

STUDIES ON THE HEPATO AND RENO PROTECTIVE EFFECT OF ALCOHOLIC SEED EXTRACT OF *CAESALPINIA BONDUCELLA* ON ALLOXAN INDUCED DIABETIC MALE ALBINO WISTAR RATSVedavyasa Sagar¹, R. Nazeer Ahamed^{2*} and Subhas C. Bijjaragi²¹Assistant Professor of Zoology, Veerashaiva College, Ballari- 583102, Karnataka, India.²PG Department of Studies and Research in Zoology, Karnatak University, Dharwad-580003, Karnataka, India.***Author for Correspondence: Dr. R. Nazeer Ahamed**

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ABSTRACT

Objective: To evaluate the efficacy of alcoholic seed extract of *Caesalpinia bonducella* (ASECB) in hepato and reno protection in alloxan induced diabetic male albino rats. **Methods:** The rats were divided into six groups, Normal-Control, Diabetic-Control, Normal+200mg ASECB, Normal+400mg ASECB, Diabetic+200mg ASECB and Diabetic+400mg ASECB. Rats were made diabetic by intraperitoneal injection of alloxan. Oral administration of different doses of ASECB to normal and diabetic rats was conducted for a period of 30 days. Histopathology of liver and kidney and biochemical estimation of urea, uric acid and creatinine was studied. **Results:** Lobular architecture of liver of control and control-treated rats was normal and undisturbed. In diabetic rats, overall lobular architecture was disturbed. Central veins appear congested, sinusoidal space dilated, hepatic chords disrupted and hepatocytes overlapped and degenerated. In diabetic-treated groups histological disturbances were improved and restored to normalcy. The kidney histology was normal in control and in control-treated rats. In diabetic-control group, the kidney section showed pathological changes in malpighian corpuscles and in urinary tubules. In diabetic-treated rats, the renal corpuscle, tubules and other segments were well maintained and returned to normalcy. The diabetic control group showed significant ($P < 0.05$) increase in renal markers, while the diabetic rats treated with ASECB showed a significant ($P < 0.05$) fall in levels of renal markers. Higher dose of 400 mg was more effective than 200 mg. **Conclusion:** ASECB has improved the liver and kidney functions by ameliorating histopathological lesions and degenerative effects of alloxan associated with diabetic state. Thus, ASECB serves as a good oral hepato and reno protective agent.

KEYWORDS: *Caesalpinia bonducella*; Albino rats; Diabetes mellitus; Liver; Kidney.**1. INTRODUCTION**

Diabetes, medically known as Diabetes Mellitus (DM) is common endocrine disorder and affects the lives of millions of people, directly and indirectly each year. It is characterized by the deficiency in production of insulin by the endocrine pancreas leading to excess glucose in blood and in urine. Chronic hyperglycemia during diabetes causes glycation of body proteins that in turn lead to secondary complications affecting kidney, heart, liver and many other vital organs. The symptoms of diabetes are increased blood sugar, bodyweight loss, weakness, frequent urination, severe thirst, blurred vision, extreme hunger, extreme fatigue, tiredness, numbness in the hands or feet, recurring gum and skin infections, etc. DM is a chronic lifelong disease that can be treated but not cured. Though both men and women can be affected by DM, the rate of DM in women has increased considerably in the recent years. DM is a silent killer. If uncontrolled, it can lead to deadly complications. A world wide survey reported that DM is

affecting nearly 10% of the population every year.^[1] According to World Health Organization (WHO), the diabetic population is likely to increase to 300 million or more by the year 2025.^[2] The number of people suffering from the disease worldwide is increasing at an alarming rate with projected 366 million peoples likely to be diabetic by the year 2030 as against 191 million estimated in 2000.^[3] This disease is the seventh leading cause of death in the world. Every eight seconds somewhere in the world someone dies from diabetes. The number of people with diabetes multiplies worldwide. It is projected to become one of the world main disablers and killers within the next 25 years.^[4]

Recent studies on geographical and ethnic influences have shown that people of Indian origin are highly prone to diabetes. It is estimated that between 10-12% of the urban population and 4-6% of the rural population of India have diabetes now. One in every five urban adult Indians now suffer from DM. As per the estimate of the

International Diabetes Federation, there are about 41 million diabetics in India alone and this figure is expected to rise to 70 million by the year 2025.^[5] India is a home to the largest diabetic population in the world. WHO has issued a warning that India will be the diabetic capital of the world. Based on etiology, DM is classified into Type-1 (IDDM-Insulin dependent DM), Type-2 (NIDDM-Non-insulin dependent DM) and Gestational Diabetes (GD). Type-1 is also called juvenile diabetes as it is commonly seen in children and teenagers. This occurs when the pancreas is unable to produce insulin and results in increase in glucose level in blood. People with Type-1 DM need to take insulin every day. Type-2 diabetes is said to occur in middle-aged and older people. In this case, either the production of insulin is low or the pancreas does produce insulin, but the body becomes resistant to insulin. It is not able to use the insulin properly, leading to the presence of glucose in the blood. Only 5 to 10% have Type-1 and 90 to 95% have Type-2 DM. Gestational diabetes occurs in pregnant women due to hormonal changes. Though GD does not stay after the baby is born, women who have GD during pregnancy are at higher risk of developing Type-2 diabetes later in their life.

Different types of oral hypoglycemic agents such as biguanides, sulphonylurea are available along with insulin for the treatment of DM.^[6] But side effects are associated with their uses.^[7,8] Synthetic hypoglycemic drugs can produce side effects including coma, disturbances of liver and kidney function. In addition they are not suitable for use during pregnancy.^[9] Further, insulin therapy has shortcomings such as need for constant refrigeration, it cannot be used orally and insulin injections are associated with the risk of fatal hypoglycemia in the event of excess dosage in which blood sugar level falls.^[10] A fall below 55mg/dL produces severe symptoms leading to insulin shock and death.

Plants are the important source of drugs, in fact many of the currently available drugs were derived either directly or indirectly from the plants. Over three-quarters of the world population relies mainly on plants and plant extracts for health care. More than 30% of the entire plant species were used for medical purposes. Of the 2,50,000 higher plant species on earth, more than 80,000 are medicinal. Herbal remedies from medicinal plant have been used traditionally. In India, drugs of herbal origin have been used in traditional systems of medicines such as Unani and Ayurveda since ancient times. Since time immemorial, plant remedies have been used to relieve diabetes. In Ayurveda a number of plants are reported to be used in curing diabetes. In the 6th century Sushruta, an Indian physician recommended plant remedies for the treatment of DM. Prior to discovery of insulin and other hypoglycemic synthetic drugs herbal medicine has been long used for the treatment of diabetic patients and they are currently accepted as an alternative therapy for diabetic treatment and control.^[11] In many

places throughout the world, DM is kept under control by the use of medicinal plant treatment, although this type of treatment has not been taken seriously by the medical field. The WHO approves the use of plant drugs for different diseases including DM.^[12] There has been increasing demand for the use of plant products with antidiabetic activity due to low cost, easy availability and lesser side effects. There are more than 1200 plants worldwide that are used in the treatment of DM. The search for more effective and safer antidiabetic agent has become an area of active research. Although herbal medicines have long been used effectively in treating DM in Asian communities and throughout the world, yet the therapeutic efficacy, mechanism of action and safety of most of the herbals used has not been worked out. Only a small number of plants have received scientific and medical evaluation to assess their efficacy.

Caesalpinia bonduc (Lin.) Roxb. is a medicinal plant belonging to the family Fabaceae/ Caesalpinaceae. Though hypoglycemic and hypolipidemic property of *Caesalpinia bonduc* (Lin.) Roxb. is reported, yet, its hepato and reno protective effect, its therapeutic efficacy and its safety has not been worked out. There is paucity of information in this regard and needs investigation on this plant.

2. MATERIALS AND METHODS

2.1 Venue

The whole experimental work was conducted at the Post Graduate Department of Studies and Research in Zoology, Karnatak University, Dharwad, Karnataka, India.

2.2 Experimental animals used

Adult male albino rats of Wistar strain developed from Norwegian rat (*Rattus norvegicus*, Family: Muridae, Order: Rodentia) aged about 80-90 days, weighing between 220-240 gms. bred in animal house of Department of Post Graduate Studies and Research in Zoology, Karnatak University, Dharwad, India were selected as an experimental model in the present investigation. They were housed in individual polypropylene rat cages. The animal house was maintained at 64.4-78.8 F temperature and 40-70% relative humidity with 12 hrs day-light/12 hrs darkness schedule. The animals were checked daily to ensure that an adequate supply of food and water is available. The health and behaviour was verified on a daily basis throughout the experimental period. The rats were fed with rat pellet feed supplied by M/s Krish Scientist's Shoppe, agents for scientist's choice laboratory animal feed, Bangalore, India, and water *ad libitum* throughout the experimental period. These are quite moderately prolific strain, rather resistant to infections. The experiments were designed as per guidelines of Institutional Animal Ethics Committee (IAEC), Department of Studies in Zoology, Karnatak University, Dharwad, Karnataka, India vide Registration No. 639/02/a/CPCSEA. CPCSEA (Committee for the Purpose of

Control and Supervision on Experiments on Animals) guidelines were followed for maintenance, use and disposal of the experimental animals.

2.3 Induction of diabetes

Alloxan monohydrate ($C_4H_2N_2O_4H_2O$) was used as diabetes inducer in rats. The purpose of choosing alloxan monohydrate as diabetes-inducing agent was that it causes a massive reduction of the beta cells of the islets of langerhans and induces hyperglycemia.^[13] Best, 1989^[14] has also opined that alloxan, a beta cytotoxin, induces 'chemical diabetes' in a wide variety of animal species by damaging the insulin secreting cells (beta cells) of pancreas. Alloxan is well known for its selective pancreatic islet cell toxicity and has been extensively used in inducing DM in animals. DM was induced in normal healthy male albino rats by single intraperitoneal freshly prepared injection of alloxan (150 mg/kg body weight) dissolved in normal saline (2 ml/kg bw).^[15] The higher dose of alloxan usually caused death before severe diabetes could develop. The animals were allowed to fast for 12 hrs prior to alloxan injection. After 3 days of alloxan injection, the glucose level was measured. Rats showing fasting glucose levels >250 mg/ dl were considered as diabetic and selected for the investigation.

2.4 Description of the plant used

Caesalpinia bonducella (Linn.) Roxb: The plant is a large dioecious thorny shrub found throughout India. The branches are armed with hooks and prickles. The leaves are 3cm long and the flowers pale yellow in color. The fruits are inflated pods with prickles having 1 or 2 seeds. The seeds of this plant are hard, globular, grey colored, shiny, smooth surfaced with yellowish white kernel. The seed coat is thick. The plant is commonly known as 'fever nut' or 'bonduc nut'. Several months to years may be required for its germination because of its hardened nature. It is a well known traditional plant used in folklore medicine around the world and especially greater parts of India. The powdered seed kernel of this is used by the local people of Assam in the treatment of diabetes.^[16] All parts of this plant have medicinal properties.^[17] Due to continuous over exploitation of the plant and destroying its habitat, its number has drastically reduced. Now it is the time to preserve this rare medicinal plant species. It will become extinct if proper steps are not taken for its conservation and are listed under endangered medicinal plant category.^[18]

2.5 Procurement of seeds of *Caesalpinia bonducella* and preparation of the extract

Seeds of the *C. bonducella* were collected from the local market in Dharwad district, Karnataka, India. The seed extract was prepared by following the method of Shukla et al.,^[19] The seeds were shade dried, coarsely powdered using a mixer and sieved to get uniform powder. 50 gm powder of *C. bonducella* was extracted with 500 ml of

95% ethanol by using a Soxhlet apparatus for 16 hrs. The crude extract obtained was filtered through Whatman paper and the filtrate was evaporated to dryness on rotary flash evaporator under reduced pressure to obtain a greenish black jelly residue. The seed extracts obtained was then stored in airtight glass containers and refrigerated till further use.

2.6 Acute toxicity studies

The plant extracts often influence toxicities. Hence, acute oral toxicity test for ASECB was carried out to know possible toxicities. When administered orally, ASECB was found to be relatively nontoxic.^[20] There was no mortality or toxic reactions were noticed with the selected doses among the rats until the end of the study period.

2.7 Experimental design

A total of 36 rats (18 normal, 18 diabetic) were used. The rats were randomly divided into 6 groups. All the experimental rats were placed on normal diet throughout the experiment. Group-I served as normal-control, Group-II served as diabetic-control and both the groups did not receive any treatment. Alloxan was administered and induced diabetes in Group-II, V and VI. The experimental animals of Group-III, IV, V and VI were administered with ASECB. Six rats were used for each group (n=6).

2.8 Blood Sample collection and biochemical estimation

At the end of the experiment, animals were kept overnight fast but the animals had free access to water and sacrificed after a short exposure to sodium pentobarbital. Blood samples were collected from each rat into sample tubes by direct cardiac puncture using a 1ml syringe and a 22 gauge needle for analysis. Urea and Creatinine were determined by the methods of Patton and Crouch^[21] and Henry et. al.,^[22] respectively. Liver and Kidney were surgically removed immediately, blotted to remove blood traces and stored in 10% neutral buffered formalin solution for histopathological studies.

2.9 Histopathological studies

After proper fixation, liver and kidney tissues were dehydrated in graded series of alcohol, cleared in benzene and embedded in paraffin wax. The paraffin blocks were sectioned at 5µm thickness by LEICA RM 2255 microtome according to the procedures of Kittel et. al.,^[23] Morawietz et. al.,^[24] and Fehlert et. al.,^[25] The tissue sections were subjected to rehydration by exposing them to decreasing concentrations of alcohol, 100-10% and then stained with haematoxylin. The sections were dehydrated by using increasing concentrations of alcohol 10-100% and then stained with eosin.^[26] The stained slides were photographed under Axio Imager M 2 microscope.

GROUP	TREATMENTS	DOSES
I	Normal-Control	Administered 1ml distilled water /rat/day orally for 30 days
II	Diabetic-Control	Administered 1ml distilled water/rat/day orally for 30 days
III	Normal-Treated	Administered 200 mg ASECB/kg bw orally/rat/1ml for 30 days
IV	Normal-Treated	Administered 400 mg ASECB/kg bw orally/rat/1ml for 30 days
V	Diabetic-Treated	Administered 200 mg ASECB/kg bw orally/rat/1ml for 30 days
VI	Diabetic-Treated	Administered 400 mg ASECB/kg bw orally/rat/1ml for 30 days

2.10 Statistical evaluation

All values were expressed as mean \pm S.E. Statistical evaluation was done using one-way analysis of variance (ANOVA).^[27] P values of < 0.05 were considered as significant.

3. RESULTS

3.1 Measurement of Urea, Uric acid and Creatinine

The data in respect of mean values of urea, uric acid and creatinine is summarized in Table 1. The alloxan produced a significant ($P < 0.05$) increase in the levels of urea, uric acid and creatinine in diabetic induced rats of Group II, V and VI. The values of renal markers of normal rats which are administered with different doses (200mg and 400mg) of ASECB (Group- III and IV) for 30 days are more or less similar to normal control animals. The diabetic rats which are treated orally with 200 mg and 400 mg ASECB (Group-V and VI) for 30 days showed a significant ($P < 0.05$) fall in the levels renal markers when compared to untreated diabetic animals (Group-II). The administration of plant seed extract to diabetic experimental animals almost normalizes the levels of urea, uric acid and creatinine. Further, the results reveal that the higher dose of 400mg is more effective than a dose of 200mg.

3.2 Histopathology of Liver

Microscopic observations of liver histology of control rats (Group-I) revealed the distinct lobule with central vein, hepatic chords and sinusoid. Hepatocytes were distinct tightly abutting to each other in a chord, well stained with well maintained cytoplasm and nucleus (Fig. 1). The portal triad, comprising of portal vein, hepatic artery and bile duct appear normal. The animals of Group-II which are diabetic showed disturbance of overall lobular architecture (Fig. 2-4). Central vein appears congested. Necrosis, fibrosis, dilation of sinusoidal space and disarray of hepatic chords was noticed. At certain regions the hepatocytes are overlapping and degenerated. Inflammation and karyolysis of hepatocytes was also noticed.

The experimental animals of Group-III and IV which are normal but treated with 200 mg and 400 mg ASECB, respectively has revealed a histological architecture (Fig. 5-6) which were similar to normal controls. There were no noticeable changes on the histological aspects. Hepatic chords, hepatocytes, sinusoids and central vein appear normal and even better than normal control. The plant seed extract on its own did not produce any morphological and histological changes in the liver.

Microscopic observations of sections of liver of diabetic animals treated with 200 mg and 400 mg ASECB respectively (Group-V and VI) for 30 days, showed the restoration of normal histoarchitecture. The liver is free from histopathological changes observed in those of untreated diabetic rats. The central vein, hepatic chords and sinusoidal space appear normal. Nuclei are distinct and well stained. Whole architecture which was disturbed in Group-II due to diabetic condition brought back to its normal state. There was marked improvement in overall liver histoarchitecture (Fig. 7-8). The histological investigations and severity degree of different areas affected in liver of various groups are depicted in Table 2. The ASECB treatment has rejuvenated the histological disturbances. Thus the restoration of normal histology is due to extract treatment. This observation implies the protective effect of ASECB on liver.

3.3 Histopathology of Kidney

Microscopic observations of histopathology of kidney in control animals revealed normal structure. The glomeruli, blood vessels and interstitium appear normal and distinct. Various segments of kidney tubules were well preserved and clearly seen (Fig. A). Morphological and pathological changes occurred in the kidney tissues of diabetic control rats (Group-II). In these rats, the kidney section showed epithelial atrophy, mild mesengial expansion, may be due to cell proliferation in the glomeruli, increased capsular space, hemorrhagic pathological lesions and moderate congestion of capillaries (Fig. B-D).

There were no significant histological alterations in the renal corpuscle or any other segment of kidney tubules of normal animals administered with 200 mg and 400 mg ASECB (Group-III and IV). The histological architecture was almost similar to normal control without any histological changes (Fig. E-F). Normal histology was maintained indicating that the plant seed extract is non-toxic and has no side effects.

Histological observations of kidney of experimental rats belonging to Group-V and VI which are diabetic and treated with 200 mg and 400 mg ASECB respectively for 30 days showed normal structure with minimal histological changes. The renal corpuscle, nephric tubules returned to normal and well maintained. Changes in the histological architecture noticed in animals of Group-II, were almost normalized (Fig. G-H). The histological investigations and severity degree of different areas affected in kidney of various groups are

depicted in Table 3. There was significant recovery with extract administration indicating the efficacy of ASECB.

The results reveal that the higher dose extract administration is more effective.

Table: 1 Effect of treatment of ASECB on serum urea, uric acid and creatinine levels in normal, alloxan induced diabetic and diabetic treated male albino rats.

Group	Treatment	Urea (mg/dl)	Uric acid (mg/dl)	Creatinine (mg/dl)
I	Normal-Control	31.55±1.32 ^a	2.72±0.11 ^a	0.81±0.06 ^a
II	Diabetic-Control	69.18±2.33 ^b	5.01±0.05 ^b	1.92±0.13 ^b
III	Normal+200mg ASECB	30.38±0.74 ^a	2.79±0.33 ^a	0.82±0.03 ^a
IV	Normal+400mg ASECB	33.51±1.66 ^a	2.91±0.54 ^a	0.83±0.08 ^a
V	Diabetic+200mg ASECB	41.37±3.17 ^a	3.02±0.38 ^a	0.91±0.11 ^a
VI	Diabetic+400mg ASECB	32.19±0.48 ^a	2.71±0.11 ^a	0.84±0.07 ^a

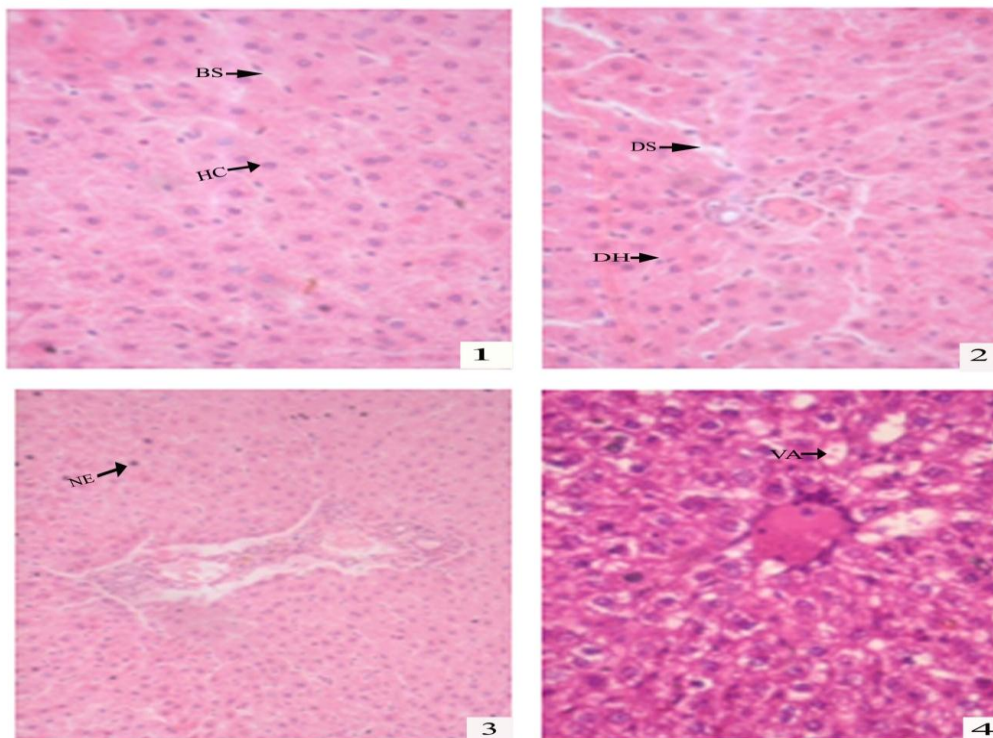
Note: Groups with similar superscript letters (a, b, c) in the given column indicates not significant. While groups with dissimilar superscript letter indicate significantly different from each other.

Table: 2 Histopathological investigations of Liver of various groups and severity degree of different areas affected.

Sl. No.	Microscopic histological regions	Group-I	Group-II	Group-III	Group-IV	Group-V	Group-VI
01	Central vein	Maintained	Moderate	Maintained	Maintained	Mild	Maintained
02	Hepatic cord	Maintained	Mild	Maintained	Maintained	Slight	Maintained
03	Sinusoid	Maintained	Moderate	Maintained	Maintained	Mild	Maintained
04	Hepatic triad	Maintained	Mild	Maintained	Maintained	Slight	Maintained
05	Hepatocytes	Maintained	Severe	Maintained	Maintained	Mild	Maintained

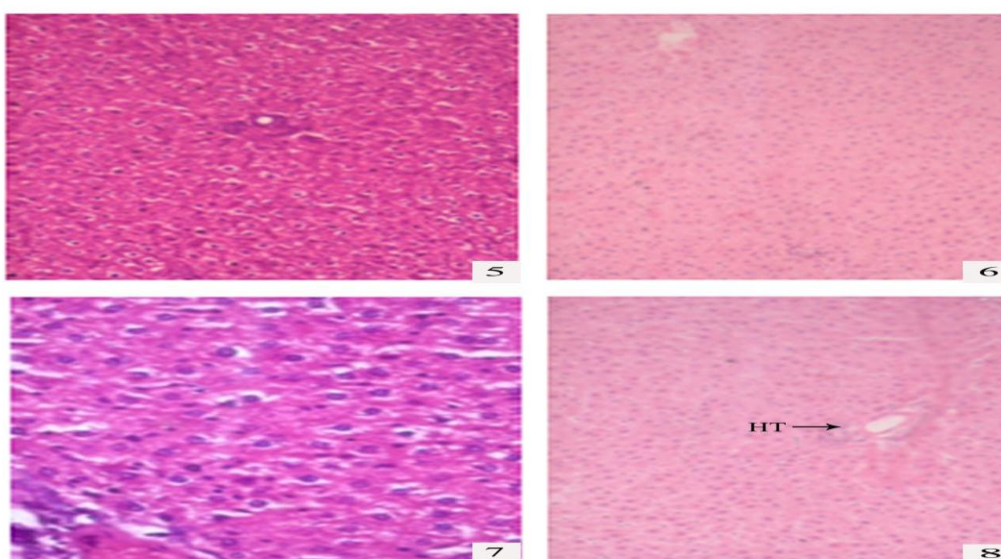
Table: 3 Histopathological investigations of Kidney of various groups and severity degree of different areas affected.

Sl. No.	Microscopic histological regions	Group-I	Group-II	Group-III	Group-IV	Group-V	Group-VI
01	Glomerulus	Maintained	Massive	Maintained	Maintained	Moderate	Maintained
02	Bowman's capsule	Maintained	Moderate	Maintained	Maintained	Slight	Maintained
03	Urinary/Nephric tubules	Maintained	Severe	Maintained	Maintained	Mild	Maintained
04	Collecting duct	Maintained	Mild	Maintained	Maintained	Slight	Maintained



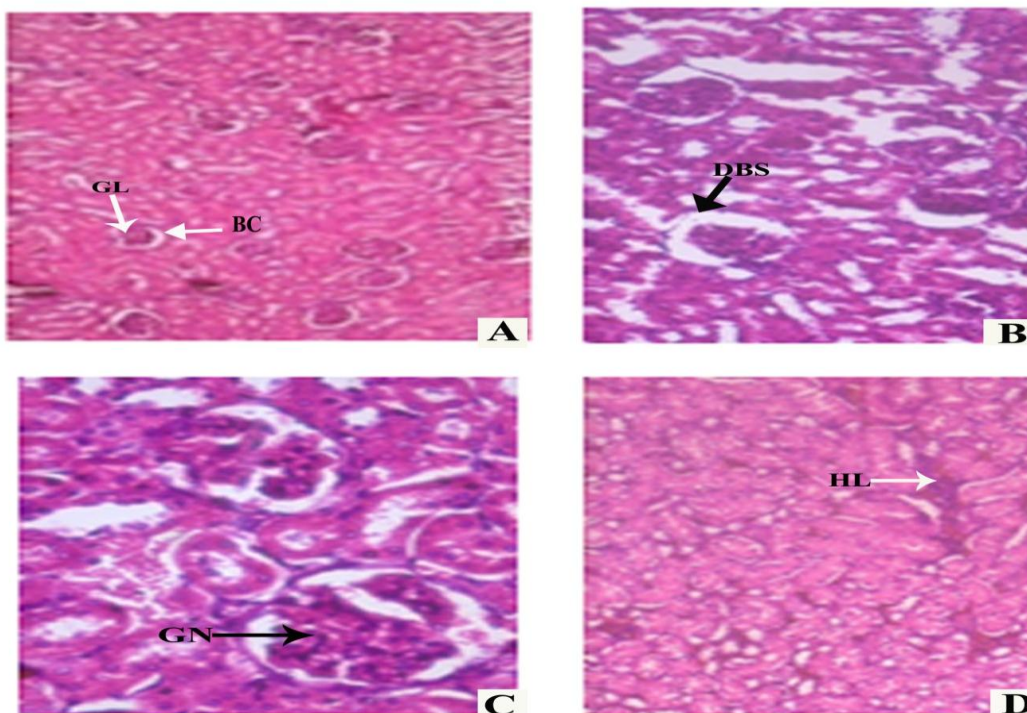
1. Photomicrograph of section of liver of control rat (Group-I) showing normal hepatic cords and hepatocytes with distinct nucleus and cytoplasm (H & E x400)
2. photomicrograph of section of liver of diabetic-control rat (Group-II) showing disarray of hepatic cords and dilation of sinusoids (H & E x400)
3. Photomicrograph of section of liver of diabetic-control rat (Group-II) showing necrosis of hepatocytes (H & E x200)
4. Photomicrograph of section of liver of diabetic rat (Group-II) showing vacuulations and degeneration of hepatocytes (H & E x400)

Abbreviations: BS= Blood sinusoid, HC= Hepatocyte, DS= Dilation of sinusoid, DH= Disarray of hepatic cords, NE= Necrosis, VA= Vacuolation, H & E= Hematoxylin and Eosin

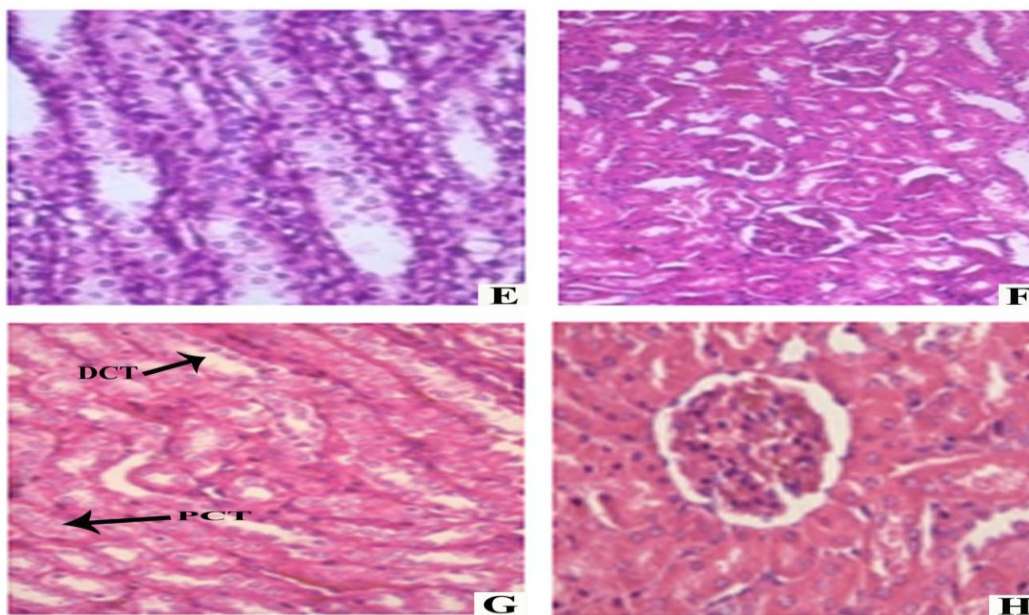


5. Photomicrograph of section of liver of normal rat treated with 200 mg ASECB (Group-III) showing normal histoarchitecture (H & E x400)
6. Photomicrograph of section of liver of normal rat treated with 400 mg ASECB (Group-IV) showing normal histoarchitecture (H & E x200)
7. Photomicrograph of section of liver of diabetic rat treated with 200 mg ASECB (Group-V) showing improvement in arrangement of hepatic cords and hepatocytes (H & E x400)
8. Photomicrograph of section of liver of diabetic rat treated with 400 mg ASECB (Group-VI) showing restoration of histological alteration to normalcy (H & E x200)

Abbreviations: HT= Hepatic triad, H & E= Hematoxylin and Eosin



A. Section of cortex part kidney of control rat (Group-I) showing normal architecture with prominent Bowman's capsule and tubules (H & E, PAS x200)
B. Section of kidney of diabetic-control rat (Group-II) showing degeneration and congestion of glomerular capillaries with increased Bowman's urinary space (H & E, PAS x400)
C. Section of cortex part of kidney of diabetic-control rat (Group-II) showing glomerular necrosis (H & E, PAS x400)
D. Section of cortex part of kidney of diabetic-control rat (Group-II) showing hemorrhagic pathological lesions of proximal uriniferous tubules (H & E, PAS x400)
Abbreviations: GL= Glomerulus, BC= Bowman's capsule, DBS= Dilated Bowman's space, HL= Hemorrhagic lesions, GN= Glomerular necrosis, H&E= Haematoxylin and Eosin, PAS= Periodic acid Schiff



E. Section of kidney of normal rat treated with 200 mg ASECB (Group-III) showing normal histoarchitecture (H & E, PAS x 400)
F. Section of cortex part of kidney of normal rat treated with 400 mg ASECB (Group-IV) showing normal renal corpuscle and uriniferous tubules (H & E, PAS x200)
G. Section of kidney of diabetic rat treated with 200 mg ASECB (Group-V) showing improvement in normal histoarchitecture (H & E, PAS x400)
H. Section of cortex part of kidney of diabetic rat treated with 400 mg ASECB (Group-VI) showing restoration of histological alterations to normalcy (H & E, PAS x600)
Abbreviations: DCT= Distal Convolved tubule, PCT= Proximal Convolved Tubule, H&E= Haematoxylin and Eosin, PAS= Periodic acid Schiff

4. DISCUSSION

C. bonducella has been reported to possess several activities. But the details about the safety of this medicinal plant on vital organs like liver, kidney, heart etc., are scarce. There are less scientific reports on this. In this scenario, in the present study the preventive/protective effect of seed extract of *C. bonducella* on the histology of liver and kidney was investigated. Diabetes has emerged as a major health care problem in all parts of the world particularly in India. Currently there are over 240 million diabetics worldwide and about 40% of them develop severe Diabetic nephropathy (DN).^[28] In India alone approximately 8,50,000 individuals are affected by DN.

In the present work the levels of urea, uric acid and creatinine concentration in diabetic induced rats are elevated. This elevation of the renal markers might have resulted in oxidative stress leading to tissue and cellular damage. Prakasam *et al.*,^[29] and Fekete *et al.*,^[30] have also reported that these parameters are considered significant markers of renal dysfunction. Halliwell and Gutteridge,^[31] also reported that alloxan induced diabetes caused increasing levels of creatinine and urea. Alloxan produces oxygen radicals and oxidative stress in the body. Supplementation of ASECB might have resulted in decrement of these parameters and thus ameliorating renal dysfunction of diabetic rats.

The alloxan causes destruction of pancreatic β -cells and cause severe hypoinsulinaemia that is responsible for hypoglycemia seen in alloxan treated animals. However, its action is not directed to pancreatic β -cells only, as other organs such as liver and kidney are also affected by alloxan administration. Biochemical parameters and studies on beta cells of islets of Langerhans reported earlier from our laboratory^[32, 33] are in correlation with the present histological observation of liver and kidney. Our report of hyperglycemia in diabetic rats may be the cause for histological disturbances seen in diabetes induced rats. Anonymus,^[34] has also reported that hyperglycemia is the principal factor responsible for structural alterations at the renal level. He has made it clear that hyperglycemia is directly associated with diabetic micro vascular complications, particularly in the kidney. Memisogullari *et al.*,^[35] also opined that hyperglycemia is an important factor for kidney damage. The increase of serum protein level in diabetic rats after supplementation of ASECB, which was reported from our laboratory, indicates the improvement of renal function. Further the hepato and reno protective activity of ASECB could be due to regeneration of liver and kidney tissues. Kumar *et al.*,^[36] also reported the hepato protective and antioxidant effects of the methanol extract of *C. bonducella* in wistar albino rats.

Experimental diabetes, induced by chemical agents like alloxan, destroys β -cells of pancreas by generating excess reactive oxygen species and produces kidney lesions that are similar to human DN.^[37] The results

indicate a primary and secondary effect of the diabetic state of the kidney of the rat. The primary effect, the diabetes factor was associated with hyperglycemia and was responsible for dilatation of proximal and distal tubules. The secondary effect, named the individual response factor was associated with inflammatory processes.^[38] In Group-II the kidney was damaged due to alloxan and histoarchitecture was almost normalized after supplementation of ASECB. The restoration of the normal histoarchitecture of the kidney may be due to regenerative ability of kidney tubules due to extract administration. Associated with progress of diabetes a state of decreased total protein concentration is evidenced, which may have resulted from either hyper filtration induced diabetic nephropathy and /or increased protein catabolism.^[29, 39]

In conclusion, the present histopathological and biochemical studies on liver and kidney suggests that ASECB has the ability to improve the liver and kidney functions by ameliorating histopathological lesions associated with diabetes and thus has hepato and reno protective effect in experimentally induced diabetic rats. Although promising results have been obtained, further investigation is required for isolation, identification, biological evaluation and mechanism of action of active principle in the extract responsible for protective effect and treating diabetes.

Conflict of interest statement

We declare that we have no conflict of interest.

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