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Antioxidants and Hepatoprotective Studyof a Purified *Bauhinia Variegate* Leaves and Flowers against Carbon Tetrachloride-Induced Toxicity in Experimental Rats

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This study was designed to investigate the hepatoprotective activity and antioxidant enzymes of purified *Bauhinia variegate* leaves extract and purified flowers extract were administered (200 mg/kg, orally once daily) to reduce the effect of carbon tetrachloride-damage in rat'sliver for three weeks. Thereafter, the levels of some serum biochemical factorssuch as alanine aminotransferase, aspartate aminotransferase, alkaline phosphatase,and the activity of three different antioxidant enzymes (glutathione, superoxide dismutase, and catalase) were investigated.Liver homogenate can used to estimated antioxidant parameters: glutathione, superoxide dismutase and catalase. The purified *Bauhinia variegate* leaves and purified flowers significantly ($p<0.05$) inhibited the carbon tetrachloride-induced increase in alanine aminotransferase (149.0± 4.40 and 133.08±6.84) unit/L, aspartate aminotransferase(114±9.28 and 117.93±1.96) unit/L, alkaline phosphatase (3.60±0.28 and 2.43±0.11)unit/100ml, levels at the tested doses, respectively after treatment. However, purified *Bauhinia variegate* leaves and purified flowers treatment noticeablyimproved the activity of antioxidant limitations:glutathione, superoxide dismutase, and catalase. The hepatoprotective activity can confirmed by histological finding.From these results it can be concludedthat the *Bauhinia variegate* leaves and flowers extracts contain remarkable of flavonoid can be used as reducing oxidative stress.

Keywords: *Bauhinia variegate*, HepatoprotectiveActivity, Antioxidant.

Liver is the most important organ in the body. Its role in the regulation of various physiological processes, and its activity is associated with its vigorous functions, such as metabolism, secretion, and storage. Its capacity to detoxify waste products of metabolites and toxic substances as well as for create good compounds. The *Bauhinia variegate* contains health promoting phenolic compounds, proteins, vitamin C and flavonoids, which have been shown good *in vitro*as anti-oxidative properties and anti-inflammatory which are able to scavenge free radicals and protect the liver from carbon tetrachloride (CCL_4)- injury[1].

In recent years, however, several studies have attempted to study the connection between antioxidant and anti-inflammatory mechanisms such as phytochemicals. The phytochemical constructions from plant sources which are able to capablymoderate oxidative and inflammatory stress to prevent diet-related diseases [2,3].

Dealing with natural produces has less their lateral properties than when equated to manufacturer medications[4].

A previous report demonstrated that plants are deliberated as prospective hepatoprotective mediators since they comprise a mixture of diversephy to medicine that are synergistic in their action [5].

Many plant nutrients like,vegetables, fruits, and legumes has abundant poly-phenolic compound and of their beneficial health effects. Mani and co-workers illustrated that nutritional poly-phenolic complexesaid to reinstate the equilibrium between the natural antioxidants and free radicals by improving the activity of regular antioxidant defenses for example, superoxide dismutase (SOD), glutathione peroxidase (GPx), glutathione reductase (GR), and glutathione-*S*-transferase (GST) and by direct scavenging of free radicals [2,6].

Thegenus *Bauhinia* (Fabaceae) comprises three hundred species and famouscow's paw plant, because of the character of their leaves [7]. There are many pharmacological actions have been stated for *Bauhinia* species, comprisinganti-inflammatory, antioxidant, and anti-hyperlipidemic, and hepatoprotective properties. Bodakhe and Ram., reported that alcoholic extract of the stem bark of *B. variegata* indicates hepatoprotective activity against CCl4 induced hepatotoxicity in rats at the dose of 100 and 200 mg/kg. Oral administration of ethanol extract reduce the level of serum alanine aminotransferase (ALT), aspartate amino transferase (AST), alkaline phosphatase(ALP), gamma glutamyl transferase (GGT), total lipids, and raise the level of total proteinthrough the hepatotoxicity [8].

The aims of the study are to carry out biochemical and histological analyses and comparative study of purified *B. variegate* leaves and flowers extractions on CCL_4–induced injury in the liver's experimental rats.

MATERIALSAND METHODS

Extraction and Purification of Flavonoids

Bauhinia leaves and flowers were collected from Garden University of Baghdad in November 2016 and flowers during May 2017. The leaves and flowers were wash and dry then crushed by electric grinder to powder then stored in an air tight container untilused. The extracts of flavonoids compounds from leaves purified *Bauhinia variegate* leaves (PBVL) extract and purified flowers (PBVF)extract were papered according to [9, 10] by using methanol solvent with ratio (7:1) by using soxhlet extractor the filtered solvent dried by rotary at 40C°.

Column Chromatography

A purification of the flavonoids is preceding using open glass column (2.5 x 16) cm was filled with lica gel G60 special for column chromatography. Five milliliters of methanol of leaves extract by flowers of Bauhinia by methanol was exposed to column and eluted with methanol solution,and the rate of flow regulated to be 60 ml / min[11]. The ferric chloride ($FeCL_3$) 1% solution were tested in all fractions as a colorimetric method for polyphenols identification [12].

General Chemical Detection Methods

All extracts of leaves and flowers were tested by lead acetate [13] and flavonoids test by Liebermann reaction and ferric chloride test[11].

Experimental Animals

Rats (n= 25); their average weight (250-300gm) were used in this experiment. Rats were individually housed in plastic cages at controlled rooms in temperature and humidity. The light/dark cycle was 12l/12d- hours. Rats were divided into 5 groups of five rats per group each as following:

Group I: Control rats received normal diet and water[14].

Group II: (CCl_4 group). Rat (n= 5) in this group treated by CCL_4 (3.2 mg/ kg)atthe 1stday and the 8th day.

Group III:(Vitamin C group). Rats(n= 5) have been treated with oral daily ingestion of vitamin C in dose 180 mg/kg for 3 weeks and treated with CCL_4 at 1stday and the 8thday.

Group IV: (Flowers purified group). Rats(n= 5) have been treated with oral daily ingestion of flowers purified extract 200mg/kg for 3 weeks and treated with CCL_4at 1stday and the 8th day.

Group V: (Leaves purified group). Rats (n= 5) have been treated with oral daily ingestion with leaves purified extract 200mg/kg for 3 weeks and treated with CCL_4at 1stday and the 8th day.

Methods of Biochemical Analysis of Blood Samples

Samples Collection

After sacrifice the animals by anesthetic ether, previous to partition either perfused or rinse tissue with a phosphate buffer saline (PBS)

solution, pH 7.4, to eliminate any red blood cells and clots. Homogenize the tissue in 5 ml of cold buffer (50 mM phosphate, pH 6-7, comprising 1mM EDTA) per gram tissue, then centrifuge at 10,000ã g for 15minutes at 4Cº, eradicate the supernatant and store on ice the supernatant will have to be deprotonated before assaying.

Estimation of Serum Glutathione

Glutathione (GSH) was estimated rendering to the method of Jollow *et al*.,[15] using Kit depending on {5,5dithio-bis(2-nitrobenzoic acid)} which reduced by sulfhydryl (SHgroup) to yellow composite.The absorbance of chromogen was identified at 412 nm for serum GSH concentration.

Determination of Serum Catalase

Catalase (CAT) is asignificant cellular antioxidant enzyme that protects against oxidative stress using ready-made kit[16].

Determination of Serum Superoxide Dismutase

Superoxide dismutase was determined using ready-made kit[17].

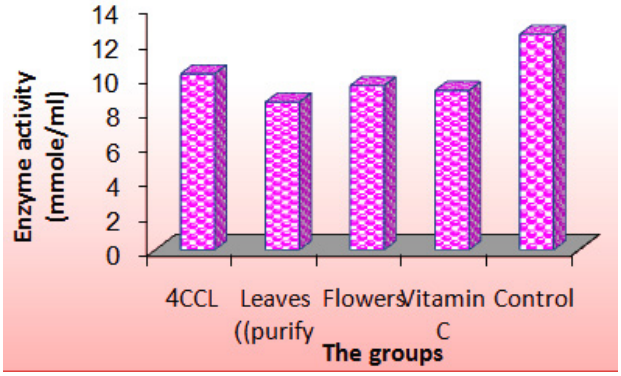


Fig. 1. The effect of difference pure of Bauhinia variegate L. leaves and flowers onCAT (unit/ml)

Table 1. The chemical identification of *Bauhinia variegate* two extracts

Type of Extract	Test	Result
PBVLPBVF	Lead acetate	White jelly residue
PBVLPBVF	Test for Flavonoids	Dark yellow color
PBVLPBVF	Liebermann Reaction	Blue-violet
PBVLPBVF	Ferric chloride	Green color

Table 2. Effect of leaves purified and flowers purified of *Bauhinia vareigata L.* on CAT, GSH, and SOD activities in different groups

Groups	Mean ± SD		
	CAT (unit/ml)	GSH (unit/ml)	SOD (unit/ml)
Control	5.60 ± 0.43 b	7.31 ± 0.33 b	1.44 ± 0.11 b
CCL_4	10.17 ± 0.51 a	11.59 ± 0.60 a	3.19 ± 0.87 a
Leaves (purify)	8.54 ± 0.33 a	11.43 ± 0.46 a	1.66 ± 0.59 b
Flowers (purify)	9.49 ± 0.10 a	13.10 ± 0.11 a	3.65 ± 1.16 a
Vitamin C	9.21 ± 0.26 a	12.59 ± 0.24 a	2.28 ± 0.43 ab
LSD value	1.632 *	2.194 *	1.625 *
* $p< 0.05$			

Different letters represent the significant difference at P< 0.05between means of column

Determination of Serum Liver Functions Tests

The serum was used for the assessment of serum ALT, AST, andALP as limitations of liver function tests. Serum ALT and AST were estimated using ready-made kit from Linear® chemicals[18]. While Serum ALP activity have been estimated using ready-made kit[19].

Histopathological Examinations

Tissue samples from liver and kidney were prepared for histopathological studies according to[20]: Using fixation and paraffin embedding technique. Liver and kidney samples were de-waxed by xylene first, dry and then washed shortly in 3 changes of absolute alcohol, then 95% alcohol and 70% alcohol. Washed in water for 5 minutes. Stain with haemotoxylin for 5-10 minutes then washed again in water for five minute. The slides were then placed in eosin for 10-15 seconds after that washed again in water for 3 minute. The sections were then dehydrated in (70, 80, and 95%) alcohol few seconds for each and 2 changes of absolute alcohol.The final step put the slides in xylene after drying and covered by covered slip with Canada balsam, and examined under light microscope.

Statistical analysis

The data were analyzed to obtain the level of significance. The least significant difference (LSD) test was used to compare between the means for the groups[21].

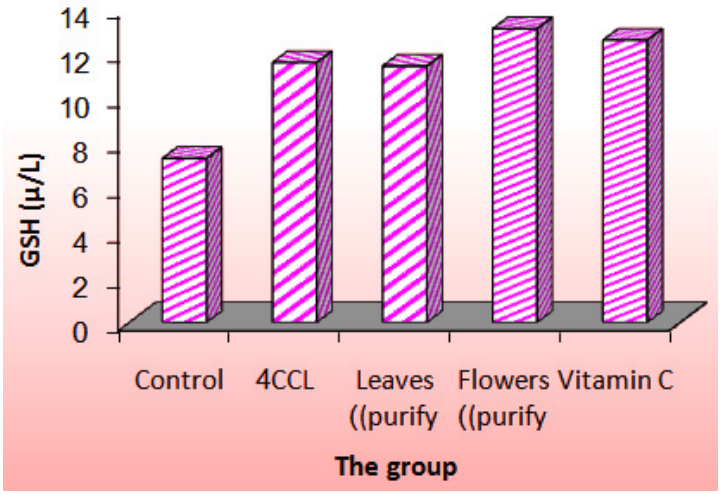


Fig. 2. The effect of difference pure of Bauhinia variegate L. leaves and flowers on GSH(unit/ml)

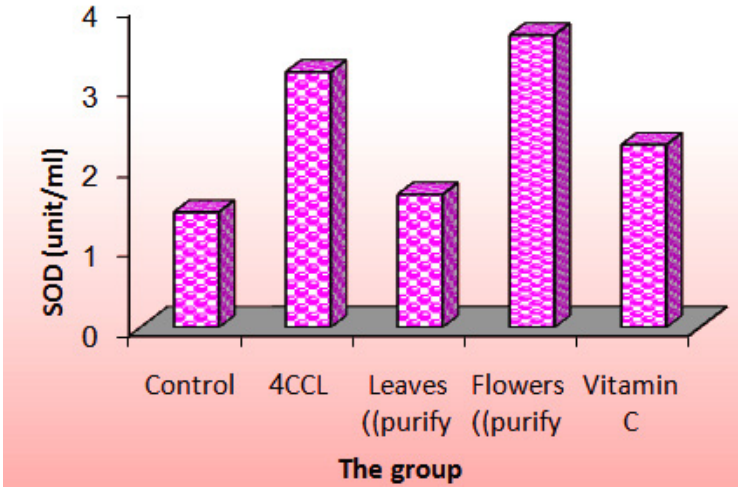


Fig. 3. The effect of difference pure of Bauhinia variegate L. leaves and flowers on SOD(unit/ml)

RESULTS AND DISCUSSION

Two types of extracts were PBVL and PBVF. These extracts were tested for general chemical identifications to verify the chemical constituents of each extract (Table 1).

The poly-phenolic fraction, the extracts were dark brown residue, and there were compatible with the positive results of phytochemical analysis. The dark brown color may be due to the presence of large amounts of poly-phenolic compounds and flavonoids [22,23].

Table (2) and Figure (1)the concentrations of serum CAT were significantly increased (p<0.05) in the treated rats group by flowers purify for 3 weeks (9.49±0.10 Unit/ml) in comparison to the control group (5.60±0.43unit/ml) which indicate that of severe hepatotoxicity.The CAT is a haemoprotein, it protects the cells from the accumulation of H_2O_2 by dismutating it to form H_2O and O_2[24]. The decrease in the level of catalase enzyme was detected in paracetamol of treated rat due to extremecreation of free radicals and triggering of lipid peroxidation [25]. The biomarkers of oxidative stress include CAT, GST, and SOD[26].

These results suggest that the leaves and flowers purify of *Bauhinia vareigata L.* may have protective activity against toxic effects of hepatic damage-inducing agents. This evidence is in agreement with the previous studies[3].

Table 3. Effect of purified leaves and flowers as hepatoprotectiveon serum ALT (U/L)

Groups	Mean ± SD (unit/L) Week 1	Week 2	Week 3
Control	151.15 ± 2.44 b	119.40 ± 9.77 b	92.04 ± 0.49 b
CCL_4	181.93 ± 4.39 a	176.56 ± 17.10 a	147.01 ± 3.17 a
Leaves (purify)	157.99 ± 8.79 ab	152.62 ± 10.75 a	149.69 ± 4.40 a
Flowers (purify)	137.23 ± 2.20 b	150.18 ± 0.98 a	133.08 ± 6.84 a
Vitamin C	158.73 ± 8.55 ab	153.11 ± 3.42 a	110.85 ± 4.15 ab
LSD value	25.839 *	27.561 *	32.677 *
* p< 0.05			

Means bearing different letters within the same column within each period are significant at p< 0.05

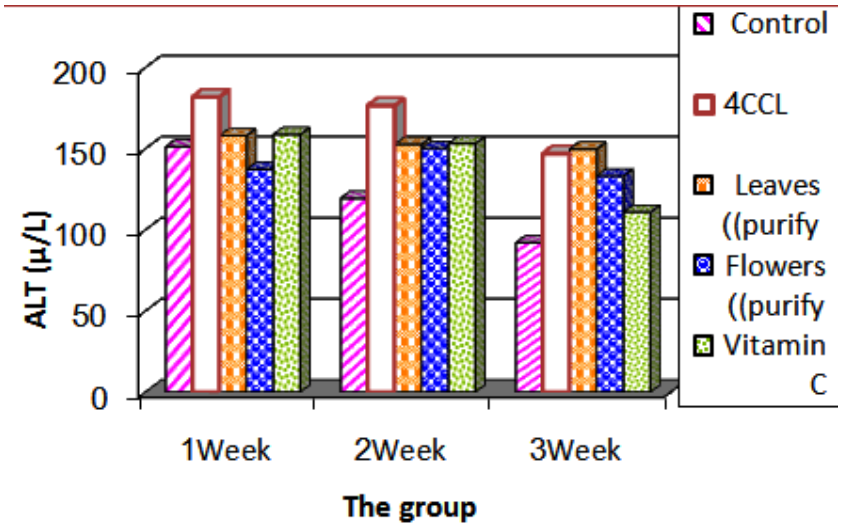


Fig. 4. The effect of difference pure of Bauhinia variegate L. leaves and flowers on serum ALT (unit/L)

The rats treated by CCL_4 showed a markedly reduction ($p < 0.05$) in the liverenzymes' level of serum GSH to (11.59 µmol/min/L) as paralleled to control rat (13.63 µmol/min/L) (Figure 2).

In the Figure (2), there was a markedly elevation($p<0.05$) in the level of serum GSH in rat treated with 200 mg/kg of flowers purified *B. variegata* for three weeks than in rats treated with leaves purified 200 mg/kg which reach (13.10 and 11.43µmol/min/L respectively) as compared to control rats. Moreover, there was a significant reduce ($p<0.05$) in the liver activity level of GSH in rats treated with 200 mg/kg of vitamin C to (12.54 µmol/min/L) for 3 weeks was significantly ($p< 0.05$) less than control treated group [27]. It has been documented that aqueous extract of *P.niruri*can be act as a heptoprotection along side paracetamol.

Figure (3) showed that the CCL_4 treated ratincrease significantly ($p<0.05$) in the liver activity level of SOD (3.19 unit/ml) as paralleled to the control group (1.44 unit/ml). Rats treated with 200mg/kg of flowers purified *B. variegata* for 3 weeks revealed a significant increase ($p<0.05$) in the level of SOD 3.65 unit/ml as paralleled to the control group. Also, rats treated with 200 mg/kg of vitamin C presented a markedly increase ($p<0.05$) in the level of SOD 2.28 unit/ml as paralleled to the controls.

In the table (3) and Figure (4) showed a significant increase ($p<0.05$) in the levels of serum ALT than the healthy control group. The treatment of rat with leaves purify improved ($p<0.05$) of elevated values. Treating rat with flowers purify in the diet was more efficient than the lower dose. Treatment with purify flowers extract (133.08±6.4 U/I) for 3 weeks showed markedly elevation($p< 0.05$) in serum concentrations of ALT in paralleled to the CCL_4 treated group. Additionally, the ALT levels were distinctly increase ($p< 0.05$) in the

Table 4. Effect of purified leaves and flowers as hepatoprotective on serum ALP(unit/100ml)

Groups	Mean ± SD (unit/100 ml)		
	Week 1	Week 2	Week 3
Control	3.04 ± 0.16 b	2.48 ± 0.15 a	2.35 ± 0.05 b
CCL_4	3.05 ± 0.13 b	1.21 ± 0.05 b	3.93 ± 0.07 a
Leaves (purify)	4.88 ± 0.66 a	2.25 ± 0.03 a	3.60 ± 0.28 a
Flowers (purify)	3.44 ± 0.06 b	2.77 ± 0.09 a	2.43 ± 0.11 b
Vitamin C	3.73 ± 0.05 b	2.50 ± 0.22 a	3.52 ± 0.14 a
LSD value	1.037 *	0.749 *	0.863 *
* $p< 0.05$			

Means bearing different letters within the same column within each period are significant at $p< 0.05$

Table 5. Effect of purified leaves and flowers as hepatoprotective on serum AST (unit/L)

Groups	Mean ± SD (unit/L)		
	Week 1	Week 2	Week 3
Control	139.23 ± 0.41 a	141.40 ± 0.37 a	140.85 ± 0.48 a
CCL_4	106.21 ± 6.36 b	108.41 ± 2.68 c	109.14 ± 0.01 b
Leaves (purify)	123.56 ± 0.24 ab	136.40 ± 4.01 a	114.02 ± 9.28 b
Flowers (purify)	119.15 ± 3.66 b	116.22 ± 12.4 bc	117.93 ± 1.96 b
Vitamin C	114.27 ± 0.24 b	129.38 ± 0.88 ab	137.38 ± 2.00 a
LSD value	19.448 NS	16.209 *	19.536 *
*$p< 0.05$			

Means bearing different letters within the same column within each period are significant at $p < 0.05$, NS: Not significant

purify leaves extract (149.69 ± 4.40 U/I) for 3 weeks, this indicate that the effective role of purify flowers and leaves as hepatoprotective, which is in accordance with the study of Nasir[28].

Biswas and his workers shown that liver enzymes levels (ALT, AST, and ALP) were significantly increase in all the groups of rats treated with different doses of diclofenac sodium as a result of injury in liver rat's tissue[29].

Rats treated by CCL_4 revealed a markedly raise(p<0.05) in serum ALP level as paralleled to control rats (Table 4) (Figure 5). While rats treated with 200 mg/kg of purified leaves extract revealed a markedly elevation(p<0.05) in serum ALP level as paralleled to the control group, but significantly less than CCL_4-only treated group. Rats treated with 200mg/kg of purified flowers extract showed a significant (p<0.05) decrease in serum ALP level as paralleled to the control group, but significantly it was less than CCL_4-treated group.

Figure (6) illustrates then in CCL_4–treated group,there was a significant increase(p<0.05) in serum AST level as paralleled to control rats (Table 5). Rats treated with 200 mg/kg of flowers purified showed a significant elevation (p<0.05) in serum AST level as paralleled to control group. Rats treated with 200 mg/kg of leaves purified extract indicateda significant increase (p<0.05) in serum AST level as paralleled to the control group, but significantly it was less from CCL_4-treated group. Rats treated with 200 mg/kg of essential vitamin C a markedly elevation (p<0.05) in serum AST level as paralleled to the control group, but significantly it was fewer than CCL_4-treated group.

Histopathological section in the kidney rat of normal animals (control group)showing the

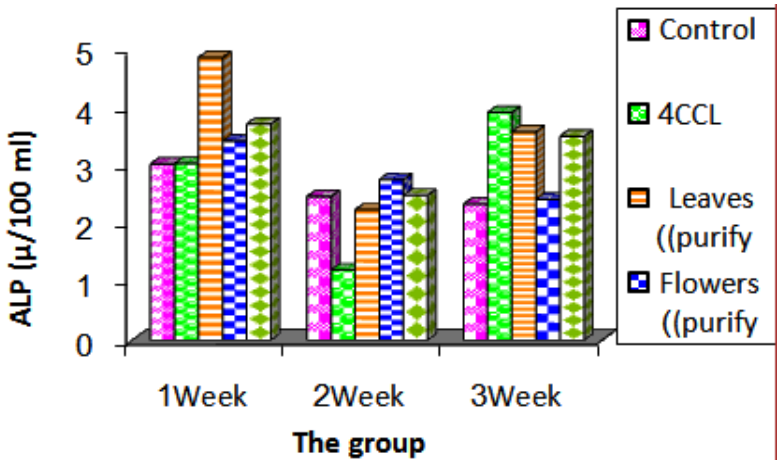


Fig. 5. The effect of difference pure of Bauhinia variegate L. leaves and flowers on serum ALP (unit/100ml)

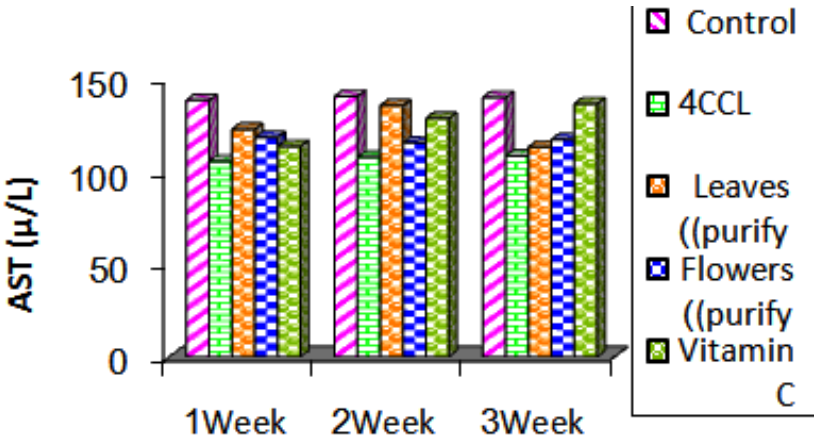
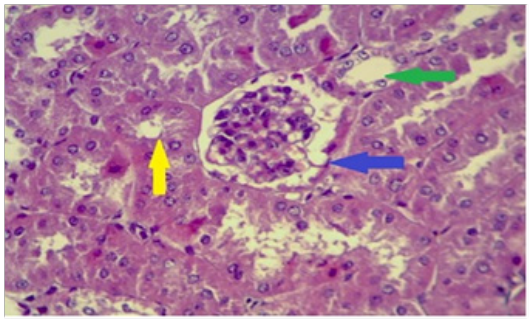
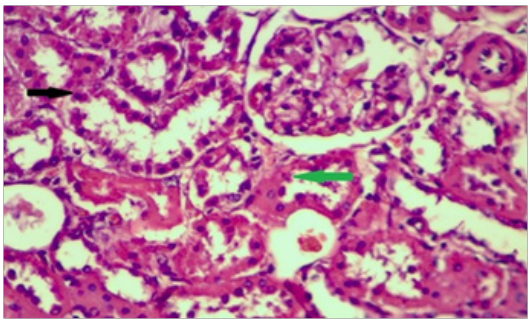


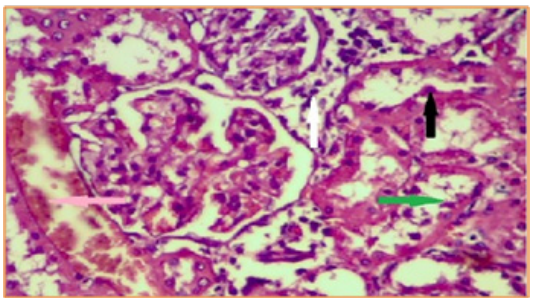
Fig. 6. The effect of difference pure of *Bauhinia variegate* L. leaves and flowers on serum AST (unit/L)

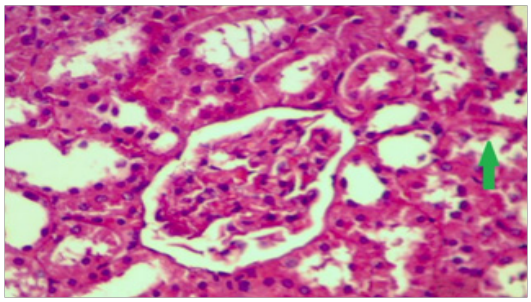
normal glomeruli, proximal convoluted tubules and distal convoluted tubules (Figure 7A). But when the rat treated with $CCL_4$the kidney section showing degeneration and necrosis of renal epithelial tubules (Figure 7B). However, when the rat treated with CCL_4 and with vitamin C



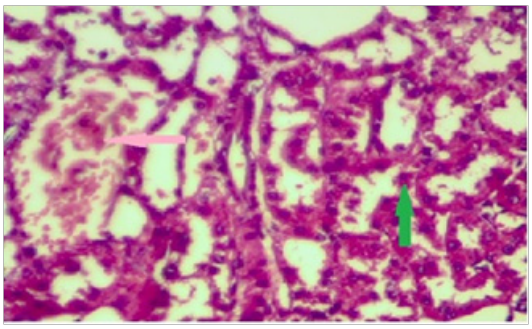


Histopathological section in the kidney Rat of normal Histopathologicalsection in the kidney rattreated animals shows Look like normal renal tissue showing with CCL4 showing degeneration ( ) and the glomeruli ( ) proximal convoluted tubules nerosis ( ) of renal eoithelial tubules ( ) and distal convoluted tubules ( ) (H&E stain 400X). (H&E stain 400X).





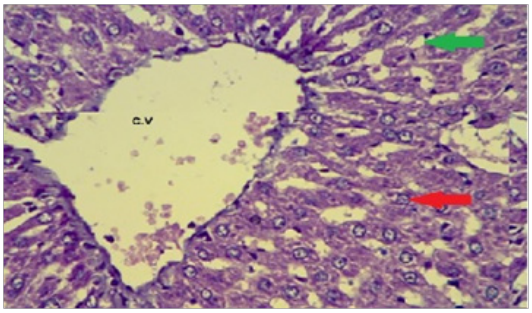
Histopathological section in the kidney rat treated Histopathological section in the Kidney rat treated With vitamin C showing degeneration ( )and with flowers purified shows look like like normal nerosis ( ) of epithelial lining epithelial cells of renal tissue withmild degenerative ( ) renal tubules withcongestion ( ) in between changes of renal epithelial cells. (H&E stain 400X).renal tubules and inside the glomeruli, with moderate inflammatory cellsinfiltration. (H&E stain 400X).



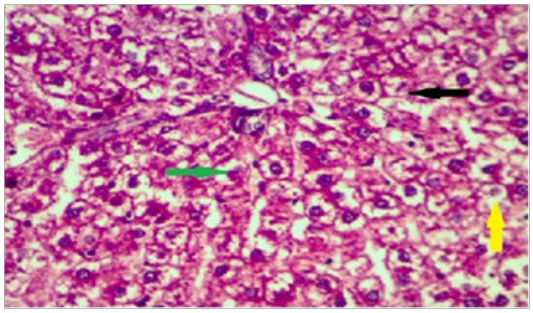
Histopathological section in the kidney rat treated with Leaves purified shows Look like Congestion ( ) of blood vessels, degeneration ( ) of renal tubular epithelial cells. (H & E stain 400X)

Fig. 7. Hisptopathological sections in the kidney rat after treated with CCl4 and treatment with leaves purified and flowers purified fromBauhinia variegate

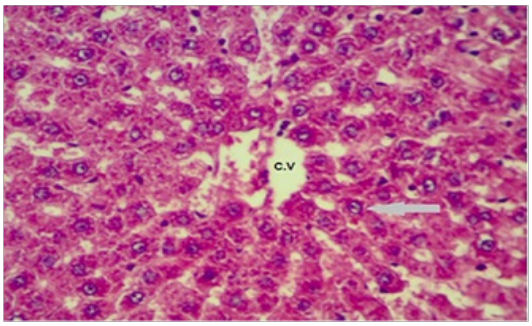
the kidney section tissue showing degenerative changes and necrosis of epithelial lining cells of renal tubules with congestion in between renal tubules and inside the glomeruli, and moderate inflammatory cells infiltration (Figure 7C). While in rat's kidney treated by $CCL_4$and with Flowers



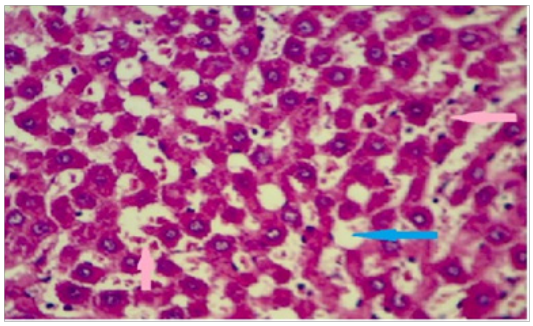
Histopathologicalsection in the liver of normal ratshows normal structure appearance which consist of central vein and threads of hepatocytes ( ) withsinusoid ( )



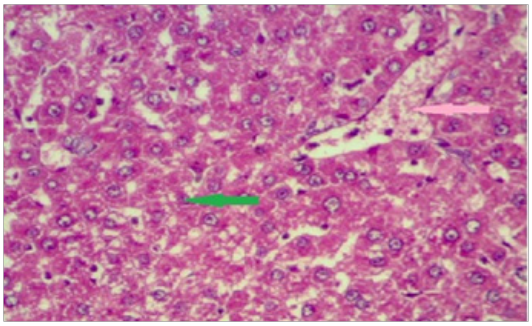
Histopathological section in the liver of rat treatedwith CCL4 shows degeneration ( )depletion glycoprotein ( ) and apoptotic cells ( )



Histopathological section in the liver of rat treated with vitamin C shows dilatation of sinusoids ( )with congestions ( ) of sinusoidal capillaries and hepatic shrinkage of hepatocyte cells.



Histopathologicalsection in the liver of rat treated with flowers purified extract showslook-like normal tissue which consists of central vein and threads of hepatocyte cells ( )



Histopathological section in the liver of animal treatment with Leaves purified extract shows Look - like normal architecture of hepatic tissue with mild congestion ( ) and certain degeneration ( ) of hepatocyte cells

Fig. 8. Histopathological sections in the liver of rat after treatment with CCl_4, leaves purified, and flowers purified from *Bauhinia variegate*(H & E stain400X)

purified shows look like normal renal tissue with mild degenerative changes of renal epithelial cells (Figure 7D). Finally the section in the kidney rat treated with CCL_4 and with leaves purified shows congestion of blood vessels, degeneration of renal tubular epithelial cells (Figure 7E).

In liver section from each animal treated with CCL_4 showed aggregation inflammatory cell particle neutrophil and macrophage in the liver parenchyma in addition to necrotic of hepatocyte which characteristic by dichotic of disappear of nuclei and also showed aggregation of inflammatory cell around a central vein (Figure 8B) as compared with control group (Figure 8A) Which shows normal structure appearance which consist of central vein and threads of hepatocyte with sinusoid. But when the rat treated with CCL_4 and treatment with vitamin C shows dilatation of sinusoids with congestion of sinusoidal capillaries and Shrinkage of hepatocyte cells. (Figure 8 C). The (Figure 8D) has shown the rat lives treated with flowers purified extract look–like normal hepatic tissue which consists of central vein and threads of hepatocyte cells. Also (Figure 8E). shown section in the liver of animal treatment with Leaves purified extract shows look–like normal architecture of hepatic tissue with mild congestion and certain degeneration of hepatocyte cells.These results agree with Biswas *et al*.,whom showed that the liver tissues histopathological changes, confirming hepatotoxicity by CCL_4[29].

The hepatotoxicity induced by $CCL_4$is owed to the creation of the active metabolite, trichloromethyl free radical (CCL_3). This readily interrelates with molecular oxygen to form the trichloromethylperoxy radical (CCL_3OO). Both radicals are capable of binding to proteins and other macromolecules with concurrent attack on polyunsaturated fatty acids to yield lipid peroxidation foremost to hepatotoxicity[30].

Shaikh *et al*., found that the measurement of marker enzymes like serum ALT and AST is a suitable method to screen oxidative cell injury[31]. Inhibition of these raised enzymes levels detected in treated groups probably due to its compensation of bio-membranes of hepatic parenchymal cells.

All the results indicate that flavonoids presented in the plant part could be an important source of antioxidant molecules. The antioxidant capacity of flavonoids is based on their molecular structure. The hydroxyl group position and other characteristics in the chemical structure of flavonoids are more vital for their antioxidant and free radical scavenging actions. Plants phenolic in general are effective free radical scavengers and antioxidants. The remarkably strong *A. nervosa* leaf extract can be used as a powerful herbal antioxidant. The antioxidant activity should be regarded as an additional health promoting value for use as phytonutrients[32].

Al-Sayed *et al*., indicated that the histological explanations established the highlyhepato protective activity[33]. The study recommend that a nutritional enhancement of BHE could employ a favorable result against oxidative stress and liver diseases by improving the antioxidant defense status, reducing lipid peroxidation, and protecting against the pathological fluctuations of the liver.Therefore, measurement the activities of serum marker enzymes like AST and ALT can make assessment of liver function and these enzymes is sensitive marker of liver injury and several fold increase in the release of these enzyme indicate severing of damage in chronic injury.

Biswas *et al*., shown that the phytochemical investigation of ethanolic extract of *vaccariapyrim adatamedik* have shown the presence of flavonoid. ethanol extract of *vaccariapyrim adatamedik* offers protective effect against CCL_4–induced hepatotoxicity in experimental rats. The study, revealed that the group treated with CCL_4 showed dramatic elevation in serum ALT, AST, ALP, (total and direct) bilirubin levels, and triglycerides levels.

CONCLUSIONS

From the current results, it can be concluded that the administration of purified PLBV and PFBV of prevented CCL_4 induced elevation in different biochemical parameters representing the hepatoprotective activity of the purified leaves and flowers extract against CCL_4 induced hepatotoxicity. This was also established by the result of histopathological examination, which revealed dose dependent diminish in prevalence and severity of histopathological changes.

Bauhinia variegate has differentiated pharmacological prospective and was used since ancient times. It has a strong future in the field of herbal medicine, thus the plant should be cultured

in a largescale principally in unutilized and wasteland which will cooperative the financial of the farmers beside with the progress of study in the field of herbal medicine. Likewise, scientific research isrequisite to discover the pharmacological impending of the plant.

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