RESEARCH ARTICLE

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Biochemical and histological changes of *Wrightia tinctoria R. BR.* extracts in alloxan induced diabetic rats.

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ABSTRACT

The aim of the current research work is to evaluate the biochemical changes of fruit extracts of *Wrightia tinctoria* in alloxan induced diabetic rats. Methanolic and ethyl acetate extracts of *W. tinctoria* were obtained by cold maceration and have been administered to alloxan induced diabetic albino rats. Parameters including Blood glucose, Triglycerides, Cholesterol, Protein, Urea, Creatinine, SGPT, SGOT were checked using standard test kits and methods after administration of extracts and found significant improvement. Histological changes in pancreas and liver of the animal were also examined. The study showed significant improvement after giving drug extracts in diabetic rats. The implications of results after administration of the extracts show their potential use in management of diabetes.

Key words: Medicinal plants, Hypoglycemia, Diabetes, *Wrightia tinctoria.*

Introduction

Interest in medicinal plants as a re-emerging health aid has been fuelled by rising costs of prescription drugs in the maintenance of personal health and well-being, and bio prospecting of new plant derived drugs. Based on current research and financial investments, medicinal plants will, seemingly, continue to play an important role as a health aid.[1]The essential values of some plants have long been published, but a large number of them have remained unexplored to date. Therefore there is a need and necessity to explore their uses and to conduct pharmacological studies to ascertain their therapeutic properties. Although herbal medicine are effective in the treatment of various ailments, very often these drugs are improperly used, and only few of them have been validated by scientific criteria.[2]so these plant drugs do need a detailed study in the light of modern medicine. Since the modern world is full of stress, the incidence of diabetes is on increasing trend. By definition, diabetes mellitus is categorized as a metabolic disease characterized by hyperglycemia resulting from defects in insulin secretion and insulin action, or both.[3] Diabetes mellitus is a potential morbid condition with high prevalence worldwide thus the disease constitutes a major health concern.[4]Currently the global prevalence of the disease is around 200 million and would increase to 300 million by 2025 as per WHO estimation [5].In view of the increasing prevalence, there is a growing need to develop integrated approaches towards the management and prevention of Diabetes mellitus by exploring the potentials offered by the traditional phytotherapies. Moreover uncontrolled diabetes appears to involve oxidative stress known to exhibit direct tissue damage properties, which may lead to many complications. It has been already been established that chronic hyperglycemia of diabetes is associated with long term damage, dysfunction and eventually the failure of organs, especially the eyes, kidney, nerves, heart and blood vessels [6].A scientific investigation of traditional herbal remedies for diabetes may provide valuable lead for the development of an alternative drug and therapeutic strategies[7].Experimental evidences suggest the involvement of free radicals in the pathogenesis of diabetes. So plants capable of neutralizing free radicals are effective in preventing diabetes and

reduce the severity of diabetic complications. Phenolics and flavonoids can exert their antioxidant activity by various mechanisms like quenching the free radicals, by chelating metal ions by inhibiting enzymatic systems responsible for free radical generation,[8] so polyphenolics and flavonoids incorporation into diet could contribute to potential management of hyperglycemia and also helps to alleviate complications of organ functions. But selection of inappropriate animal models has been identified as one of the common problem associated with ethno botanical researches [9].

Alloxan and its reduction product dialuric acid establish a redox cycle with the formation of superoxide radicals. These radicals undergo dismutation to hydrogen peroxide. Thereafter, highly reactive hydroxyl radicals are formed by fenton reaction. The action of reactive oxygen species with a simultaneous massive increase in cytosolic calcium concentration causes rapid destruction of beta cells [10] Thus alloxan induced diabetes mellitus served as a pathological bio model for testing a substance with supposed antioxidant activities *in vivo* [11].One of the target s of reactive oxygen species is DNA of pancreatic islets .Its fragmentation takes place in beta cells exposed to alloxan. The increase in oxygen free radicals in diabetic conditions is mainly because of the effect of the diabetogenic agent alloxan. The simplistic argument often made against the use of alloxan to induce type II diabetes mellitus is that, alloxan administration produces beta cells damage and thus leading to type I rather than type II Diabetes mellitus. Alloxan administration in experimental animals has been reported to produce pancreatic lesion which is proportional to the dose of the drug administered. And the size of the lesion also correlates with the pancreatic insulin content [12].This perhaps explains why the drug at a low or medium dose does not produce absolute but insufficient insulin deficiency in experimental animals. Experimental dose of the drug must be carefully selected in order to avoid excessive pancreatic tissue damage [13].

Wrightia tinctoria R.Br. (Apocynacea) is extensively used in the indian system of medicine[14]. This plant is externally used indigenously in indian system of traditional medicine as a remedy for various ailments as stomachic ,skin diseases ,antidiarrhoel, antihaemorrhagic [15],almost every part of plant is useful-leaves pungent chewed for relief from tooth ache ,bark and seeds are antidysenteric, antidiarrhoel and antihaemorrhagic [16]. Oil emulsion of leaves and pods is used to treat psoriasis [17,18].Five flavanoids from leaves indigotin, indrubin, isatin, tryptanthin and rutin were isolated [19].ursolic acid and iso ricinolic acid has been also isolated from the seed pods and seed oil[20].The plant is also reported for its antimicrobial activity, wound healing, hepatoprotective activity[21,22,23,24].

This study was carried to clarify the effect of *W.tinctoria* extracts (Methanol and ethyl acetate) beneficial in the treatment of diabetes on blood glucose, biochemical parameters and their possible effects on pancreatic tissue and liver. The most fascinating phytonutrients flavanoids and phenolics in plant pods that give strong antioxidant activity made this plant to be more concern about the study. Active solvent extracts of the plants are commonly used because they may contain more than one active ingredient and less expensive than a purified single compound. Keeping these facts in view the present study has been undertaken to identify the active antidiabetic extract of *Wrightia tinctoria.*

MATERIALS AND METHODS:

Plant material

Matured fruits of *W. tinctoria* (Family: Apocyanacea) were collected in the month of April 2011 from village Keesara gutta, Ranga Reddy district, Hyderabad, Andhra Pradesh, India. The plant was identified and authenticated by Professor Badraiah, Department of Botany, Osmania University, Hyderabad, India. A voucher specimen-0573 was deposited at herbarium of the Osmania University, Hyderabad for future reference.

Preparation of extracts

The fruits were dried in shade at room temperature and powdered to coarse powder. Extracted using methanol, ethyl acetate, chloroform by simple maceration technique for seven days. The excess solvent was removed using Rotary flash evaporator. The obtained crude extract was stored in air tight container in refrigerator below 10°c for further studies. Ethyl acetate and methanolic extracts were selected for the study based on the preliminary phytochemical evaluation.

The Extracts were administered orally daily to different groups of rats at a dose of 300mg/kg and 200mg/kg body weight of methanolic and ethyl

acetate extracts respectively. The dose was fixed according to the toxicity studies (OECD guidelines).In toxicity studies no mortality observed up to 3000mg/kg, for methanolic and 2000mg/kg for ethyl acetate extract, so these doses were considered as the maximum tolerated dose. From this 1/10 of the dose was selected for further pharmacological studies.

Alloxan Monohydrate: Alloxan monohydrate was used in physiological saline. The solution was injected as a single dose of 120mg/kg intra-peritonially with in 50-75 seconds.

Experimental animals: Albino male rats (wistar strain) weighing between 150 - 200g were used in this investigation. Animals were purchased from the National Institute of Nutrition C/O Teena biolabs Pvt. Ltd. for the experimental purpose. Experimental procedure was approved by the Institutional Animal Ethical Committee (IAEC) of Gokaraju and Rangarju College of Pharmacy, Osmania University, Hyderabad. Constituted for the purpose of CPCSEA Govt. of India (Registration number 177/99 /CPCSEA 6th Aug 2011) and all the procedures were followed as per rules and regulations. All the animals were kept for acclimatization for 2 weeks under laboratory conditions and fed with pellet diet and tap water *ad libitum*.

Induction of Experimental diabetes: The diabetes was induced in rats as described by Trivedi et al. (25) animals were allowed to fast for 18 hrs prior to injection with freshly prepared solution of alloxan monohydrate 120 mg/kg, I.P. The rats were given 5% glucose solution in the cages to prevent hypoglycemia. After 5 days, the rats with fasting serum glucose levels more than 250mg/dl were considered as diabetic and were used in subsequent experimental procedures.

Experimental Design: The rats were divided in to two groups normal animals and hyperglycemic induced animals. The hyperglycemic rats were divided into 4 groups consisting of six animals each.

Group 1: Normal control
Group 2: Diabetic control
Group 3: Diabetic rats treated with 10mg/kg of glibenclamide orally
Group 4: Diabetic rats treated with 300mg/kg (body weight) methanolic extract orally.
Group 5: Diabetic rats treated with 200mg/kg (body weight) ethyl acetate extract orally.

Group 1 and 2 animals were fed with 2% carboxy methyl cellulose. Group 3 animals were treated with standard drug glibenclamide dissolved in 2% carboxy methyl cellulose. Group 4 and 5 animals were treated with methanolic and chloroform extract of plant drug dissolved in 2%carboxy methyl cellulose daily from the day 1 to 14.

Blood glucose level and body weight were monitored at regular intervals for 2 weeks. Animals were sacrificed after 14 days of treatment four hours after dosing. Blood was collected and serum was separated by centrifugation at 5000 rpm for 20 min. collected serum was used for biochemical analysis.

Biochemical estimation:

Serum glucose level was determined according to Trinder et al [26], total protein was determined according to Lowry et al [27].Triglycerides, Cholesterol, Urea, Creatinine were determined according to the procedures given in the kits. SGPT and SGOT were evaluated using Reitman and Frankel [28]

Histopathological Examination:

Pancreas and liver isolated were subjected to histopathological examination fixing in 10% Formalin. Sections were cut at 5µm thickness and stained with Haematoxylin and eosin [29]. Microscope examination of sections was then carried out for histological changes [30]

Statistical analysis:

The values were expressed as mean ± SEM. The obtained results were statistically analyzed using One-Way analysis of varience (ANOVA) followed by student-Newman Keuls Multiple comparison test. values with $p<0.05$ were considered as significant.

Results:

1. Effect of Methanolic extract and Ethyl acetate extract of pods of Wrightia tinctoria (WTM and WTEA respectively) on body weight profile of alloxan induced diabetic rats.

Group	Initial body weight(gms)	Final body weight(gms)
Diabetic Control	229.16±10.03	178.33±8.72
Diabetic+Standard	229.16±10.03	206±8.21*
Diabetic+WTM	225±9.12	215±9.12*
Diabetic+WTEA	222.5±9.46	222.5±9.46***

***p<0.001**p<0.01*p<0.05 NS-Non significant

Body weight:

There was a significant reduction in body weight of the animals in diabetic group. After administration of methanol and ethyl acetate extract of W.tinctoria

for 14 days, the body weight was recovered significantly (p<0.05)by methanolic extract and (p<0.001) by ethyl acetate extract compared to diabetic control.

Some biochemical parameters in alloxan diabetic rats 14 days from the beginning of the experiment (Mean±SEM)

2. Effect of Methanolic extract and Ethyl acetate extract of pods of Wrightia tinctoria (WTM and WTEA respectively) on glucose levels of alloxan induced diabetic rats

Group	Initial glucose level 0f diabetic rats	Final glucose level 0f diabetic rats	Percentage reduction
Diabetic Control	292.489±3.95	258.296±3.415	11.6%
Diabetic+Standard	329.313±8.226	187.106±6.00***	43.18%
Diabetic+WTM	316.533±5.82	122.263 ±3.33***	61.37%
Diabetic+WTEA	300.391±8.35	132.672±2.99***	55.83%

***p<0.001**p<0.01*p<0.05 NS-Non significant

3. Effect of Methanolic extract and Ethyl acetate extract of pods of *Wrightia tinctoria* (WTM and WTEA respectively) on lipid profile of alloxan induced diabetic rats

Group	Total cholesterol(mg/dl)	Total protein(g/dl)	Triglycerides(mg/dl)
Diabetic Control	117.3±3.29	5.15±0.18	185.6±8.22
Diabetic+Standard	72.55±2.09***	6.62±0.14***	110.78±2.18***
Diabetic+WTM	90.06±1.81***	7.09±0.06***	111.76±5.29***
Diabetic+WTEA	61.03±1.98***	7.12±0.04***	68.05±5.42***

***p<0.001**p<0.01*p<0.05 NS-Non significant

4. Effect of Methanolic extract and Ethyl acetate extract of pods of Wrightia tinctoria (WTM and WTEA respectively) on SGPT and SGOT levels of alloxan induced diabetic rats.

Group	SGPT(U/L)	SGOT(U/L)
Diabetic Control	75.33±2.02	186.08±5.57
Diabetic+Standard	27.4±1.47***	60.14±4.69***
Diabetic+WTM	55.23±0.97***	105.91±9.92***
Diabetic+WTEA	45.78±0.61***	130.28±4.64***

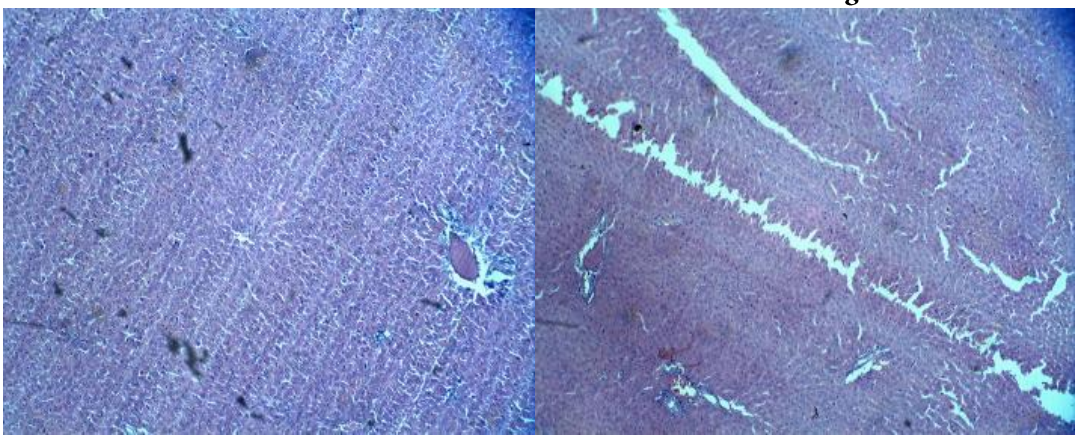
*****p<0.001**p<0.01*p<0.05 NS-Non significant

5. Effect of Methanolic extract and Ethyl acetate extract of pods of *Wrightia tinctoria* (WTM and WTEA respectively) on Urea and Creatinine levels of alloxan induced diabetic rats.

Group	Urea(mg/dl)	Creatinine(mg/dl)
Diabetic Control	37.15±2.88	0.96±0.05
Diabetic+Standard	18.38±0.89***	0.62±0.01***
Diabetic+WTM	17.25±0.49***	0.84±0.02**
Diabetic+WTEA	26.01±1.35***	0.58±0.02***

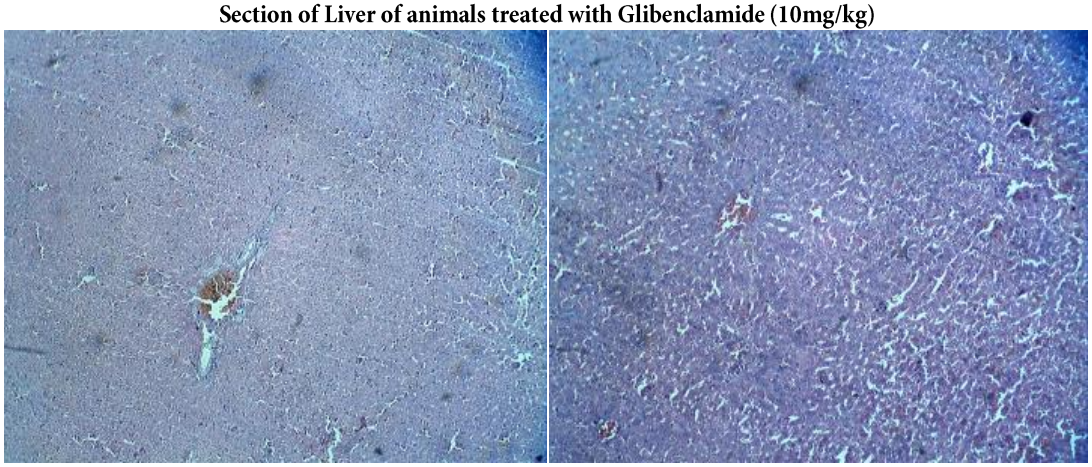
***p<0.001**p<0.01*p<0.05 NS-Non significant

Sections of the liver tissue of animals treated with extracts of *Wrightia tinctoria*.

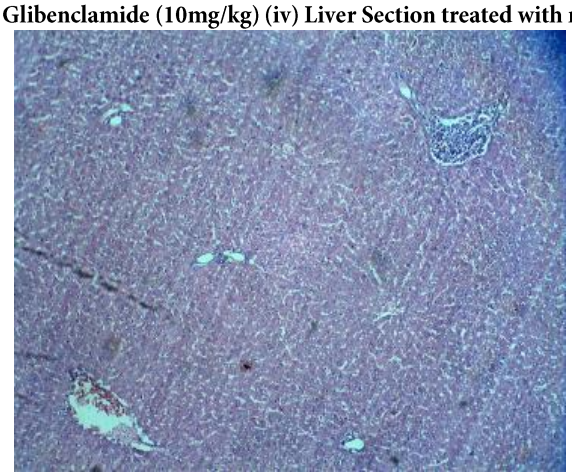


(i)Liver Section control showing normal islets (ii) Liver Section alloxan treated necrosis of islets (120mg/kg i.p)

Section of Liver of animals treated with Glibenclamide (10mg/kg)



(iii)Liver section treated with Glibenclamide (10mg/kg) (iv) Liver Section treated with methanolic (300mg/kg) extract

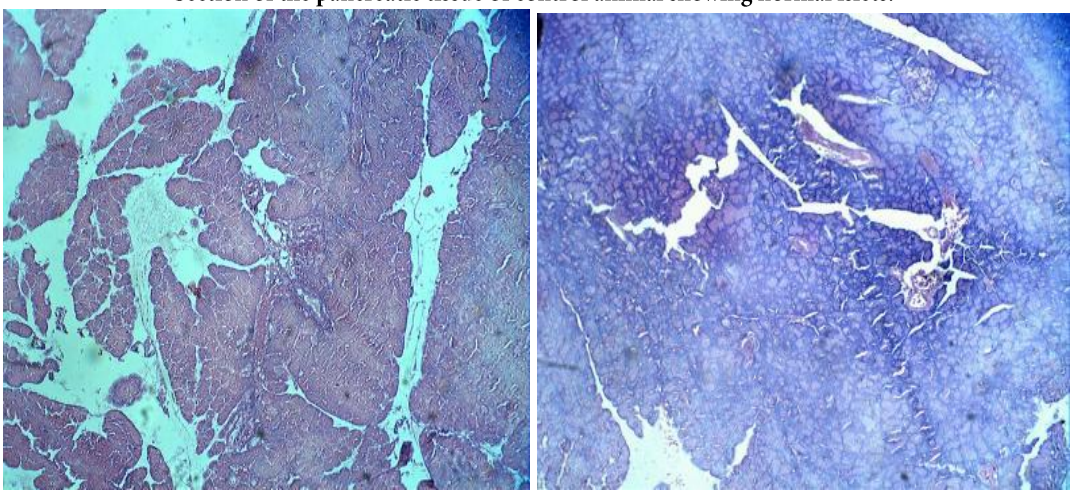


(v) Section of the liver of animals treated with ethyl acetate (200mg/kg) extract.

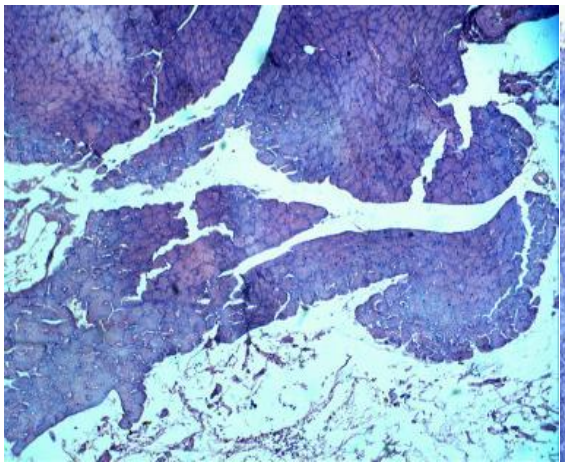
Sections of the pancreatic tissue of animals treated with extracts of *Wrightia tinctoria.*

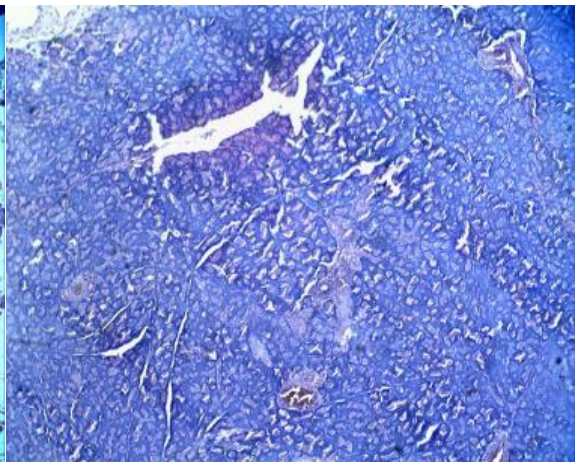
Pancreas fig:

Section of the pancreatic tissue of control animal showing normal islets.

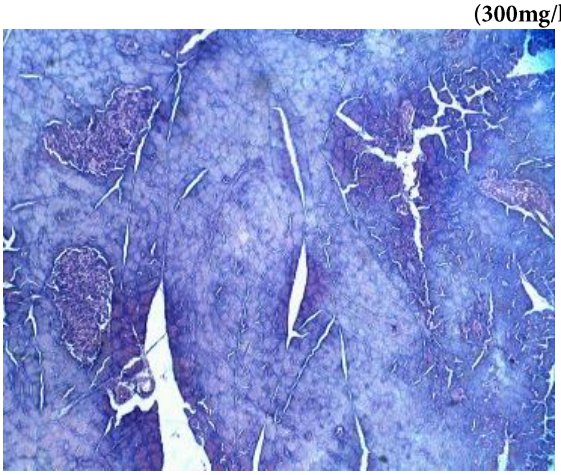


(i) Pancreatic tissue section control showing normal islets (ii) Pancreatic tissue alloxan treated showing necrosis of islets (120mg/kg i.p)





(iii)Pancreatic tissue treated with Glibenclamide(10mg/kg) (iv) (Pancreatic tissue treated with methanolic (300mg/kg) extract



(v) Section of the pancreatic tissue of animals treated with ethyl acetate (200mg/kg) extract.

DISCUSSION:

Body weight:

In diabetic rats, decrease in body weight was observed. This indicates the polyphagic condition and loss of weight due to excessive breakdown of tissue proteins. The decrease in body weight in diabetic rats could be due to dehydration and catabolism of fats and proteins, which further leads to muscle wasting might also be the reason for the reduced body weight in diabetic rats.

Oral administration of extracts for consecutive 14 days to diabetic rats improves body weight .This could be due to better control of hyperglycemic state in the diabetic rats. Reversal of weight loss in extract treated diabetic group indicates the restorative effect of the extract which may be due to reversal of gluconeogenesis and glycogenolysis.

Bio chemical parameters:

In diabetic animals, insulin deficiency leads to various metabolic alterations in animals viz., increased blood glucose, increased cholesterol, Triglyceride, SGPT, SGOT levels. Treatment with extracts reduced glucose levels and also reduced Triglycerides, Cholestrol, SGPT, SGOT levels thus reducing the complications caused due to diabetes. Confirms the possibility that major function of the extract is on the protection of vital tissues liver and pancreas, thereby reducing the causation of diabetes in the experimental animals. The present study also indicates that *W. tinct*oria can partially inhibit alloxan renal toxicity as observed from serum urea and creatinine levels.

Histological changes:

Histopathological Evaluation of pancreas:

Diabetic rats revealed degeneration and lytic changes in islets of langerhans of pancreas. It was also observed that the islets were shrinken, inflammatory cellular infiltration with fibrosis. Treatment of these diabetic rats with glibenclamide inhibited alloxan induced shrinking of islets of langerhans of the pancreas, inflammatory cellular infiltration and enlarged pancreatic cells. Treatment with *Wrightia Tinctoria* extracts (Both methanolic and ethyl acetate extracts) reversed all the effects of alloxan linear and dose dependently and supports biochemical tests.

Histopathological Evaluation of Liver:

Liver sections showed necrosis and reduction in the number of cells due to decrease in the antioxidant defense after inducing diabetes with alloxan. Treatment with *Wrightia Tinctoria* extracts (Both methanolic and ethyl acetate extracts) reversed all the effects of alloxan linear and dose dependently and supports biochemical tests.

Conclusion:

The results obtained from current investigation revealed that methanolic and ethyl acetate extracts of matured fruits of *Wrightia tinctoria* possesses significant anti-diabetic activity and these extracts have shown major function on the protection of vital tissues like liver and pancreas, inhibiting renal toxicity thereby reducing the causation of diabetes in the experimental animals.

Hence the presence of natural anti oxidants in both the extracts of fruits of *W. tinctoria.* are known to be bioactive anti-diabetic principles, which strengthen the endogenous antioxidant defenses against the Reactive Oxygen Species and restore the optimal balance. In this context *W. tinctoria* can be mentioned as a plant of considerable importance for Further studies to fractionate the active principles and to elucidate the exact mechanism of action are, therefore, required to be undertaken.

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