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THERAPEUTIC POTENTIAL OF PARTIALLY PROCESSED *CAMELLIA SINENSIS* (L.) LEAVES TO TREAT DIABETIC HEPATO-RENAL COMPLICATIONS

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Abstract

Hepato-renal toxicity is a common complication of diabetic hyperlipidemia due to disease and its therapeutic drugs. Increased intake of tea (*Camellia sinensis L.*) is associated with a lower risk of hyperlipidemia and its related disorders. Aim of current study was to evaluate hepato-renal therapeutic effects of intraperitoneally induced 25mg/ml/kg b.w. of n-Hexane, ethyl acetate, methanolic and water extracts of partially processed *C. sinensis*(L.)leaves against streptozotocin-induced(55mg/ml/kg b.w.) diabetic hyperlipidemia in albino Wistar rats (both genders, 300- 400 g), by keeping metformin (250 mg/ml/kg b.w.) as positive control and high fed diet as negative control, followed by the estimation of biochemical, hormonal (by kit method) and histological profile of blood, hepatic and renal tissues of animals. Statistically analyzed results (at p≤ 0.05) showed that *C.sinensis*(L.) extracts significantly improved ALT (52.2±4.8 U/L), AST (57.14U/L), ALP (148±25U/L),bilirubin (0.13±0.02 mg/dl), albumin (2.4±0/dl), globulin (1.8±04mg/dl), A/G ratio (0.8±0.6mg/dl), proteins (4.9±0mg/dl), urea (41.1±1.8mg/dl), creatinine (1.0±0.1mg/dl) and uric acid (2.16±16mg/dl), as compared to negative control group[ALT (85±5.8U/L), AST (162.1±10.1U/L), ALP (150.3±41U/L), urea (66mg/dl), creatinine (1.2±0.2mg/dl) and uric acid(5.1±4mg/dl)].Effects of plant extracts were almost similar to positive control group as their ALT (81 U/L), AST (183±3U/L), ALP (338U/L), urea (56.2±25mg/dl), creatinine (0.81mg/dl) and uric acid (4.42±9mg/dl) were nearly equal. Histopathological analysis showed recovery of the glomerular structure of the renal and hepatic tissues, along with a reduction in mononuclear cell infiltrate and improvement in steatosis changes. Current results can be used for the isolation of active compounds behind the study through *in silico*, *in vivo* and *in vitro* approaches.

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Keywords: *Camellia Sinensis* (L.), Diabetic Hyperlipidemia, Hepato-Renal Protective

INTRODUCTION

Medicinal plants have been used for a long time for various diseases (Jamshidi-Kia et al.2018). There are many reasons for increasing the use of medicinal plants because herbal formulations are very efficient and have very little or no side effects (Rabiei et al.2016). World health organization (WHO) reported different medicinal plants for treatment based on their low cost, efficacy, and little or no side effects (Viktorinova et al.2016).

Hyperlipidemia is one of the most common non-communicable diseases which is present globally and the most lethal complication developed in a few years is a cardiovascular event that is hard to control and it's not possible to revert its effects. Both diseases are present in Pakistan and drastically affect the hepatic and nephric systems and their prevalence is very high all over the world. Treatment modalities that are already present cause derangement of biochemical markers of the liver and kidney and drugs have many side effects and huge monetary expenditure. Polyphenols can lower the risk of these diseases. There is a paucity of information in the literature on the effect of green tea extracts on the hepatorenal systems (Mardeen et al.2018).Cardiovascular diseases affect the people of both develop and developing countries and it's due to abnormalities in carbohydrate and lipid metabolism which is due to low insulin level and ultimately cause hyperglycemia, a change in lipid metabolism. Defects in carbohydrate metabolism cause the efforts of the physiological system of the body to maintain glucose and electrolyte imbalance and it causes overexertion in the endocrinal system and ultimately causes hyperglycemia (Rahimi-Madisehet al.2017). There are almost more than 150 million people with diabetic hyperlipidemia around the world which seems to reach almost 300 million by 2025 (Mardeenet al.2018).

Since old-time plants have been the source of medicine and used for various diseases. WHO reported different medicinal plants for treatment based on their low cost, efficacy, and little or no side effects (Islam et al. 2019). Presence of quelene in aqueous extracts of *C. sinensis.*(L.) leaves showed improvement in kidney and liver biomarkers after carbon tetra chloride injury in rats (Caglaret al. 2019).

Liver is the main metabolic organ that plays important role in human body due to its fundamental role while hepatic disorders remain the major health problem in the world therefore discovery of less toxic and most effective treatment for liver diseases gaining more intention and for that many natural products have been used for their strong hepatoprotective role, out of which*C. sinensis.* (L.) is the major one (Suhayla et al., 2022). People suffering from diabetes mellitus are at high risk of complications and most lethal is nephropathy because it leads to end stage renal failure and this developed due to increased production of reactive oxygen species and imbalance between oxidant and anti-oxidant levels. Uncontrolled hyperglycemia because development of microvascular

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disorders that includes changes in endothelial renal cells, smooth muscle cells, mesangial cells, podocytes, tubular and ductal cells, and myofibroblasts (Maezawa et al. 2015).

Liver is also affected by diabetes mellitus and lead to the development of non-alcoholic fatty changes that starts with simple steatosis and ultimately cause liver fibrosis (Lucchesi et al. 2013). The main component of *C. sinensis.*L is catechins that has strong antioxidant activity and it activates many antioxidant enzymes and remove free radicals from the body (Prasanth et al. 2019). It also has hepatoprotective activity. Many studies showed that regular intake of *C. sinensis* for several weeks has a potent hypoglycaemic effect, and may eliminate or reduce the risk of diabetic complications (Moodley et al. 2015, Betonico et al. 2016, Hirata et al., 2017).

It is important to investigate alternative therapy that cures diabetic hepato renal complications. This alternative therapy may include use of *C. sinensis.*(L.) extracts as it contains alkaloids, theaflavins, flavonols and catechins, which plays important role in controlling diabetic hepato renal complications. So objective of current study was to demonstrate the potential protective effect of *C. sinensis*(L.) by using n-hexane, ethyl acetate, methanolic and distilled water extracts to treatdiabetic hyperlipidemia.

MATERIAL AND METHODS

Collection, identification and preparation of plant

Partially processed leaves of *C. sinensis* (L.) has been collected from National Tea Research Institute, Shinkiari, and Khyber Pakhtunkhwa, Pakistan, identified by a renowned taxonomist and deposited at university herbarium with botanical number.

Leaves were shade dried at room temperature and grinded into 80 mesh powder by mechanical means, followed by the addition of n-hexane (in 1:10 ratio) with shaking for 24 hours in shaker incubator (K-J-201BD), centrifugation for 15 minutes at 5000 rpm (SIGMA 203,43191) and filtration through What man filter paper 1.0. Filtrate had been shade dried at room temperature while next solvent (ethyl acetate, methanol and dist. water respectively) has been added in residue with repetition of previous procedure. Dried filtrate has been re- dissolved in 15 % DMSO to prepare stock solution (100 mg/ ml) (Asma et al.2016).

Animal grouping

Healthy and disease free albino Wistar rats(300- 400 g)were divided into eight groups (Table 1) (each having half male and half female) in animal house of IMBB, The University of Lahore in standard lab conditions (at 20-26 ^{0}C/ 68-78.8 ^{0}F, 30%–70% humidity)and fed with standard diet (mixture of 23 % crude protein,3.0% crude fat, 7.0% crude fiber, 8% acid insoluble ash, 1-2.5 % calcium, 0.9% Phosphorus, 0.5-1% sodium and 12% moisture) has been given during the period of whole experiment.

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Table 1: Animal groups

Animal groups		Treatments
Control groups	Vehicle	Only normal saline
	Negative/ High fed diet treated	High fed diet treated
	Negative/ Hyperlipidemic	Only 55mg/Kg/b.w. STZ induced
	Positive/ Hypolipidemic	250 mg/Kg/b.w. metformin induced*
Experimental groups (Treated with *C. sinensis* L. leaves extracts @25mg/ml/Kg b.w.).	n-HE	n-hexane extract treated*
	EAE	Ethyl acetate extract treated*
	ME	Methanolic extract treated*
	Dwe	Distil. water extract treated*

* Drug and extracts has been induced after the induction of diabetic hyperlipidemia through 55 mg/Kg/b.w. STZ

Induction of diabetic hyperlipidemia, plant extracts and Hypolipidemic drug

Before the induction of diabetic hyperlipidemia and to check which animal can become diabetic rapidly, OGTT was performed prior to the induction of STZ in 8-10 hour fasted rats, by the administration of 10% glucose solution, followed by the measurement of blood glucose level after 0, 30, 60, 90, and 120 minutes and those animals were considered to develop diabetes whose blood glucose level was more than 200mg/dl (Asma et al., 2016).

Rats were made hyperlipidemic by intraperitoneal induction of 55mg/Kg body weight streptozotocin (STZ), followed by the oral administration of 10%glucose solution to prevent severe hypoglycemic effect of STZ(Brian et al., 2021).

25mg/ml/Kg b.w. of n-hexane, ethyl acetate, methanolic and distilled water extract of *C. sinensis*L. Leaves (in experimental groups) and 250 mg/ml/kg b.w. metformin (in positive control group) had been injected intraperitoneally in rats after STZ induction (Zaabi et al., 2021)

Effect of drugs and plant extracts on hormonal, biochemical and histological profile

Rats were anesthetized by giving inhalant anesthesia (chloroform, halogenated ether and placed in closed container for 2 to 3 minutes). After sedation, rats were removed from container, placed on a slab (pre-cleaned with spirit, to avoid from any contamination of skin) and made a small cut in the middle of abdomen to the snout anteriorly and the genital opening posteriorly by making transverse incisions along the length of the limbs. Blood sample were taken in EDTA and non-EDTA blood vacutainers and centrifuged at 2000 rpm for 5 minutes to separate the serum and blood cells for the estimation of urea, creatinine, bilirubin, uric acid, albumin, globulin total protein, A/G ratio, alkaline aminotransferase (ALT), aspartate transferase(AST), Alkaline phosphatase (ALP) by using kit methods. Urea, creatinine and uric acid level has been measured by standard method *(*Bamanikar et al., 2016) Bilirubin level has been examined by using kit method *(*Jendrassik and Grof, 1938) AST, ALT level has been estimated the by using kit method

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(Pezeshki et al., 2016), ALP level has been measured by the kit method (Sundaramet al., 2013) and Albumin, globulin and A/G ration was measured by the kit method (Yokozawa et al., 2005).

Liver and kidney were preserved in 10% neutral formalin for the histopathological analysis of hepatic and renal tissues at 400 um (Alkiyumi et al., 2012).

Statistical analysis

The data was analyzed by using Two-Way ANOVA by considering $P<0.05$ level of significance through Garph Pad prism 8.0 while results has been expressed as± SEM.

RESULTS

Plant has been identified by the botanical number of GC.Herb.Bot.3779

Content of different metabolites in animals

Statistically observed results showed that urea content in both male and females of the negative control group (193.6±6 and 195.6±14mg/dl respectively), high fed diet group (67.6±4 and female 40.3±3mg/dl) and positive control group (34±1 and 32.6±1mg/dl respectively) has been increased as compared to vehicle (46.3±1and 45.3±4 mg/dl respectively), while it decreased in animals treated with n-hexane(43.6±5 and 36±2mg/dl respectively), methanolic (45.3±3 and 33.6±3mg/dl respectively), ethyl acetate (68±0 and 41.6±0.06 mg/dl) and distilled water (48.6±4 and 34±0mg/dl respectively) extracts of *C. sinensis*(L.) as compared to positive control group (Figure 1a). Creatinine content in males and females of the negative control group has been same (0.7±0.1 mg/dl in both) as compared to vehicles (0.63±0.08 and 0.8±0.2mg/dl), but it decreased as compared to animals in high-fed group (1.5±0.2 and 2.8±0.1 mg/dl). Male rats of the positive control group has less amount of creatinine (0.56±0.06 mg/dl), while females have increased amount (2.8±0.1 and 0.7±0.1mg/dl) as compared to vehicle rats. Creatinine levels of both male and female rats treated with n-hexane (0.7±0.1mg/dl and 2±0mg/dl respectively), methanolic (1.9±0.06and 0.8±0.2mg/dl respectively) and distilled water extract (0.63±0.1 and females 0.8±0.2mg/dl respectively) extracts of *C. sinensis* (L.) leaves has been decreased as compared to the negative control group (Figure 1, b).

Urea content was least in both males and females of vehicle (1.6±0.1 and 1.8±0.1mg/dl respectively) as compared to animals in negative control group (3.03±0 and 3±0mg/dl respectively) and high fed diet group (2.16±0.1 and 2.83±0.16 mg/dl respectively), while it was almost similar to animals in positive control group (1.5±0.26 and 1.33±0.16mg/dl respectively) and n-hexane extract treated group (1.7±0.1 and 1.6±0.4mg/dl respectively). Least value of uric acid was observed in male and female rats treated with the methanolic (1.8±0.3 and1±0.05 mg/dl respectively) and distilled water extracts (1.26±0.1 and 1.16±0.1mg/dl) (Figure 1, c).

Statistically analyzed results showed that bilirubin content in male and females of the negative control group (2.3±0.1 and 2.6±0.2mg/dl respectively) has been increased as

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compared to the vehicle (0.2±0 and 1.6±0 mg/dl). On the other hand, in males and females of the positive control group, it has been increased (1.6±0 and 1.1±0 mg/dl respectively) and decreased in animals treated with high-fed diet (1.83±0.1 and 2.2±0.05mg/dl) as compared to vehicle. Content of bilirubin in both males and females treated with n-hexane (0.13±0.02 mg/dl and 0.13±0.03 mg/dl respectively), methanolic (1.33±0.1 and 1.7±1mg/dl respectively), ethyl acetate (0.13±0.03 and 0.36±0.1mg/dl respectively) and distilled water (0.16±0.03 and 0.53±0.2 mg/dl respectively) extract of *C. sinensis* (L.) leaves has been decreased more prominently as compared to a positive control group (Figure 1, d). Albumin content was same in both males and females of high fed diet treated group (2.66±0.3 and 3.76±0.1 respectively), hyperlipidemic (2.9±0.5 and 3.36±4 g/dl respectively) and hypolipidemic (2.6±0.3 and 3.7±0.2g/dl respectively) control groups and it was also same in animals treated with n-Hexane (2.6±0.1 and 3.06±0.08 g/dl respectively), ethyl acetate (2.3±0.2 and 2.4±0 g/dl respectively), methanolic (2.6±0.2 and 2.7±0.1g/dl respectively) and distilled water (2.4±0.1 and 2.8±0.2g/dl respectively) extracts of *C. sinensis* (L.) leaves (Figure 1, h).

Statistical analysis of globulin content showed that it has been raised slightly in both males and females rats treated with high fed diet (2.6±0.1 and 1.8±0.1 g/dl respectively), methanolic (3.3±0 and 2.3±0.1 g/dl respectively) and distilled water (3.2±0.1 and2.7±0.05 g/dl respectively) extracts of *C. sinensis*(L.) leaves and decreased in rats treated with n-Hexane (2.8±0.06 and 2.7±0.06 g/dl respectively) and ethyl acetate (2.4±0.1 and 2.3±0.2 g/dl respectively) extracts of *C. sinensis* (L.) leaves and these results were significantly similar to that of hyperlipidemic (1.03±0.03 and 2.4±0.2g/dl respectively) and positive (1.8±0.2and1.7±0.1g/dl respectively) control groups as compared to vehicle (2±0.05and2.06±0.2g/dl respectively)(Figure 1, i)

After statistical analysis of total protein content, results showed that protein level of males and females in negative control (6.56±7and 6.46±.2mg/dl respectively) has been significantly increased as compared to vehicle (6±0 and5.16±0 mg/dl respectively) and it slightly elevated in rats treated with high fed diet (6.16±0.4 and5.23±0.1 mg/dl respectively) and in positive control group (6.56±7and 6.46±.2 mg/dl respectively). But it has been decreased significantly in rats treated with n-Hexane (5.53±0.3 and 3.76±0.1mg/dl respectively), ethyl acetate (4.33±0.3 and 5.83±2mg/dl respectively), methanolic (5.93±0.3 and 5.96±0.6mg/dl respectively) and Distilled water (5.63±0.2 and 5.1±0.1mg/dl respectively) extracts of *C. sinensis* (L.) (Figure 1, j).

Statistically analyzed results of A/G ratio showed A/G ratio of males and females treated with high fed diet (1.23±0.03 and 1.33±0.03 % respectively) has been increased as compared to vehicle (1.26±0.1 and 1.4±0.2 % respectively), and slightly decreased in hyperlipidemic (1.1±0.1 and 1.1±0.08 % respectively) and positive control(1.1±0.5 and1.1±0.09 % respectively) groups, while it has been decreased significantly in rats treated with n-Hexane(1.1±3 and 1.03±0.03 % respectively), ethyl acetate (1.02±0.01and1.06±0.02 % respectively), methanolic (1.02±0.01and1.06±0.02 %

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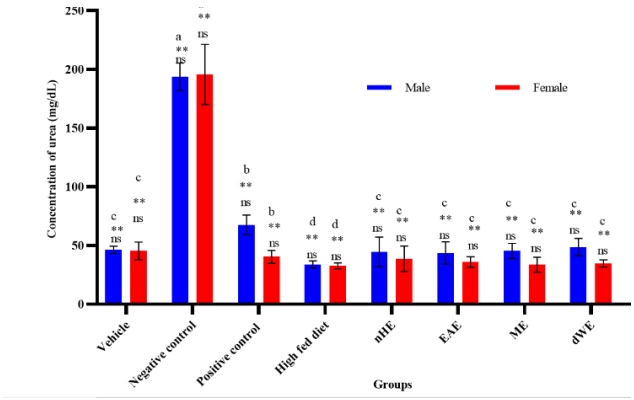
respectively) and distilled water (1.04±0.02 and 1.23±0.6 % respectively) extracts of *C. sinensis*(L.) leaves has as compared to the negative control group(Figure 1, k).

Concentration of enzymes in animals

Statistically observed results showed that AST level in both males and females of high fed diet treated group (63±1 and 73±3 U/L respectively), hyperlipidemic control (72.3±2 and77±2 U/L respectively) and anti-hyperlipidemic control (88.4±1 and 94±2 U/L respectively) has been increased as compared to vehicle (40±0.5 and 43±2 U/L respectively), while its levels in male and females rats treated with n-Hexane (41.3±1 and 33.3±7U/L respectively), ethyl acetate (32.6±1 and 40±0.5U/L respectively), methanolic (28.6±4 and 27±3U/L respectively) and distilled water (44±2 and 35.5±4U/L respectively)extracts of *C. sinensis*(L.) leaves has been decreased as compared to positive control group(Figure 1, e)

ALT content in both males and females animals of high fed diet treated group (63±1 and 73±3 U/L respectively), hyperlipidemic (72.3±2 and 77±2 U/L respectively) and positive control (88.4±1 and 94±2U/L respectively) has been increased as compared to vehicle (40±0.5 and 43±2U/L respectively), while it has been decreased in male and females rats treated with n-Hexane (41.3±1 and 33.3±7U/L respectively), ethyl acetate (32.6±1 and 40±0.5 U/L respectively), methanolic (28.6±4 and 27±3U/L respectively) and distilled water (44±2 and 35.5±4 U/L respectively) extracts of *C. sinensis*(L.) leaves as compared to positive control group(Figure 1, f).

Statistically analyzed results of ALP content showed that in both males and females of rats treated with high fed diet (108.3±1 and 122.3±2U/L respectively) has been decreased as compared to vehicles (111±5 and 128±4U/L respectively) and but still it was more than the animals in negative (95±3 and89±1 U/L respectively) and positive control (95.4±2 and104±3 U/L respectively) groups. n-Hexane extract of *C. sinensis* (L.) leaves has decreased ALP content (98±8 and127±3 U/L respectively) as compared to the negative control group, while rats treated with ethyl acetate (147.3± 4 and118±4 U/L respectively), methanolic (128.6±4 and 27±3 U/L respectively) and distilled water (106.6±4 and119±1 U/L respectively) has elevated levels of ALP as compared to vehicle (Figure 1, g).



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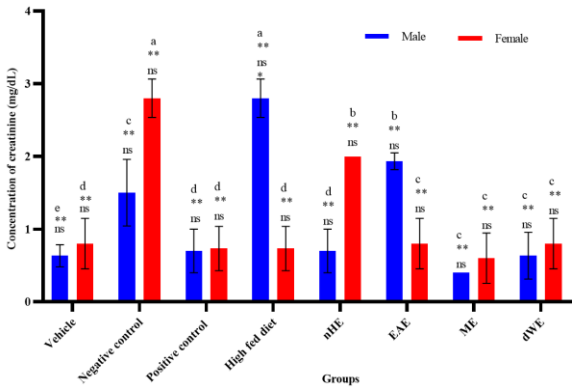

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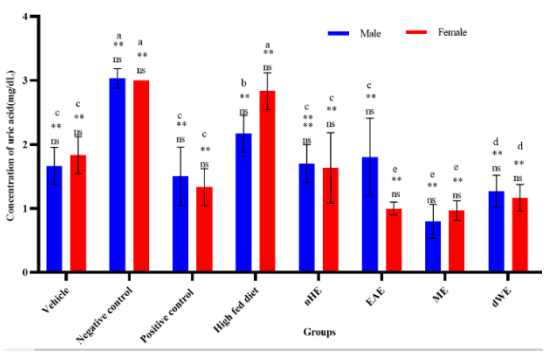

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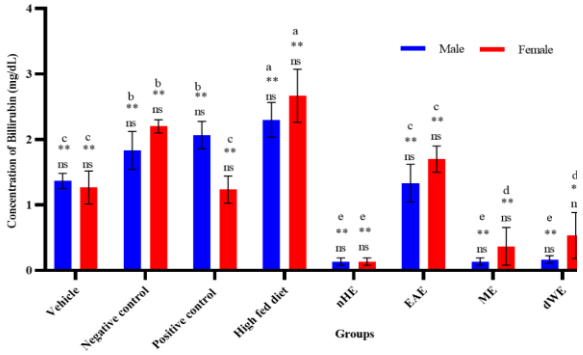

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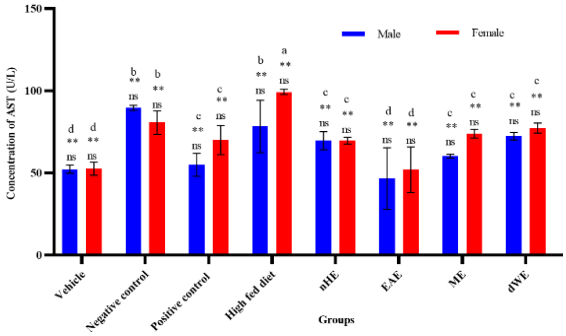

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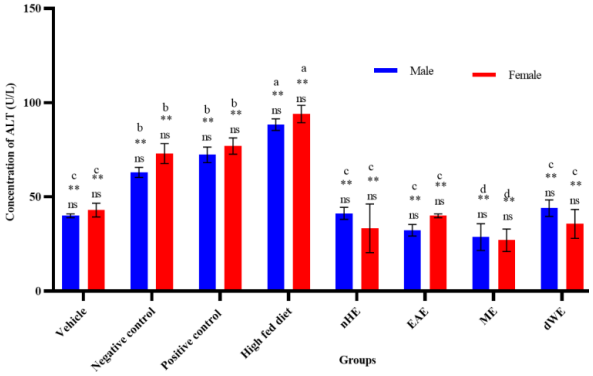

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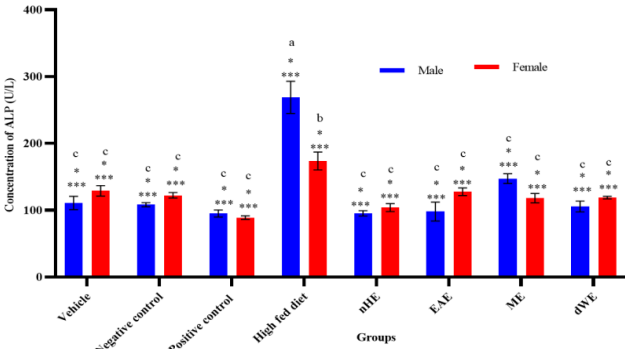

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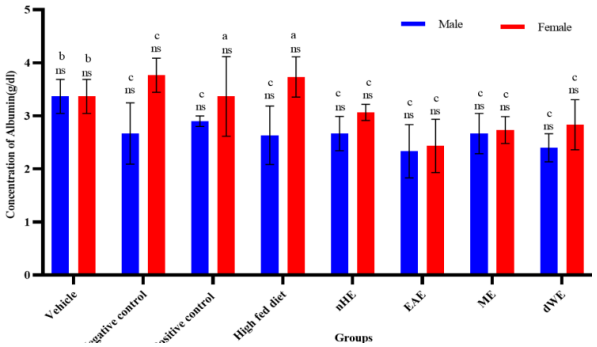

(h)

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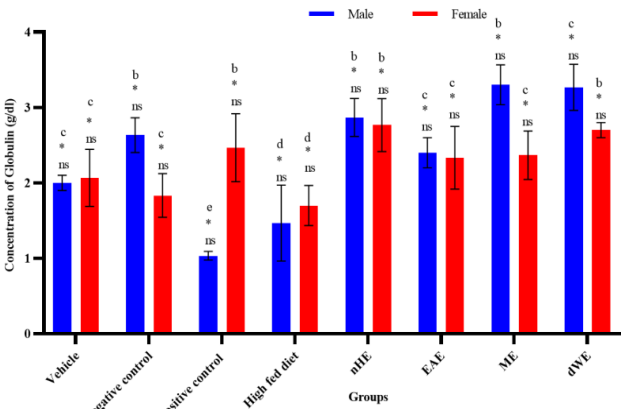

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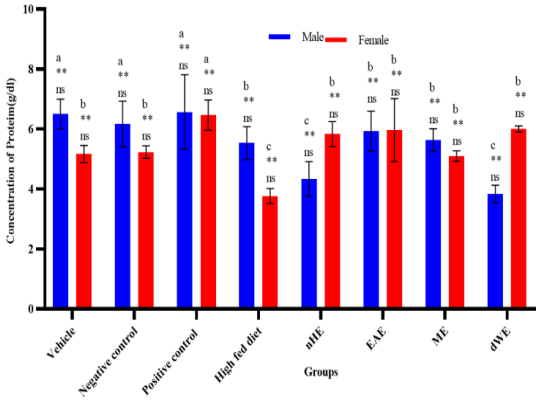

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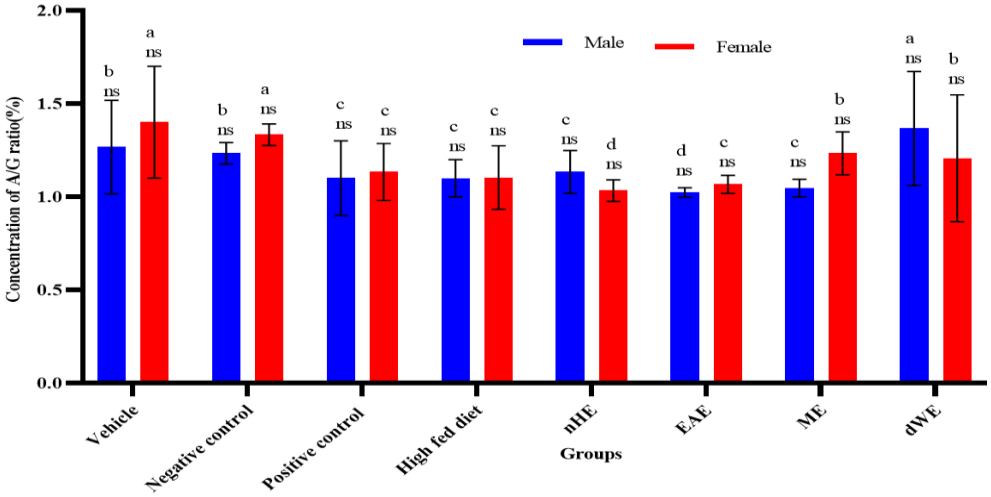

(k)

Figure 1: Concentration of urea (a), creatinine (b),uric acid (c), bilirubin (d), AST (e), ALT (f), ALP (g), albumin (h), globulin (i), total protein (j) and A/G ratio (k) in albino Wistar rats

HFD= high fed diet, nHE=-n-Hexane extract, EAE= ethyl acetate extract, ME= Methanolic extract, dWE= distilled water extract. a-e =Comparison of animal groups from most significant results to less significant. Highly significant ***= P- 0.0001, Most significant**= P<0.01(0.0010-0.0092), Significant*= P<0.05(0.0392-0.0471), ns=non-significant

Histopathological profile of renal and hepatic tissues

In a vehicle control group, histological features of renal tissue showed normal glomerular structure, and structure of tubules, while vascular hemorrhage, congestion, hyperplasia,

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and edema were unremarkable. Renal tissues in high fed diet group showed mild dilation in blood vessels and increased space in Bowman capsule. Mononuclear cells infiltrate was present, while increased glomerular sclerosis was seen in very few segments, along with increased connective tissue. Hyperlipidemic control group showed abnormal kidney structure in the form of mesangial cell expansion with dilated glomerular blood capillaries, while main pathology was tubular regeneration. Positive/hypolipidemic group showed recovery of pathological changes in a few segments of renal tissues. n-hexane extract treated group showed intact architecture of renal tissues in all segments. Congestion and severe chronic inflammation was present in all segments, while Glomeruli were unremarkable, and necrosis was also present. No RBCs and no sign of chronic pyelonephritis in any segments has been observed. Ethyl acetate extract treated group showed intact architecture of renal tissues, with congestion and chronic inflammation in all renal segments. Glomeruli were unremarkable and necrosis, RBCs casts and sign of chronic pyelonephritis were absent. Methanolic extract treated kidney cells were found with intact architecture, with the presence of congestion and chronic inflammation in all renal segments. Glomeruli were unremarkable, while necrosis was absent. RBCs casts and sign of chronic pyelonephritis were also are present. In rats treated with distilled water extract showed presence of congestion and chronic inflammation, with unremarkable glomeruli and necrosis, while RBCs casts and signs of chronic pyelonephritis were present (Figure 2 A).

In vehicle, histological features of hepatic tissue showed normal lobular structure, while fatty changes, sinusoidal dilation, chronic venous congestion, and fragments of lobules were maintained and were unremarkable. Hepatic tissues of high fed diet treated group showed appearance of typical liver steatosis with lipid droplets, while in negative/hyperlipidemic control group, abnormal interruption in the radiating pattern of hepatocytes cords has been observed. Hepatocytes showed degeneration with loss of architecture and cytoplasmic vacuolation, while nuclei were heterogeneous in shape and size along with blood vessel hemorrhages in multiple hepatic segments. Hepatic tissue of positive/ hypolipidemic group showed interruption in radiating pattern of the hepatocytes cord, but with reduced cytoplasmic vacuolation and fatty changes as compared to only STZ treated group, which shows mild recovery in pathological aspects, with no vascular hemorrhages. n- hexane extract treated hepatic tissues showed intact architecture of liver cells and moderate venous congestion and chronic inflammation. Necrosis was absent while central vein dilation, hemorrhage, and microvascular steatosis were present. Ethyl acetate extract treated hepatic tissue showed intact architecture of liver cells, moderate venous congestion, while chronic inflammation, necrosis, central vein dilation, hemorrhage, and microvascular steatosis were not seen. Methanolic extract treated hepatic tissue showed intact architecture of hepatic tissues, with the presence of central vein dilation, severe hemorrhage, and microvascular steatosis. Distilled water extract treated hepatic tissue showed partly intact architecture of hepatic tissues, with moderate venous congestion, mild chronic inflammation and necrosis, presence of central vein dilation and hemorrhage, while microvascular steatosis was absent (Figure 2 B).

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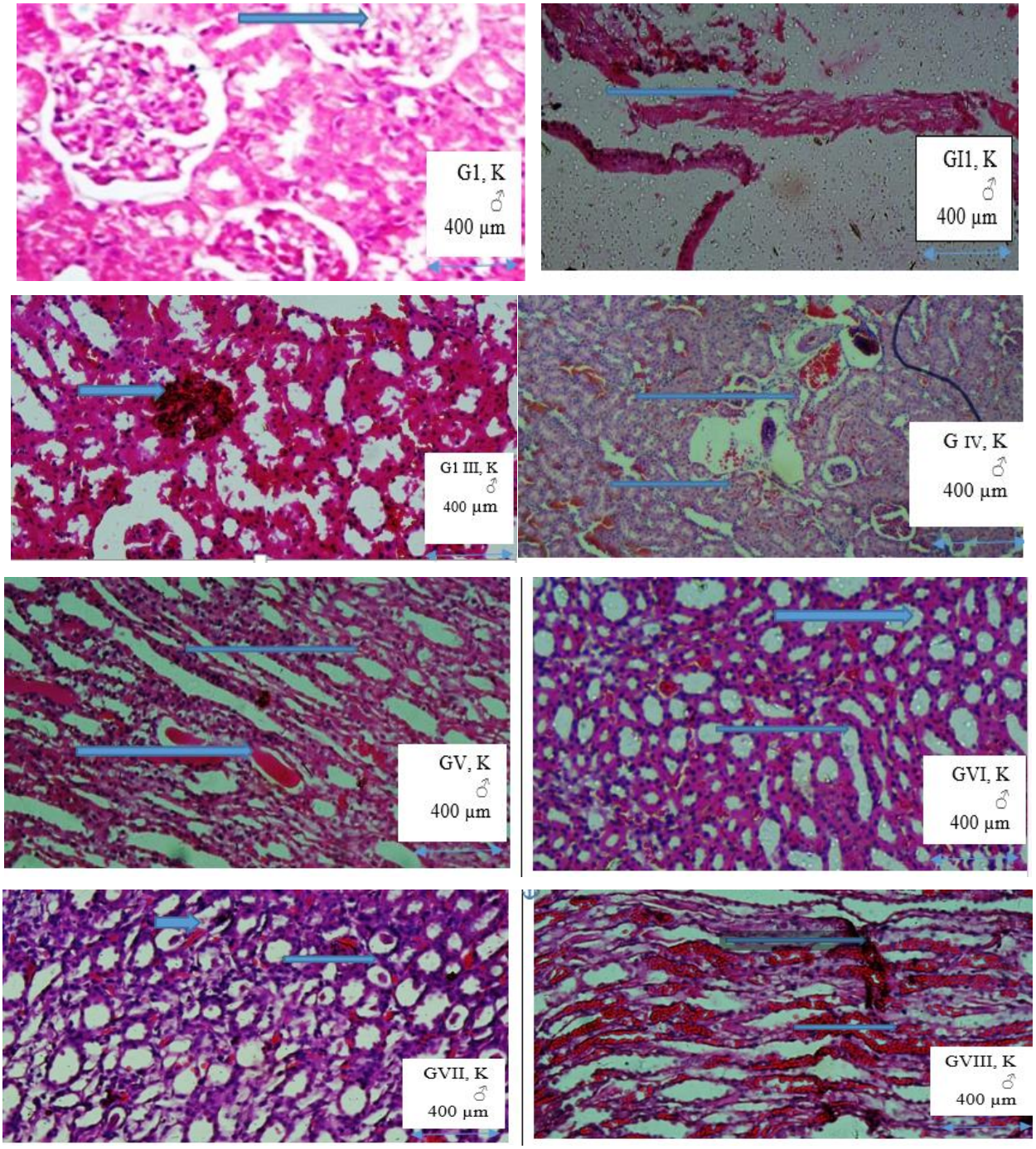

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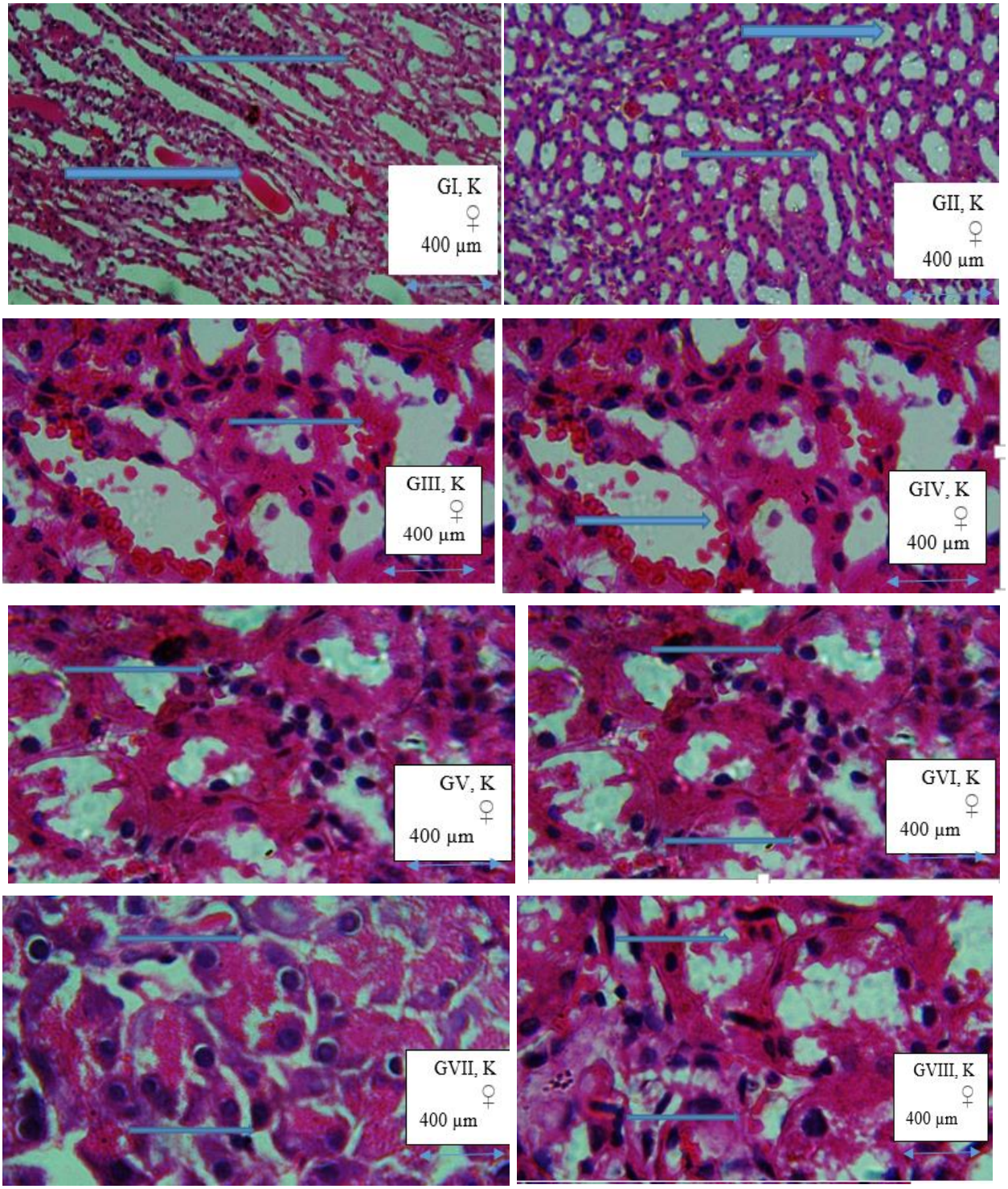

(A)

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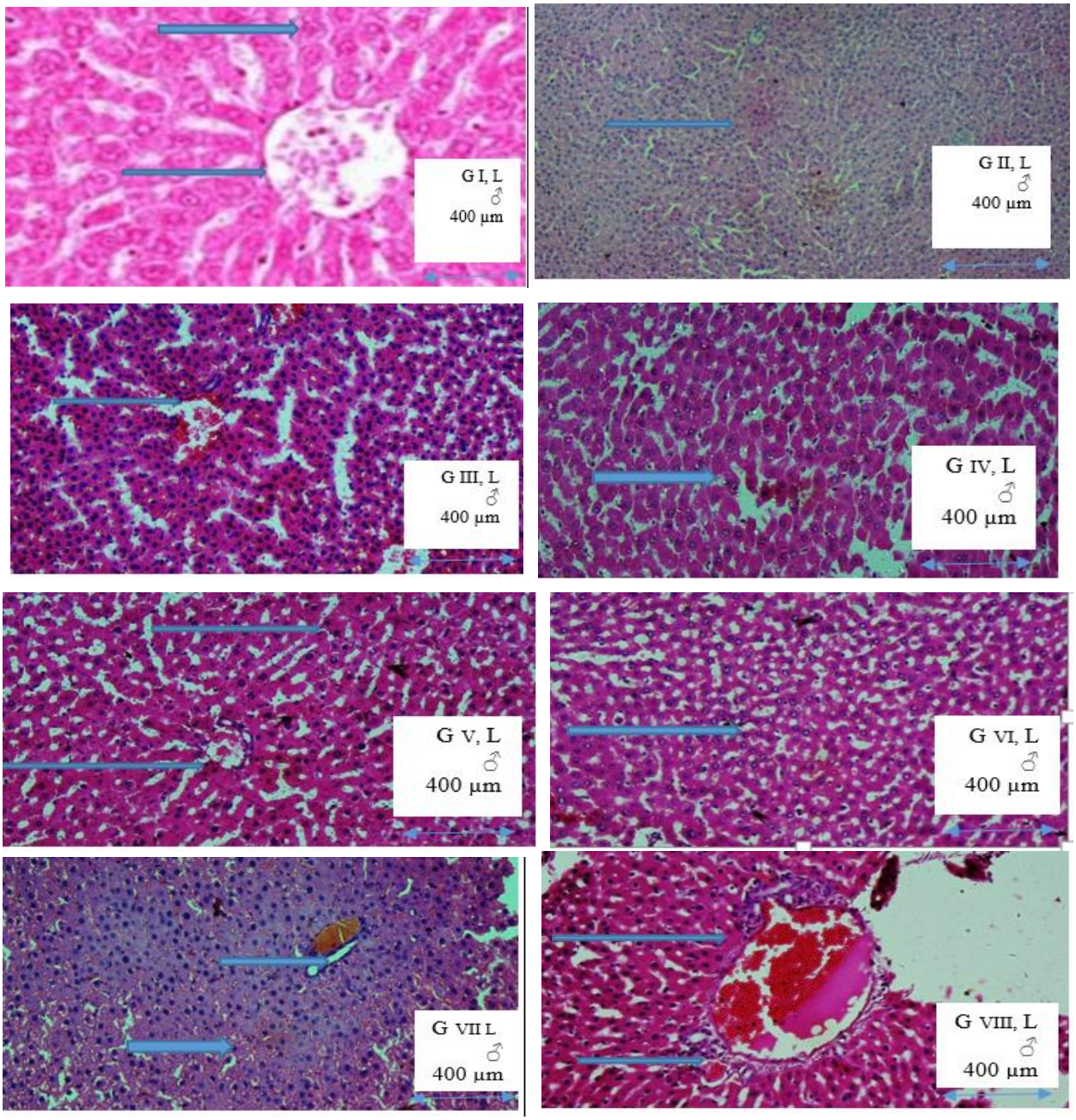

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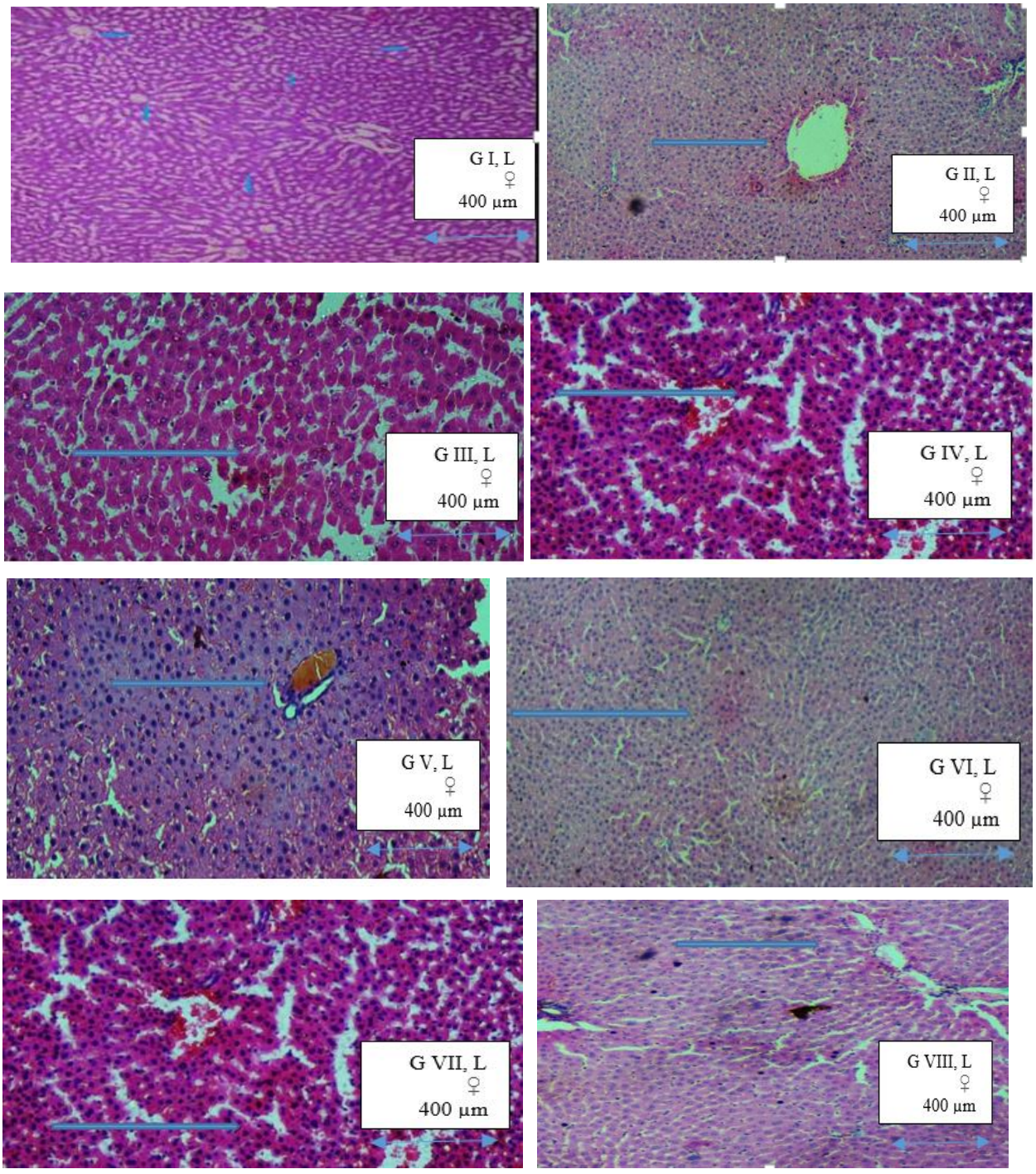

(B)

Figure 2: Histological Features Kidney/ Renal (K) and Liver/ Hepatic (L) Tissues of at400 µm

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G I= Vehicle, G II= High fed diet group, G III= Negative control group, G IV= Positive control group, G V= Rats treated with n-hexane extract of *C. sinensis* (L.) leaves, G VI= Methanolic extract of *C. sinensis* (L.) leaves, G VII= Ethyl acetate extract of *C. sinensis* (L.) leaves, G VIII= Distilled water extract of *C. sinensis* (L.) leaves. ♂ = Male, ♀= Female

DISCUSSION

Liver and kidney diseases are most common diseases in world. Current available medications are not organo-protective as compared to natural products. *C. sinensis.*(L.) is most commonly used drink as green or black tea and have many health-preserving properties. Extracts of *C. sinensis.*(L.) have been used as an herbal medication in the treatment of many diseases. Safety and tolerability of long-term use of *C. sinensis.*(L.) extracts have been well defined. Hepato and nephro toxicity due to diabetic hyperlipidemiais considered a good model for the study of effects of synthetic and natural drugs on liver and kidney as streptozotocin (STZ) causes destruction of liver and kidney cells (Rezagholizadehet al.2016).

Nephropathy developed by long-term hyperglycemia, while liver failure develops by long-term complications of hyperlipidemia and other metabolic disorders of the liver.STZ induced toxicity of organ lead to many complications over a period of time such as hepatopathy, nephropathy and hyperglycemia, while protective effects of *C. sinensis*(L.) extracts in STZ-induced rats on the liver and kidney are due to its anti-oxidant and anti-inflammatory properties by affecting the genes expressions of CAT, GPX1, MT-I, MT-II, SOD-I, SOD-II, and SOD-III genes (Al-Awaida et al,2019). Moreover polarity based*C. sinensis*(L.) extracts reduced the levels of serum creatinine and ALT, without affecting other parameters due to MDA(Malondialdehyde), SOD(superoxide dimutase, and GSH(glutathione hydrogenase)(Opuwari ET AL. 2020).*C. sinensis* (L.) leaves contain anti-oxidant and anti-inflammatory properties and it by affecting the genes expressions of CAT(catalase), GPX1(glutathione peroxidase 1), MT-I and II (mitochondrial encoded gene 1 and II) (Ziamajidi et al. 2017).Therefore *C. sinensis*leaves is probably an effective agent for treatment of liver and kidney diseases, which are attributed to phytocompounds identified in *C. sinensis.*(L.) (Figure 3).

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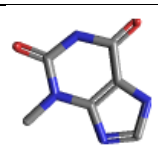
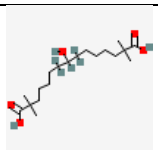
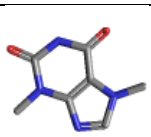
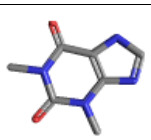
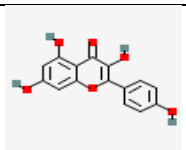
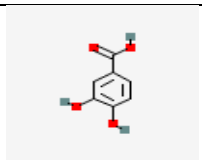
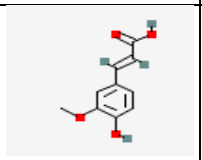
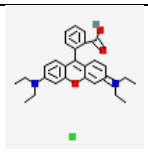
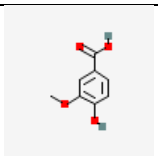
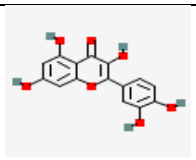
Compounds					
IUPAC names	***Methylxanthines***	***Bempedoic acid***	***Theobromine***	***Theophylline***	***Kampherol***
Molecular formula	$C_{19}H_{36}O_5$	$C_7H_6O_4$	$C_7H_8N_4O_2$	$C_7H_8N_4O_2$	$C_{15}H_{10}O_6$
Molecular Weight	*152*	349.51	*180.16*	*180.17*	*286*
Pubchem ID	*70639*	150503445	*5429*	*2153*	*5280863*
Compounds					
IUPAC names	***Protocatechuic acid***	***Ferulic acid***	***Diabasic acid***	***Vanillic acid***	***Quercetin***
Molecular formula	$C_7H_6O_4$	$C_{10}H_{10}O_4$	$C_{28}H_{31}ClN_2O_3$	$C_8H_8O_4$	$C_{15}H_{10}O_7$
Molecular Weight	154	*194*	*479*	*168*	*302*
Pubchem ID	72	*445858*	*6694*	*8468*	*5280343*

Figure 3: Phytocompounds of *C.sinensis* (L.) with their physiochemical properties

Therapeutic potential of *C.sinensis*(L.) is attributed to active chemical constituents such as glycosides, terpenoids and steroids, flavonoids, reducing sugars, tannins 3-4% of alkaloids ormethylxanthines (caffeine, theobromine, and theophylline), phenolic acids (gallic acid and chlorogenic acids), characteristic/ essential amino acids (glycine, serine, valine, leucine, threonine, andtheanine), polyphenols (flavanols, flavandiols), flavonoids(quercetin, kaempferol, myricetin), proanthocyanidins (prodelphinidin), polysaccharides, vitamins (B, C, E), minerals and trace elements (calcium, magnesium, manganese, copper, zinc, selenium, potassium)(Rishi et al., 2018). Thea Flavin plays an important role in the expression of pancreatic lipase which is the main enzyme involved in obesity and deranged lipid metabolism by regulating AMPK–FoxO3A– Mn-SOD pathway in 3 T3-L1 adipocytes (Lijun et al.,2016).Phenolic compounds constitute 30% of total dry weight, which includes Epigallocatechin (EGC) (19%), Epicatechin–3–gallate (ECG) (13.6%), Epicatechin (EC) (6.4%) (Waheed et al., 2020) (Figure 4).

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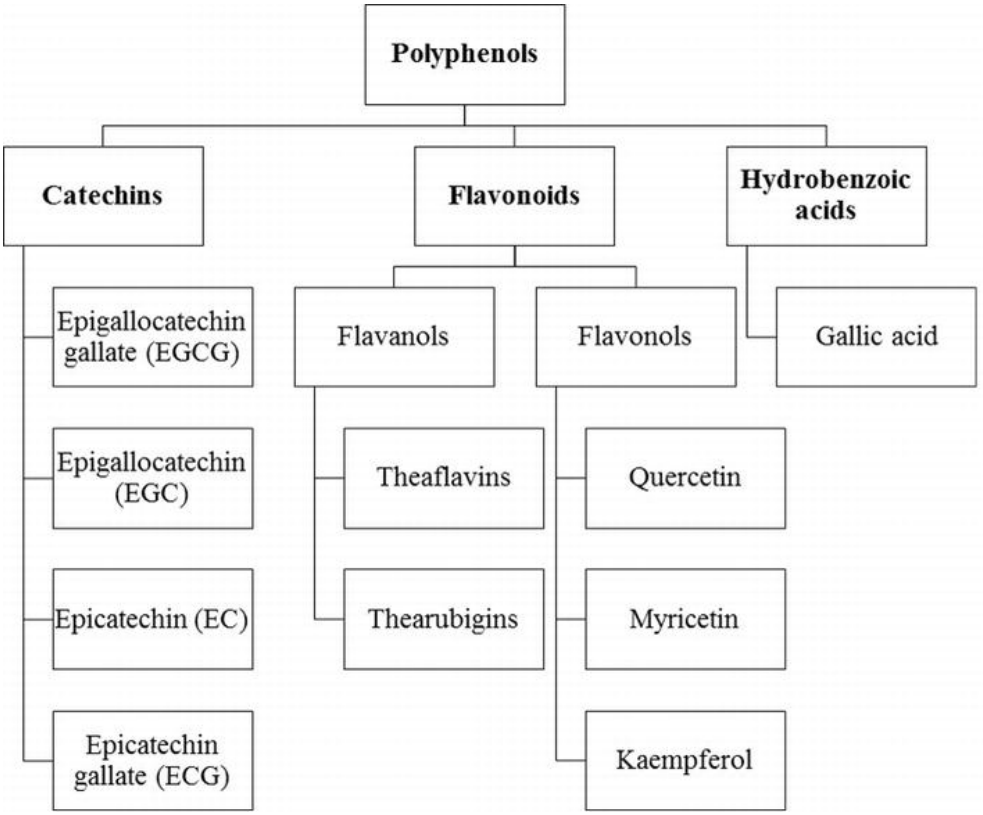

Figure 4: The main hypolipidemic, hypoglycemic hepato and nephro protective components of *Camellia sinensis (L.)* (Ali Imran 2017)

Streptozotocin induces diabetes and has toxic effects on beta cells of pancreas, thus it decreases insulin levels and cause hyperglycemia and chronic hyperglycemia, with impaired function of kidney and liver (Giri et al. 2018). In current study diabetic control rats showed significant effects on urea, uric acid, creatinine, bilirubin, AST, ALT, ALP and globulin and strongly effected alkaline phosphate, but doesn't showed any effects on albumin and A/G ratio and similar results have been reported in previous studies (Chawla et al. 2016, Duwaerts and Maher 2019, Meng et al. 2019).Metformin can decrease glucose production from hepatocytes (Proks et al. 2018) and also stimulates beta cells to bind to the sulfonylurea receptor-1 and block the ATP-sensitive channels that stimulates the release of insulin from pancreatic b-cells (Pandarekandy et al. 2017).Alcoholic green tea extracts significantly improved hepatorenal syndrome in rats, as all biochemical parameters of HRS (hepatorenal syndrome) were controlled except for ACE-1(angiotensin converting enzyme inhibitor-1) and creatinine clearance, which showed significant reduction (Youssef et al 2019).Flavonoids of green tea extracts inhibit glucose absorption by inhibiting glucosidase activity and by stimulating glucose transporter 4 (GLUT4) which stimulate glucose uptake in muscle and cause hypoglycemic effects (Ueda-Wakagi et al. 2019). Epigallo catechingallate (EGCG) effects by inhibiting inflammatory factors and reducing ROS *in vitro* (Pastoriza et al. 2017). Another study

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showed that combined treatment of *C. sinensis*(L.) extracts and metformin had a potent synergitic effects on hepato renal complication of diabetes (Duwaerts and Maher 2019) (Figure 5).

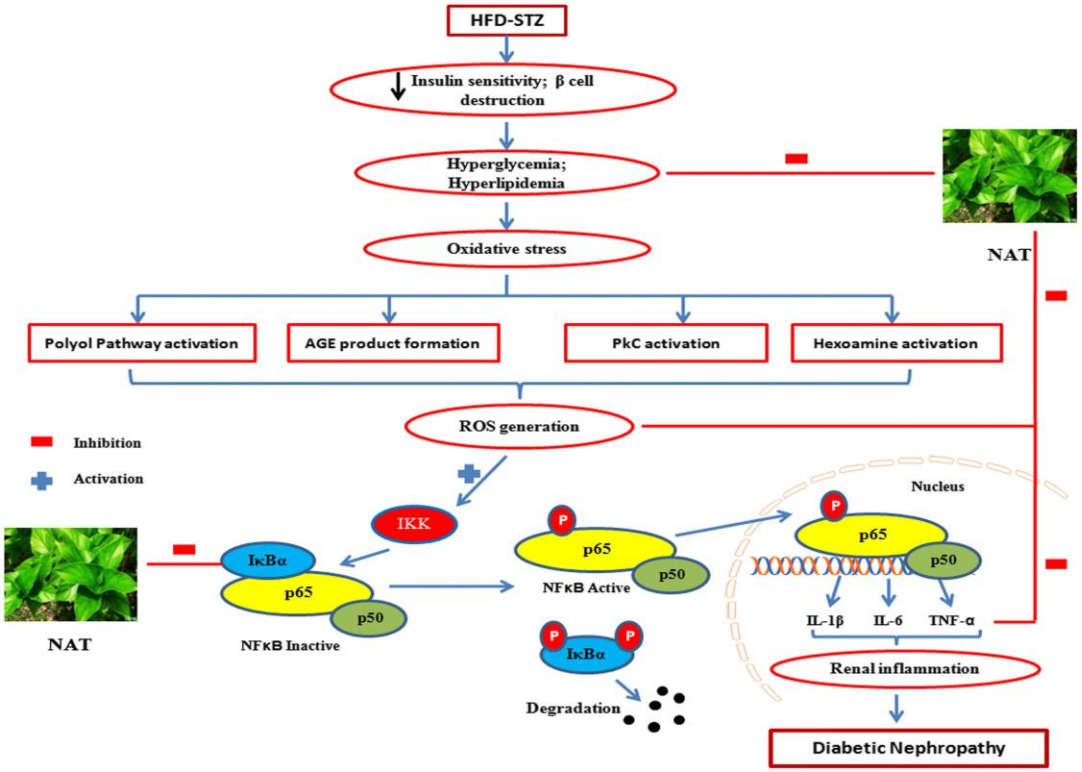

Figure 5: Mechanism of action of STZ and HFD in the development of ROS generation (Mousum *et al.*, 2018)

In current study blood urea and serum creatinine did not show significant improvement with metformin therapy but they decreased significantly after treatment with plant extracts as several hepato-renal pathological changes have been observed in the diabetic control group. These extracts decreased diabetic complications by inhibiting advanced glycation end product (AGE) formation and cutting (Zhu et al. 2014). EGCG and epicatechin gallate in the extracts might be responsible to improve the thickening of the basement membrane by relieving the damage of matrix metalloproteinase, which could degrade extracellular matrix and fibrosis (Sarkar et al. 2016; Yazdi et al. 2019).

In histopathological examination of diabetic control group, interruption in the radiating pattern of hepatocyte cords, fatty degeneration of hepatocytes with heterogeneous nuclei were seen, which are also already reported in the similar manner (Asokan et al. 2019) and these changes incompletely improved in all extracts treated groups, which might be due to the improvement of liver inflammation and decrease in oxidative stress due to levels of malondialdehyde and glutathione (Caro-Ordieres et al. 2020).

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CONCLUSION

Streptozotocin caused organ toxicity of kidney and liver and led them to work abnormally. AST, ALT, ALP serum creatinine, urea and uric acid contents showed that n-Hexane, ethyl acetate, methanolic and distilled watere xtracts of *C. sinensis* (L.) leaves had a very strong organo-protective effects, which might be due to their anti-oxidant properties, thus may lead to improvement in hepato-renalprotection.

Competing Interest

Authors have declared that no competing interest exist.

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