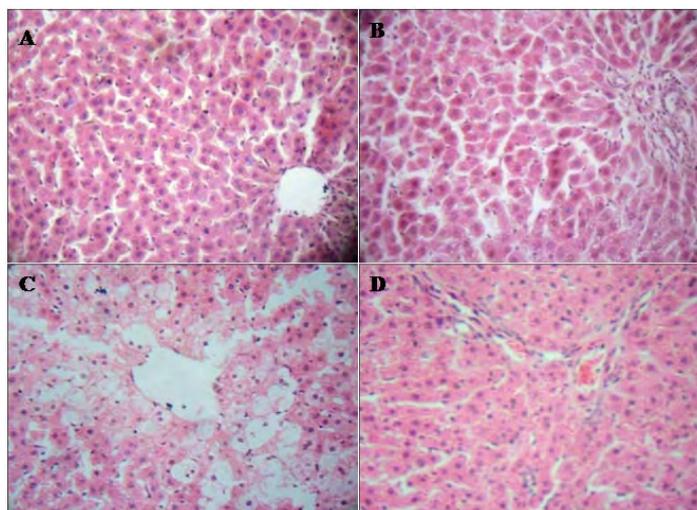


Figure 1. Effect of CCl₄ on centrolobular and periarterial region of normal rat liver: Centrolobular region showing normal histological architecture with radially arranged hepatocytes, distinguished sinusoidal and Kupffer cells, clear bile canaliculi (Fig. A). Periarterial region showing distinct hepatic cords with normal sinusoids, Kupffer cells and sinusoidal cells, clear bile canaliculi (Fig. B). CCl₄ induced fatty necrosis in centrolobular region showing distorted the hepatic cords, vacuolated cytoplasm (Fig. C). CCl₄ treated periarterial region showed normal histological architecture (Fig. D).

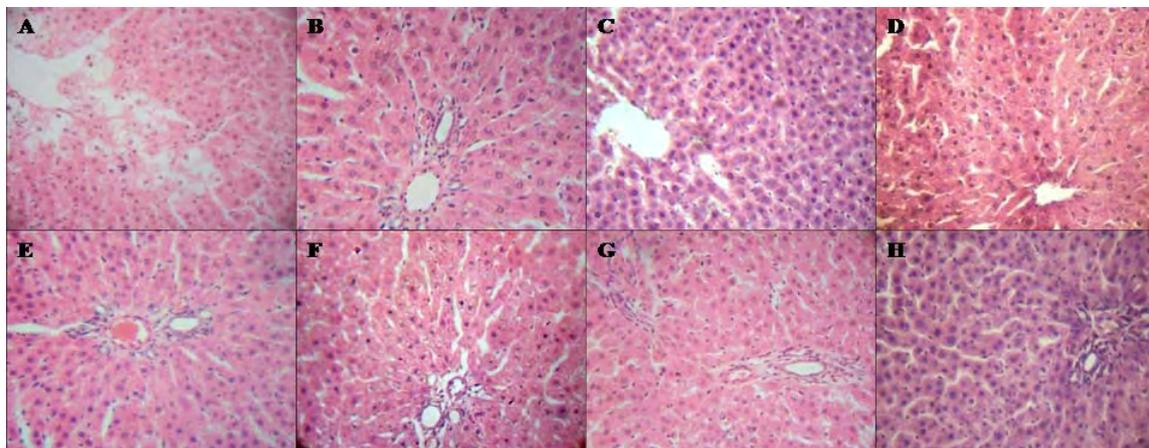


Figure 2. Effect of abhrak bhasma on CCl₄ intoxicated rat liver: Treatment of CCl₄+10mg AB showing reduced necrotic area in centrolobular region (Fig. A). Periarterial region with distinct hepatic cords, normally distributed sinusoidal cells and Kupffer cells (Fig. E). Centrolobular region treated with 20mg AB+CCl₄ showed no necrosis, clear bile canaliculi, normally distributed Kupffer cells and sinusoidal cells (Fig. B); normal periarterial region (Fig. F). Treatment of CCl₄+30mg AB/40mg AB showing normal centrolobular region with distinct hepatic cords, sinusoidal and Kupffer cells identified (Fig. C & D) and periarterial region (Fig. G & H).

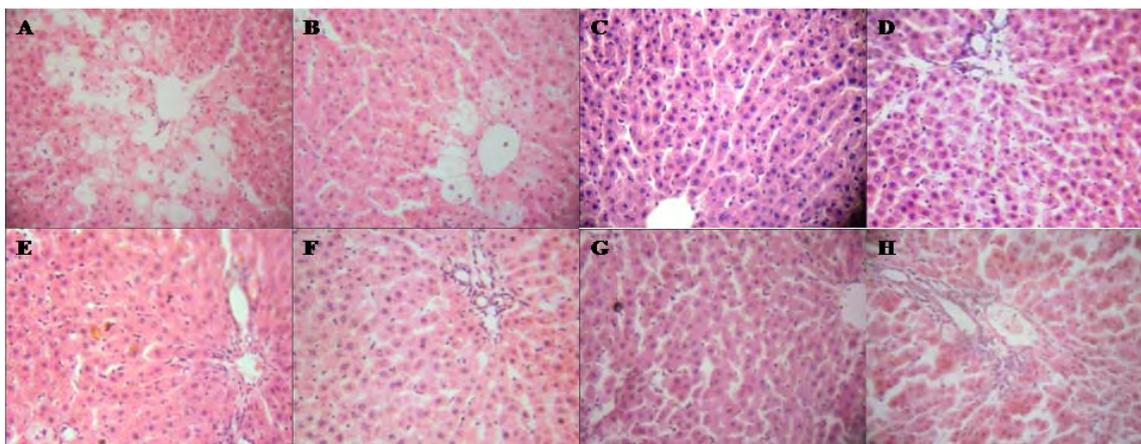


Figure 3. Effect of SiO₂ on CCl₄ intoxicated rat liver: Treatment of CCl₄+10mg SiO₂ showing reduction in centrolobular necrotic region with very small fatty accumulations in some necrotic hepatic parenchymal cells, partially displaced hepatic cords and dilated sinusoids (Fig. A). Periarterial region showed distinct but moderately foggy parenchymal cells alongwith perinuclear empty spaces. Kupffer cells and sinusoidal cells are identified. Bile canaliculi partially obliterated (Fig E). Treatment of CCl₄+20mg SiO₂ showing normal hepatocytes away from the central vein, shrunked nuclei, obliterated bile canaliculi in some portion of centrolobular region (Fig. B). Periarterial region showing distinct hepatic cords, normal sinusoids, clear bile canaliculi with normally distributed sinusoidal and Kupffer cells (Fig. F). CCl₄+30mg SiO₂ dose reduced necrotic zone in the centrolobular region (Fig. C) and periarterial region (Fig. G). Treatment of CCl₄+40mg SiO₂ showed normal centrolobular region with distinct hepatic cords, normal hepatocytes with dilated sinusoids and bile canaliculi (Fig. D) and periarterial region (Fig. H).

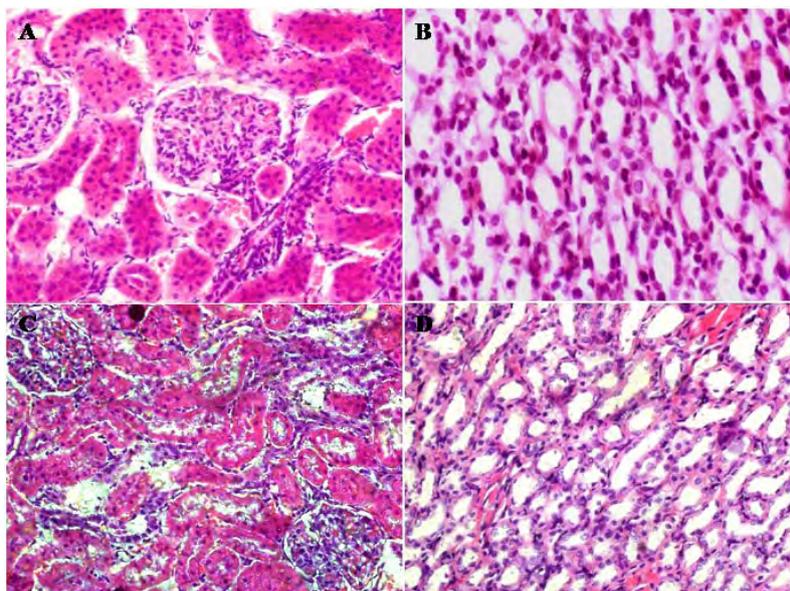


Figure 4. Effect of CCl₄ on cortex and medulla region of normal rat kidney: Cortex showing normal Glomeruli with Bowman's capsule, proximal and distal tubules were with clear lumina (Fig. A). Medulla region observed with normal stroma and interstitial tissue with intensely stained nuclei (Fig. B). CCl₄ administration showed normal architecture of both the cortex and medulla region of kidney with normal Glomeruli and Bowman's capsule. The proximal and distal tubules were observed normal with clear lumina (Fig. C). The stromal tissue and interstitial tissue can be identified (Fig. D).

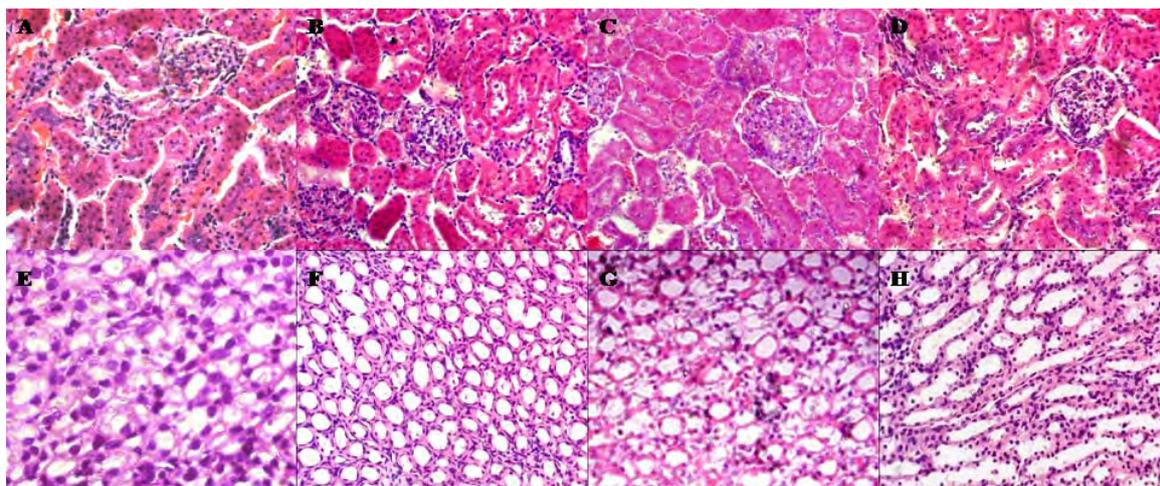


Figure 5. Effect of abhrak bhasma on cortex and medulla region of CCl_4 intoxicated rat kidney: Treatment of CCl_4 +10mg AB showing normal glomerulus and Bowman's capsule in cortex region. Proximal and distal tubules were normal with brush borders of all the proximal tubules (Fig. A). Medulla region with normal stromal tissue and interstitial tissue (Fig. E). Cortex and medulla of kidney appeared normal with 20mg AB+ CCl_4 dose treatment (Fig. B & F). Treatment of CCl_4 +30mg AB/40mg AB showing normal cortex and medulla region exhibiting normal Bowman's capsule with lumina. Proximal and distal tubules were with clear lumina and normal brush border (Fig. C & D). The stromal tissue, interstitial tissue was thin (Fig. G & H).

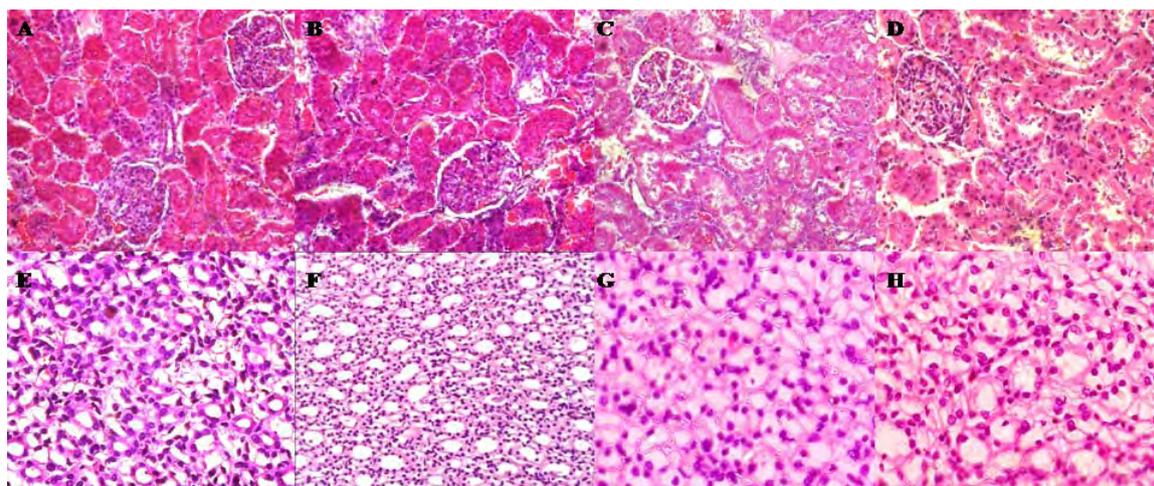


Figure 6. Effect of SiO_2 on cortex and medulla region of CCl_4 intoxicated rat kidney: Treatment of CCl_4 +10mg SiO_2 showing normal glomerulus and Bowman's capsule in cortex region, proximal and distal tubules were normal with clear lamina (Fig. A). Medulla region with normal stromal tissue and interstitial tissue (Fig. E). CCl_4 +20mg SiO_2 showed normal cortex and medulla region (Fig. B & F). Treatment of CCl_4 +30mg/40mg SiO_2 showing normal cortex region with normal Glomerulus, Bowman's capsule with clear lumina (Fig. C & D). The stromal tissue, interstitial tissue was thick (Fig. G & H).