HISTOPATHOLOGICAL STUDIES OF AQUEOUS METHANOLIC LEAF AND BARK EXTRACT OF DOLICHANDRONE ATROVIRENS

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ABSTRACT

The present study was aimed to investigate the complications of untreated diabetes on histomorphology of rats. Diabetes mellitus was experimentally induced in male wister rats by administration of streptozotocin. The establishment of diabetes mellitus was confirmed by fasting blood glucose levels. The animal was divided into five groups. Group one rats served as healthy controls that received vehicle in a similar manner other four is treated by the aqueous methanolic leaf and bark extract Dolichandrone atrovirens given in the dose of 200 mg/kg, p.o and 400 mg/kg, p.o. For histomorphological study of different organs, 50% of the animals were sacrificed after 14 days. The blood glucose level of diabetic rats was raised significantly throughout the experimental period. Further, histomorphological alterations were registered in kidneys and liver. The results show gross examination of liver and kidney did not show any abnormalities. It is clear from these results that Dolichandrone atrovirens leaves extract and bark is free from hepatic and renal toxicity.

INTRODUCTION

Diabetes Mellitus (DM) is a chronic disorder of carbohydrate, fat and protein metabolism characterized by increased blood glucose Level (hyperglycemia) resulting from defects in insulin secretion, insulin action, or both. It is one of the alarming worldwide health problems at present leading to micro vascular (retinopathy, neuro-pathy and nephropathy) and cardiovascular (heart attack, stroke and peripheral vascular disease) complications.[1-4] Diabetes mellitus is increasingly common metabolic disorder and one of the five leading causes of death in the world. It is projected that more than 300 million people will have the
disease by the year 2025 to 2030.$^{[5-7]}$ Diabetes increase the oxidative stress by several mechanisms resulting in cell injury and is implicated in the pathogenesis of vascular disease which are the mainly cause of morbidity and mortality this will leads to one of the five leading causes of death in the world.$^{[8, 9]}$

Herbal medications have a long history to treat people and recent two decades the use of natural health products as complementary or alternative approaches to existing medications is growing in popularity.$^{[10-12]}$ Traditional medicines derived mainly from plants have plays major role in the management of diabetes mellitus$^{[13-15]}$ and many herbal medicines possess considerable anti-diabetic potentials.$^{[16, 17]}$ The World Health Organisation survey indicated that about 70-80% of the world’s population rely on non conventional medicine, mainly of herbal source in the primary health care.$^{[18]}$ Experimental screening method is, therefore, important to ascertain the safety and efficacy of herbal products as well as to establish the active components of these herbal remedies.$^{[19]}$ To determine the safety of the plant products for human use, toxicological evaluation such as hepatotoxicity, CNS toxicity and renal toxicity to be carried out in various experimental animals to predict the toxicity and to provide guidelines for selecting a ‘safe’ dose in humans.$^{[20]}$ Interspecies differences in the pharmacokinetic parameters make it difficult to translate some adverse effects from animals to humans. Nevertheless, the evaluation of adverse effects in experimental animals may be more relevant in determining the toxicity of the plant preparation.$^{[21]}$ Histopathology the microscopic study of diseased tissue is an important tool in anatomical pathology, since accurate diagnosis of cancer and other diseases usually requires histopathological examination of samples. Histopathology makes two important contributions to clinical medicine and in drug discovery Histopathology makes two important contributions to clinical medicine and in drug discovery.$^{[22]}$

*Dolicandrone atrovirens* (Bignoniaceae) is a medicinal plant that grows throughout tropical parts of Indian subcontinent, particularly in sandy soils of river beds in south India and other parts of Tamil Nadu. *Dolicandrone atrovirens* leaf and bark are an important medicinal plant used widely in Indian folk medicine. Since the aqueous methonalic extract showed remarkable antioxidant and antidiabetic activity,$^{[23,24]}$ the aim of this experiment was to identify histopathological abnormalities that occur with the prolonged Streptozotocin-induced diabetes in rats.
MATERIALS AND METHODS

Plant material
The leaves and bark of *Dolichandrone atrovirens* were collected from Chitheri hills, Salem in the month of November 2009. The plant was then authenticated and a voucher specimen is kept for further reference.

Preparation of extract
The shade dried coarse powders of the plant material (1.5 kg) were extracted with 80 %v/v aqueous methanol by maceration at room temperature for 72 h and the extract was filtered, concentrated to dryness in a rotavapor under reduced pressure and controlled temperature (40-50 °C). The extractive values and nature of the leaf and bark extracts of *Dolichandrone atrovirens* were administered orally to the experiments.

Experimental animals
Healthy Adult male albino rats (150-160g) were used for sub acute toxicity and were fed with standard animal pellet diet and water ad libitum. The experiment protocols received clearance from the Institutional Animal Ethical Committee (IAEC) and CPCSEA, Chennai, India.

Subacute toxicity
Current study follows the OECD guideline 407. Development of induced diabetes mellitus was confirmed on the fifth day after Streptozotocin administration by examining the glucose level in the blood taken from the tail vein. An experiment was conducted in male albino rats (150-160g) of uniform age. The rats consisted of five groups of six each.

Group 1 - Control treated with 0.3% sodium CMC (2 ml/kg, p.o)
Group 2 – Methanolic leaves extract of *Dolichandrone atrovirens* (200 mg/kg, p.o)
Group 3 - Methanolic leaves extract of *Dolichandrone atrovirens* (400 mg/kg, p.o)
Group 4 – Methanolic barks extract of *Dolichandrone atrovirens* (200 mg/kg, p.o)
Group 5 - Methanolic barks extract of *Dolichandrone atrovirens* (400 mg/kg, p.o)

After completion of the study period, blood samples were harvested from overnight fasted animals after 24 h of the last dose of plant extracts and analyzed the hematological parameter. The animals were sacrificed and the vital organs such as liver, kidney, heart, lungs and spleen were subjected to gross examination and later weighed. Since liver and kidney are organs of metabolism and excretion, potentially toxic agents are likely to have an impact on them. So, portions of these organs were fixed in buffered 10% formalin and 5 µm thick paraffin
sections were made and stained with haemotoxylin and eosin for histopathological examination.

RESULT

Figure 1: Liver of control group showing normal architectural pattern of hepatic cords. H&E x 400

Figure 2: Liver of Dolichandrone atrovirens leaves extract 200 group showing normal architectural pattern of hepatic cords. H&E x 400

Figure 3: Liver of Dolichandrone atrovirens leaves extract 400 group showing normal architectural pattern of hepatic cords. H&E x 400

Figure 4: Liver of Dolichandrone atrovirens barks extract 200 group showing normal architectural pattern of hepatic cords around portal triad. H&E x 400

Figure 5: Liver of Dolichandrone atrovirens barks extract 400 group showing normal architectural pattern of hepatic cords around portal triad. H&E x 400
Figure 6: Kidney of control group showing normal architectural pattern of glomerulus and tubules. H&E x 400

Figure 7: Kidney of Dolichandrone atrovirens leaves extract 200 group showing normal architectural pattern of glomerulus and tubules. H&E x 400

Figure 8: Kidney of Dolichandrone atrovirens leaves extract 400 group showing normal architectural pattern of glomerulus and proximal tubules with brush borders. H&E x 400

Figure 9: Kidney of Dolichandrone atrovirens barks extract 200 group showing normal architectural pattern glomerulus and tubules. H&E x 400

Figure 10: Kidney of Dolichandrone atrovirens barks extract 400 group showing normal architectural pattern of glomerulus and proximal tubules with brush boarders. H&E x 400
DISCUSSION AND CONCLUSION

Herbal drugs are prescribed widely because of their effectiveness, less side effects and relatively low cost. Therefore, investigation on such agents from traditional medicinal plants has become more important. These studies show that, the liver of animals treated with Dolichandrone atrovirens leaves extract and Dolichandrone atrovirens bark extract showed normal histopathological features and the absence of both degeneration of hepatocytes and focal steatosis. Also there was absence of congestion of the central vein and inflammation of portal tract when compared with controls. The kidney of treated animals showed normal glomeruli, with no necrosis of tubular epithelium when compared with controls. At the same
time the gross examination of liver and kidney did not show any abnormalities. It is clear from these results that *Dolichandrone atrovirens* leaves extract and bark are free from hepatic and renal toxicity.

**REFERENCE**


