

**Short Communication**

**HISTOMORPHOMETRIC ANALYSIS IN KIDNEY TISSUE OF MICE TREATED WITH *INDIGOFERA SUFFRUTICOSA* MILL**

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**ABSTRACT**

**Objective:** To investigate by histomorphometry the effect of aqueous extract of *Indigofera suffruticosa* leaves on proximal convoluted tubules and renal corpuscles of mice with sarcoma 180.

**Methods:** Male swiss albino mice (*Mus musculus*) with 60 days of age were divided into 3 groups of 6 animals: Group 1-sarcoma 180 treated with aqueous extract (50 mg/kg, intraperitoneally (i. p.)), Group 2-sarcoma 180 treated with saline solution (15 ml/kg, i. p.) and Group 3-without sarcoma 180 treated with saline (15 ml/kg, i. p.). After 48 hours of tumor implantation, the treatment was initiated during 7 days. Histomorphometric analysis of kidney tissue was performed using IMAGEJ software.

**Results:** Antitumoral treatment with aqueous extract of *I. suffruticosa* leaves at a dose of 50 mg/kg has not caused mice renal tissue histological changes.

**Conclusion:** After treatment with aqueous extract of leaves of *I. suffruticosa*, renal architecture was preserved, suggesting its safety use as an antitumor agent.

**Keywords:** Histomorphometry, *Indigofera suffruticosa*, Kidney, Sarcoma 180.

According to the World Health Organization (WHO), about 80% of the world's population makes use of medicinal plants as the main treatment alternative in primary care [1]. Throughout human history, natural products have been used as a resource to cure diseases. Knowledge about plant popular use and the effectiveness significantly contributes to therapeutic properties spread, although chemical constituents sometimes are not known [2].

*Indigofera suffruticosa* Mill (Fabaceae) occurs in abundance in Northeastern Brazil and has intense popular use in treating infections, inflammation and other processes, with no reports of harmful side effects to humans. This species has compounds in its chemical constitution such as flavonoids derived from quercetin glycosides and alkaloids [3].

Pharmacological studies have shown that aqueous extract of leaves of *I. suffruticosa* have the following properties: anti-inflammatory [4], antiparasitic [5], embryotoxic [6], antimicrobial [7], anti-tumor [8], cytotoxic [9], embryotoxic on *Aedes aegypti* eggs and larvae and repellent to *Aedes aegypti* mosquitoes [10].

Many pharmaceutical products are lipophilic, which permits the drug to go through cellular membranes or to the target tissue. The same chemical property which enhances the drugs bioavailability can also hinder their renal excretion, since kidney debugging requires these drugs to become more hydrophilic. Thus, biotransformation reactions often increase compounds hydrophilicity, in order to make them more susceptible to renal excretion [11].

Computer new methods are being developed to evaluate tissue micro-architecture [12], such as histomorphometry, which is a technique that allows assessing tissue structure by quantitative analysis [13]. It is considered a well-established and valuable research tool for metabolic diseases study [14]. Its measurement and quantification, through statistical parameters, gives information that enables operation and organization understanding of the tissue or organ.

This study analyses the effect of aqueous extract of *I. suffruticosa* on renal tissue of mice, and it shows the importance of investigating

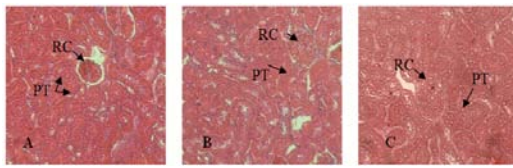
pharmacological effects of natural compounds in metabolizing organ like kidney. The lack of toxicological studies relating the effect of *I. suffruticosa* on morphological changes mainly using quantitative parameters as histomorphometry analysis highlights the importance of this proposal, suggesting its safety use as an antitumor agent.

*Indigofera suffruticosa* species was collected in Nova Cruz city, state of Pernambuco, in July 2013. The sample is cataloged under the number 43694 in Federal University of Pernambuco (UFPE) Herbarium. Leaves were reduced to small fragments (300g) and macerated for extract preparation. After 24 hours, the plant material was subjected to aqueous extraction, and the extract was concentrated through a lyophilizer. After total water extraction, dry weight and yield were determined. Swiss albino mice (*Mus musculus*) male young adults with 60 days of age and weighing on average 30 to 40g were used. Mice were kept in polypropylene cages in central air conditioning, with 12 hours light/dark cycle. Experiments were conducted with the approval of the ethics committee on animal experimentation of the Federal University of Pernambuco (UFPE), process No. 500/12, October 2012.

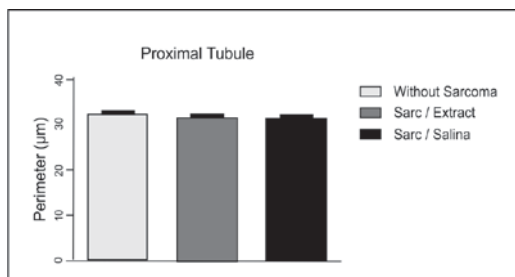
Sarcoma 180 ascitic form was induced in the experimental group (G1 and G2) by injection into animals' right axillary area containing 0.3 ml (approximately  $3 \times 10^6$  cancer cells). After 48 hours of tumor implantation, treatment was initiated. *Indigofera suffruticosa* aqueous extract (50 mg/kg) was administered intraperitoneally (i. p.) for 7 consecutive days in group G1, while groups G2 and G3 received saline 15 ml/kg i. p. for the same period.

At the end of treatment, animals were sacrificed and renal tissues fragments were fixed in 70% alcohol and stained by Hematoxylin and Eosin (H. E). Photomicrographs of histological preparations were taken through an optical microscope with a camera attached. Images were projected and enlarged, having a final increase of 790  $\mu$ m. Histomorphometric analysis of proximal convoluted tubules perimeters and renal corpuscle diameter was performed using the IMAGEJ software. For statistical analysis, the Kruskal-Wallis test ( $p < 0.05$ ) was used and data were expressed as mean and standard deviation.

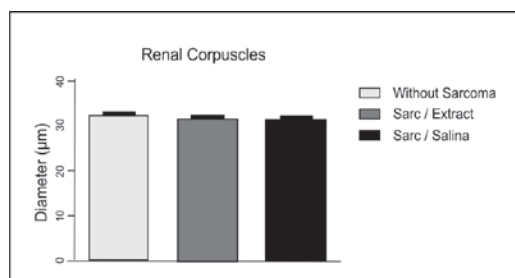
Fig. 1 (A, B and C) shows the histomorphology and fig. 2 and 3 show histomorphometric analysis of proximal tubules and the renal corpuscles of mice. Sarcoma 180/aqueous extract of *I. suffruticosa* (50 mg/kg i. p) (A); Sarcoma 180/saline (15 mg/kg i. p) (B); without Sarcoma 180/saline (15 mg/kg i. p) (C).



**Fig. 1: Histomorphology of renal tissue. G1 (A) Sarcoma 180/aqueous extract (50 mg/kg) i. p.; G2 (B) Sarcoma 180/saline i. p.; G3 (C) without sarcoma 180/saline. Proximal tubules-PT; RC-Renal Corpuscle. Magnification of 400X**



**Fig. 2: Histomorphometry of renal proximal convoluted tubules of mice (n=150). Data are expressed as mean and standard deviation and analyzed through Kruskal-Wallis test ( $p < 0.05$ )**



**Fig. 3: Histomorphometry of renal corpuscles of mice (n=150). Data are expressed as mean and standard deviation and analyzed through Kruskal-Wallis test ( $p < 0.05$ )**

The histomorphological analysis of renal tissue shows the maintenance of cellular architecture in group treated with aqueous extract and the group without sarcoma/saline; however, sarcoma 180/saline group presents cellular disarray with hypertrophied nuclei. The histomorphometric analysis of the perimeters of proximal tubules and renal corpuscles did not show statistical difference between the groups. Histological studies are important tools to detect histophysiological changes in gastrointestinal tract functions. Studies on histology and renal morphology are common in literature, revealing important role especially when it is wanted to assess new substances with nephrotoxic potential. However, no published articles with renal tissue histomorphological assessment of mice treated with *Indigofera suffruticosa* extracts were found. Similar studies using the same species of *Indigofera* demonstrated that the plants contain pharmacological substances with hepatoprotective properties [17, 18]. The kidney is a very important organ and plays an important role in blood pressure regulation and body fluid volume in acid-base balance, and in certain hormones formation and release. [15, 16].

In this study, no histological alterations were identified in the renal tissue of sarcoma 180-bearing mice treated with daily doses of aqueous extract of leaves of *I. suffruticosa* (50 mg/kg i. p.) when

compared to the control. There were no changes in the proximal convoluted tubules perimeter and renal corpuscles diameter.

This study showed that the aqueous extract obtained by maceration, when used in mice with sarcoma 180 treatment, did not induce any histomorphometric changes in the proximal convoluted tubules and renal corpuscles, suggesting a low nephrotoxic effect order.

#### ACKNOWLEDGEMENT

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#### COMPETING INTERESTS

The authors declare no competing interests.

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