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*Full Length Research Paper*

## **Antioxidant and cicatrizing activity of the species *Abarema cochliacarpus* (Gomes) Barneby & J. W. Grimes**

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In equine clinics, skin injuries are some of the most frequently treated injuries, this research evaluated the healing potential and antioxidant activity of *Abarema cochliacarpus* in order to develop a pharmaceutical formulation for treating skin lesions in horses. Firstly, the antioxidant activity of several fractions was evaluated using the 2,2-diphenyl-1-picrylhydrazyl radical method and the thiobarbituric acid test. The determination of phenolic compounds was done by means of the Folin-Ciocalteu test. To assess the wound healing activity the dry powdered stem bark and the aqueous, ethanolic and propylene glycol extracts from the same plant, material were incorporated in a gel formulation and evaluated in equines with induced wounds. Results show that all obtained extracts and their fractions have antioxidant action. Regarding the healing evaluation in equines, the extracts tested and the dry powdered stem bark showed statistically significant activity in the contraction of wounds. Histological analysis revealed the powdered stem bark as the best treatment to induce healing of cutaneous lesions in horses. Experimental studies in animals with different dosages and formulations, besides the isolation of its chemical components, as a tool in the discovery of new healing agents or optimization of the existing ones is recommended.

**Key words:** *Abarema cochliacarpus*, antioxidant activity, wound healing activity.

### **INTRODUCTION**

*Abarema cochliacarpus* (Fabaceae) is a native tree from Brazil (popularly called in this country by "barbatimão") found mainly in the Atlantic forest and Caatinga. Many

traditional communities in northeast Brazil use its bark in folk medicine (Agra et al., 2008; Pereira et al., 2013). An ethnopharmacological survey in a Brazilian traditional

community has documented popular uses of this species to treat inflammatory disorders, including ulcerations, and for wound healing purposes (Tenório et al., 2016).

The stem bark used in the preparation of herbal medicines, comprise chemical compounds that include alkaloids, flavonoids, terpenes, stilbenes, steroids, protease inhibitors, and tannins. The latter class of secondary metabolites comprises the major components of *A. cochliacarpus* and have been regarded as the main responsible compounds for its antioxidant activity, as they can scavenge free radicals (Shimizu et al., 2009).

Plant extracts thus represent an important alternative to traditional medicine for the treatment of wounds, because they can act on the surface of the lesion, and through anti-inflammatory activity, create a micro-environment that facilitates fibroplasia. The lesions may heal faster because the wound is decontaminated by the extract's antimicrobial effects, or through changes of pH in the wound, which create an unfavorable environment for the growth of micro-organisms (Dias et al., 2013).

In equine clinics, skin injuries are some of the most frequently treated injuries, affecting mainly the locomotor limbs. They occur due to the animals' active behavior and rapid reactions, especially in horses involved in sporting activities (Paganela et al., 2009). Horses have sudden reactions that can lead to limb trauma. In addition, inadequate facilities and grazing are also crucial factors for the occurrence of these lesions. Incorrect treatments and the tendency to chronicity of the lesions also hamper the cicatricial process (Viana et al., 2014). Given the interest in development of herbal medicines for the treatment of wounds, and the high incidence of equine skin lesions. The research aims to assess the phytochemical profile, antioxidant activity, and effects of powdered stem bark, natrosol gel formulation obtained from aqueous, ethanolic and propylene glycol extracts of the stem barks of *A. cochliacarpus*, on healing of skin wounds in horses.

## MATERIALS AND METHODS

### Study type and setting

The experiment involved both *in vitro* and *in vivo* laboratory evaluation. The research was conducted in the multidisciplinary research laboratory of the Centro Universitário Cesmac, located in Maceió, Alagoas; Brazil, and in the Clinical Veterinary School of the same institution, which is located in the city of Marechal Deodoro, Alagoas, Brazil.

### Ethical aspects

This study was approved by the Ethics Committee for the use of

animals (CEUA) of the Federal University of Alagoas with opinion substantiated n° 43/2012.

### Collection and identification of botanical material

The specimen was collected in the municipality of Marechal Deodoro, Alagoas, Brazil. Three samples were collected for botanical identification the specimen was identified at the local of collection by a botanist and samples from stem and leaves were stored in the Herbarium of the Environment Institute, in Alagoas, under MAC registration number: 25.370. The stem bark was dried in the shade at room temperature and powdered afterwards by means of a mill that was used in the preparation of the extract. The stem bark powder was then stored in a tightly sealed dark container. Aliquots of this material were used for incorporation into the gel of natrosol, production of extracts, and phytochemical screening.

### Preparation of extracts

#### *Aqueous extract*

Extracts from the bark of the stems of *A. cochliacarpus* were obtained by decoction over 30 min, in a proportion of 100 g of dry powdered bark for each 1000 ml of distilled water. The aqueous extract was then stored at room temperature until it was incorporated into the natrosol gel via the method of Verza (2006).

#### *Propylene glycol extract*

To obtain this extract, the powdered material was macerated with an 80% mixture of propylene glycol in distilled water for 24 h. After this period, the extract was filtered in a filter paper (Whatman No 1). The propylene glycol extract was further stored at room temperature until its incorporation into the natrosol gel via the method of Verza (2006).

#### *Ethanolic extract*

The ethanolic extract from the barks of the stem of *A. cochliacarpus* was prepared by a steeping method, in which the plant material was first crushed, then placed in a percolator tube containing absolute ethanol for 72 hours. After this period, the extract was filtered in (Whatman No 1 filter paper). This procedure was repeated until the plant material was exhausted. A portion of the liquid sample was then concentrated in a rotary evaporator under reduced pressure until a crude ethanolic extract (BSE) was obtained (Weber, 2013). The other part of the liquid sample was stored at room temperature until it was incorporated in the natrosol gel.

#### *Preparation of natrosol gel and obtaining gel from A. cochliacarpus*

The natrosol gel was prepared according to previously described methodology (Wettasinghe and Shahidi, 1999). After the

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preparation of the nanosol gel, each extract was incorporated into the nanosol gel in a 10% v/w concentration for a seven-day stability test.

### **Chemical study of the ethanolic extract**

The Crude Ethanolic Extract (CEE) of the *A. cochliacarpus* stem bark was fractioned through a liquid-liquid partition method. The extract was dissolved in mixture of methanol and water at a ratio of 6:4, respectively. From the methanol/water phase, the process of partition was initiated using the solvents hexane, chloroform and ethyl acetate, resulting in Hexane Fraction (HF), Chloroform Fraction (CF) and Ethyl Acetate Fraction (EAF), respectively. The fractions were then subjected to HPLC-DAD (High Performance Liquid Chromatography-Diode Array Detector), TLC (thin layer chromatography), phytochemical prospecting, evaluation of antioxidant activity, and determination of phenolic compounds.

### **Phytochemical screening**

Phytochemical screening was performed for searching the following metabolites: pyrogalic tannins, phlebotonic tannins, phenols, anthocyanins, anthocyanidins, flavonols, xanthenes, flavonones chalcones, aurones, flavononols, leucoanthocyanidins, catechin, flavonones, flavanonols, xanthenes, steroids, triterpenoids, and saponins (Matos, 1997).

### **Analysis of antioxidant activity by radical 2,2-diphenyl-1-picrylhydrazyl**

In the analysis of antioxidant activity by radical 2,2-diphenyl-1-picrylhydrazyl (DPPH), vegetable samples, HF, CF and AEF were dissolved in 95% ethanol, applied in chromatoplates (Gel silicic F254, disabled) and eluted in solvent system containing ethyl acetate/formic acid (8:2). After elution, chromatoplates were immersed in ethanolic solution of DPPH  $0.3 \mu\text{g mL}^{-1}$  for 10 seconds. The appearance of yellow spots underneath the purple coloration is indicative of antioxidant activity.

Absorbance values were converted to antioxidant activity percentages (AAO%) using the formula:  $\text{AAO}\% = 100 - \{[\text{ABSA} - \text{ABSB}] \times 100\} / \text{ABSC}$ , where ABSA represents the absorbance of the sample, ABSB is the absorbance of the blank, and ABSC is the absorbance of the control sample. Quantitative assessment of antioxidant activity was done by monitoring the free radical DPPH consumption and by measuring the decrease in absorbance of solutions at different concentrations (Eurides et al., 2007). Samples from BSE, HF, CF and AEF were diluted in triplicate in ethanol, with final concentrations of 250, 125, 50, 10 and  $5 \mu\text{g/ml}$ . Reactions were carried out at room temperature for 30 minutes. Readings of the absorbances were subsequently made at 518 nm.

### **Evaluation of antioxidant activity by the Thiobarbituric acid method**

For evaluation of the antioxidant activity by the Thiobarbituric acid method (TBA), homogenized egg yolk solution was used as a rich medium in lipids. The procedure was performed triplicate, in accordance with the methodology described by Ferreira (2010). Five test tubes were set up containing 0.5 ml of egg yolk (10% w/v) and 0.1 ml of the plant samples was dissolved in the least amount of methanol and the volume diluted to 1 ml with water. Three concentrations were tested for each BSE solution: 100, 500 and

1000  $\mu\text{g/ml}$ .

Next, 2,2-chloride-1-azo-bis (2-amidinopropano)-ABAP (0.07 mol/L) was added to each tube to induce lipid peroxidation, followed by a 20% solution of acetic acid (pH 3.5) and 1.5 ml of TBA (0.8% w/v) solution in sodium dodecyl sulfate-SDS (1.1% w/v). To allow observation of the complete lipid peroxidation, the antioxidants 3,5-di-tert-butyl-4- $\alpha$ -tocopherol and butylated hydroxytoluene BHT were used as positive standards in the same conditions to which plant extracts were subjected. The vials were placed in a water bath at 95°C under stirring over 60 min. After cooling, 5 ml of 1-butanol were added to each tube, and the tubes were centrifuged at 3000 rpm for 10 min.

To allow observation of the complete lipid peroxidation, the antioxidants 3,5-di-tert-butyl-4- $\alpha$ -tocopherol and butylated hydroxytoluene BHT were used as positive standards in the same conditions to which plant extracts were subjected." Should be changed to: The antioxidants, 3,5-di-tert-butyl-4- $\alpha$ -tocopherol and butylated hydroxytoluene (BHT) were used as positive standards in the same conditions to which plant extracts were subjected, allowing hence, the observation of the complete lipid peroxidation. Absorbance of organic supernatant layer was measured in a spectrophotometer at a wavelength of 532 nm. The values obtained were applied in the following formula to determine the antioxidant content of plant samples in percentage (IA%):  $\text{IA}\% = (1 - A/C) \times 100$ , where C is the absorbance of the control and the fully oxidized, the arithmetic mean of the sample tested absorbances.

### **Determination of phenolic compounds**

The vegetable samples CEE, HF, CF, and EAF were evaluated at a concentration of 2.0 mg/ml. 0.5 ml of a 2N Folin-Ciocalteu reagent solution was added to 0.5 mL of each sample, followed by 1.0 ml of water (in triplicate). After stirring for 2 min, 0.5 ml of 10% (w/v) aqueous sodium carbonate ( $\text{Na}_2\text{CO}_3$ ) was added to the tube. Then the samples were incubated for 2 h at room temperature (while protected from light). Absorbance values were obtained by readings at 750 nm, using the Folin-Ciocalteu reagent in methanol as blank sample. In order to build a calibration curve, gallic acid solutions were used at concentrations of 0.15, 0.1, 0.05, 0.025, 0.01, and 0.005  $\mu\text{g/ml}$ . Total phenol values were expressed as gallic acid equivalents (Junqueira, 1999).

### **EC calculation**

Results of *in vitro* tests were expressed in  $\text{EC}_{50}$  values (Nascimento et al., 2014), which is the concentration required to produce half (50%) of maximum effect estimated at 100% for the plant extract.

### **In vivo assay**

#### **Animal selection**

Two male and three female horses were selected from the farm of the CESMAC Veterinary Medicine School. All animals were of mixed race, aged between 2.5 and 3 years, weighing from 270 to 320 kg, and were clinically healthy and well-nourished. The animals were fed with a balanced diet (corn + wheat + soy) at a rate of 2.5 kg/day, bulky (Tifton Grass) 28 kg/animal/day and water *ad libitum*. The animals were accommodated in individual stalls covered with sand.

### **Surgical procedure**

After anesthesia, administered intravenously using 10% xylazine (0.5 mg/kg/EV), the animals were trichotomized, and a skin antisepsis was made with 1% povidone-iodine. Then, five circular and symmetrical wounds were made aseptically in the thoracic region, at the lateral to dorsal midline of each animal with the help of scalpel blade No. 23, after local anesthesia with lidocaine hydrochloride 2%, according to the method of Andriquetto and Perly (2002). The five wounds were made with the help of a punch of 3 cm, measuring approximately 3.0 × 3.0 cm, with a distance of 5 cm between them. The damage included the epidermis, dermis, and subcutis. After the surgical procedure, the wounds were measured with Vernier Calipers 200 × 0.05 mm/8 × 1/128, France.

### **Clinical evaluation of wounds**

Clinical evaluations were performed visually by the same individual every 72 h, observing the presence of hyperemia, edema, pain, discharge, itching, crust, contraction, granulation tissue, and scar tissue in accordance with Ardisson et al. (2002).

### **Morphometric analysis of wounds**

For the acquisition of the area of wounds, measurements of diameters at the time of biopsy to 0, 3, 7 and 14 days post-op were carried out. All wounds were measured with the aid of calipers (Andriquetto and Perly, 2002) and from these elements, the following equation was formulated: where A represents the area (cm<sup>2</sup>), and r the radius. The degree of contraction, expressed in percentage, was measured by the equation proposed by Ramsey et al. (1995) where  $W_o$  = initial wound area and  $W_i$  = wound area on the day of the biopsy:  $100 \times (W_o - W_i) / W_o = \% \text{ contraction}$ .

### **Treatment for the wounds**

Wounds were treated daily at 24 h intervals, using powdered stem barks as well as aqueous, ethanolic and propylene glycol extracts of *A. cochliacarpus* in a gel formulation. Four wounds were treated with *A. cochliacarpus* and the remaining wound was treated with Dakin (0.5% NaOCl). Treatments were applied on wounds of different positions for each animal. Throughout the experiment, bandages were not applied to the wounds.

### **Histological procedures**

The animals underwent incisional biopsy of the skin for bilateral microscopic analysis of the healing process. Microscopic assessments were carried out at the end of the trial period. Material collection was performed for histopathological analysis, with tissue removal four square measuring about 2 × 2 cm, in order to include the entire diameter of the wound, as well as tissue adjacent to injury. The collected material was fixed in 10% buffered formalin for 24 h, being subjected to routine histological processing, included in paraffin and then cut into 6 cm. set microtome. Then, the cuts were stained by hematoxylin and eosin and then examined using a light microscope (Barroso et al., 2010).

Microscopic analysis of the healing of each of the wounds was descriptive, qualitative evaluating the morphology of the inflammatory process, granulation tissue, cellularity, neovascularization and fibroplasia, as well as the evolution of the healing process throughout the ages (Barroso et al., 2010).

### **Statistical analysis**

The results of the area and contraction of the wounds had been expressed on average ± shunting line standard, submitted to analyses of variance, and also to Tukey's test, considering significant comparative values to the level of 5% of significance. Statistical analysis was performed using SPSS to determine the linear regression and the coefficient of determination (R<sup>2</sup>), which best explain the antioxidant potential of plant samples.

## **RESULTS**

### **Preparation and fractionation of the ethanolic extract**

From the crude extract (598 g) of stem bark, approximately 35 grams (5.85% yield) of the CF, 15 grams of EAF (2.50% yield) and 2 g of HF (0.33% yield) were obtained through primary fractionation. Fractionation of the crude extract with solvents of increasing polarity allows inferring the classes of substances extracted in the different fractions, in accordance with the known solubilities and polarities presented by various substances (Müller, 2006).

### **Phytochemical prospection**

The phytochemical screening revealed different classes of secondary metabolites including phlebotonic tannins, flavonols, xanthones, flavones, catechins, steroids, and saponins, as shown in Table 1.

### **Quantitative assessment by DPPH assay**

A significant radical scavenging activity was found for the CEE extract at low concentrations as shown in Figure 1. Furthermore, fractionation of the crude extract showed that the activity was retained by the EAF (Figure 2) since higher scavenging of radicals could be attained at lower concentrations. In contrast, similar scavenging activity for CF (Figure 3) and HF fractions (Figure 4) were achieved with higher concentrations and indicates that the compounds responsible for the activity in the crude extract had greater affinity by ethyl acetate solvent.

### **Determination of phenolic compounds**

Total phenolic content found in the screening was of 181.8 mg Gallic acid equivalents/g of the sample with regard to CEE extract, and 50% of this content was found in AEF.

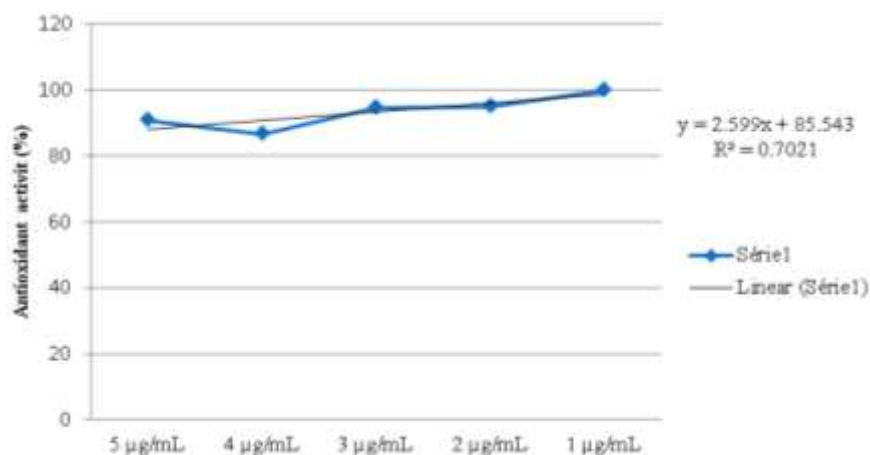
### **Antioxidant content percentage**

The most common technique used to measure lipid peroxidation is the test of Thiobarbituric Acid (TBA), a

**Table 1.** Prospection of the chemical constituents of stem bark of *A. cochliacarpus*.

Secondary metabolite	CEE	EAF	CF	HF
Pyrogalic tannins	N	N	N	N
Phlebotonic tannins	P	P	P	N
Phenols	N	N	N	N
Anthocyanins and anthocyanidins	N	N	N	N
Flavonols, xanthonés and flavonones	N	P	P	N
Chalcones and aurones	N	N	N	N
Flavononols	P	P	P	N
Leucoanthocyanidins	N	N	N	N
Catechin	N	P	P	N
Flavonones	P	P	N	N
Flavanonols and xanthonés	N	P	P	N
Steroids	N	N	N	P
Triterpenoids	N	N	N	N
Saponins	P	P	P	N

(P) indicate the presence of compounds; (N) indicate the absence of compounds. CEE, Crude Ethanolic Extract; EAF, Ethyl Acetate Fraction; CF, Chloroform Fraction; HF, Hexanic Fraction.

**Figure 1.** Antioxidant activity percentage of CEE from stem bark of *A. cochliacarpus*.

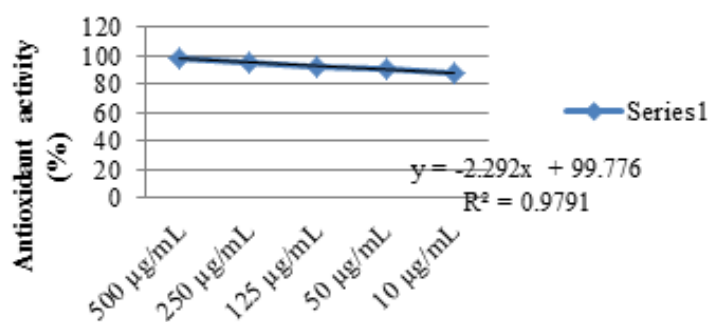
spectrophotometric method that measures the concentration peroxidation products. The final product measured is the malondialdehyde or reactive substances formed from barbituric acid. In TBA test, the CEE extract presented the highest antioxidant capacity when compared to BHT at concentrations of 1000, 500 and 100 µg/ml, as shown in Table 2.

### Evolution of the wound areas

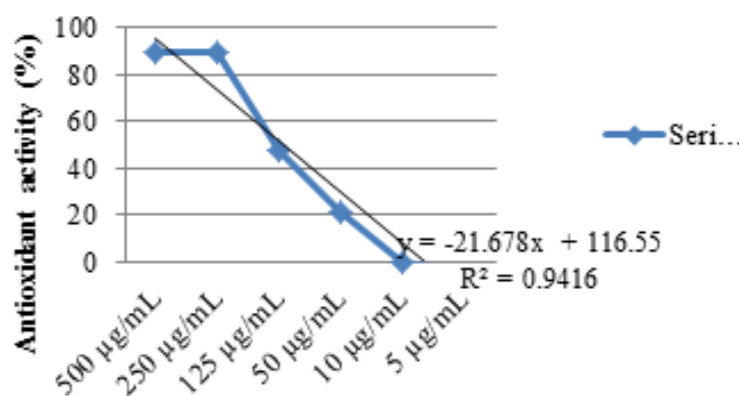
The wound areas gradually decreased with the evolution of time over the course of 14 days of treatment (Figure

5). After 24 h from the beginning of the treatment, hyperemic areas were observed in the wounds, with zones of intense vascularization. The edges were well defined geometrically; however, the presence of edema and crusting areas were sometimes displayed at the outer regions of the wounds. Throughout the course of the experiment, those wounds treated with *A. cochliacarpus* presented irregular thick crusts and dryness.

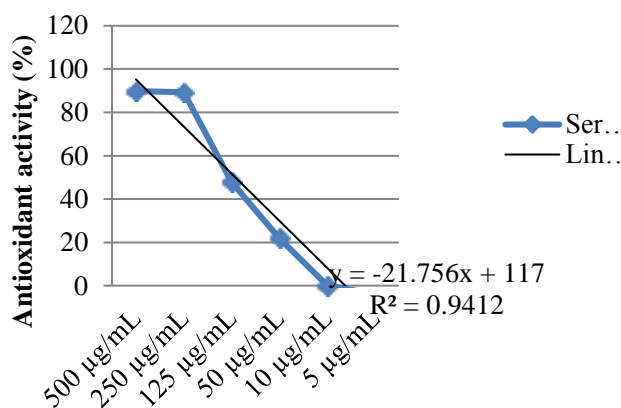
The reduction of the wound area occurred due to the mechanism of contraction and the centripetal movement of the wound border towards the center, in order to reduce the area to be covered by the proliferating



**Figure 2.** Antioxidant activity percentage of EAF from stem bark of *A. cochliacarpus*.



**Figure 3.** Antioxidant activity percentage of CF from stem bark of *A. cochliacarpus*.



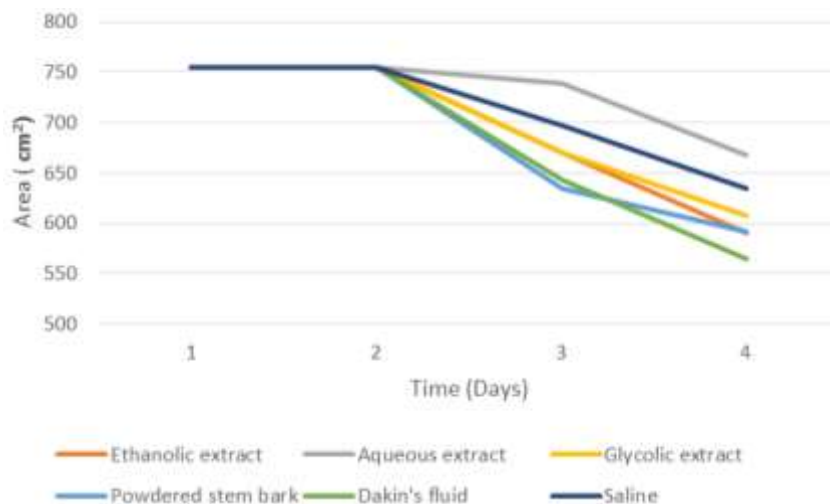
**Figure 4.** Antioxidant activity percentage of the HF from stem bark of *A. cochliacarpus*.

epithelium. The diameter and contraction data as a function of the different treatments are shown in Table 3.

Based on results shown in Table 3, it was attested that the dry powdered stem bark as well as the ethanolic and

**Table 2.** Antioxidant activity of CEE extract from stem bark of *A. cochliacarpus*.

Sample	Concentrations of the samples/AI Values%		
	1000 µg/ml	500 µg/ml	100 µg/ml
CEE	30.00	23.33	15.02
Control BHT	28.66	20.31	10.09



**Figure 5.** Wound areas of different groups following 14 days treatment.

**Table 3.** Mean and standard deviation of the diameter and contractions of the lesions according to the application of the different treatments.

Treatment	Area	Diameter	Contraction (%)
Ethanolic	692 ±78	30± 2 <sup>b</sup>	22± 9 <sup>a</sup>
Aqueous	729 ±103 <sup>a</sup>	30± 2 <sup>a</sup>	12± 6 <sup>b</sup>
Propylene glycol	697±72 <sup>c</sup>	29± 2 <sup>b</sup>	19 ±9 <sup>a</sup>
Powdered bark	683±85 <sup>c</sup>	30± 2 <sup>b</sup>	22 ±10 <sup>a</sup>
Dakin's solution	679±88 <sup>c</sup>	29± 2 <sup>b</sup>	25 ± 7 <sup>a</sup>
Saline	710 ±57 <sup>b</sup>	30± 1,23 <sup>a</sup>	16 ± 7 <sup>b</sup>

Means followed by the same letter in a column do not differ statistically at the 5% probability level by the Duncan or Scott-Knott test.

propylene glycol extracts had the best performances in relation to reducing diameters and areas of induced wounds, with a wound healing activity comparable to Dakin's solution ( $p < 0.05$ ).

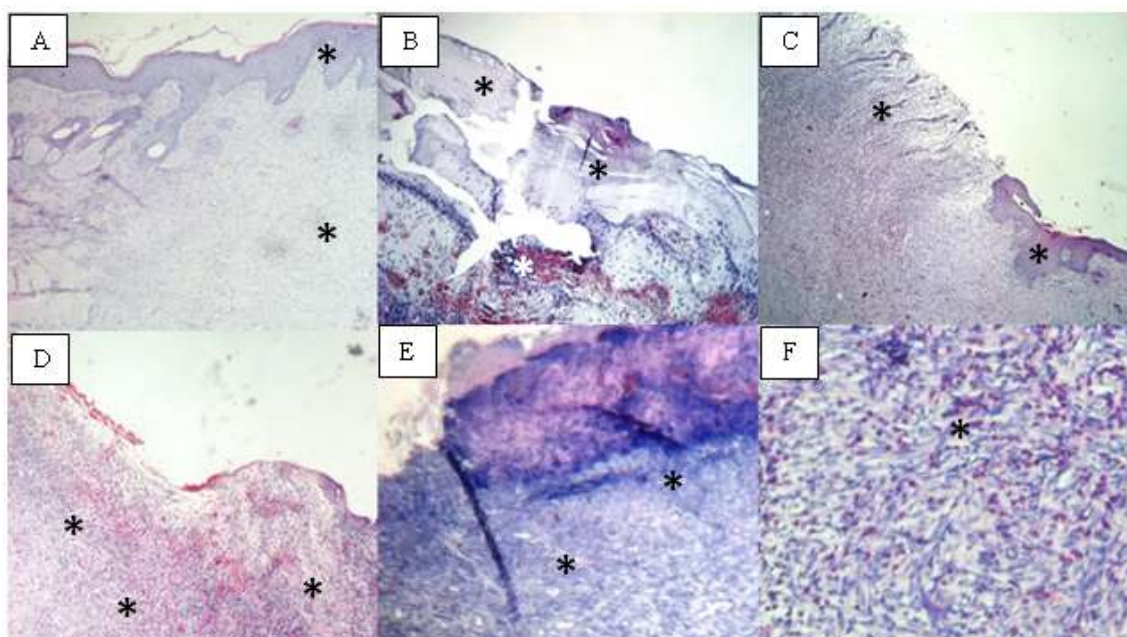
### Histological analysis

Histological evaluation of wounds on the 14th day revealed significant differences regarding the

inflammatory response and deposition of granulation tissue between the aqueous, ethanolic, propylene glycol extracts and powdered bark of *A. cochliacarpus*. The best healing occurred using the bark powder, followed by propylene glycol and ethanolic extracts. Because the treated animals exhibited less ulceration associated with neutrophilic inflammation and a greater organization of connective tissue, as illustrated by Figure 6A, B and C.

The water extract and the Dakin fluid were less effective treatments. Wounds treated with these





**Figure 6.** (A):Histopathological appearance of the lesion treated with: A. powdered stem bark. Re-epithelialization process. Presence of granulation tissue HE organized. 10 x objective; (B) Propylene glycol extract. Edema and fibrin. Bacterial colony. Ulceration with edema and fibrin. 40 x objective; (C) Propylene glycol extract. Injury with ulceration and inflammatory neutrophilic infiltrate. Irregular re-epithelialization. 40 x lens; (D). Propylene glycol extract. Ulceration associated with neutrophilic infiltration. Eosinophilic inflammation. Granulation tissue. 10x lens; (E). Dakin fluid. Ulcer associated with neutrophilic infiltrate. Granulation tissue with infiltrated mononuclear cells; (F). Powdered stem bark (Granulation tissue). Eosinophilic inflammation. 40 x lens.

substances presented greater inflammatory response, sharp ulceration associated with moderate neutrophilic inflammation, and disordered of connective tissue. These changes are not expected with that intensity on the 14<sup>th</sup> day. Histological features of the healing provided by these extracts are shown in Figure 6D and E. The presence of mononuclear infiltrates (macrophages and lymphocytes) and granulomas was observed as expected. The inflammatory phase was characterized by the presence of inflammatory cells in scar tissue, especially polymorphonuclear leukocytes (PMN) and macrophages. The wound treated with powdered stem bark accelerated re-epithelialization by the end of 14<sup>th</sup> day, which indicates the end of the healing process and suggesting greater efficacy of the product. All lesions presented a moderate to severe eosinophilic inflammatory reaction Figure 6F. Probably due to the response to induced tissue injury in the dermis of horses.

## DISCUSSION

The DPPH assay is a widely used antioxidant procedure that asserts the ability of a compound to scavenge free radicals (Bendini et al., 2007). Several advantages have

contributed to its dissemination as a first choice method of evaluation for antioxidant potential of foods (Floegel et al., 2011), plant extracts (Mensor et al., 2001) or isolated compounds (Villeño et al., 2007) which include its low cost, ease of handling and valid accuracy (Kedare and Singh, 2011).

The extracts obtained herein were able to scavenge the DPPH radical and act as potential antioxidants. This property correlates to the presence of some classes of secondary metabolites in the stem barks of *A. cochliacarpus*. As we have shown by the preliminary phytochemical screening, tannins and flavonoids are present in the stem bark of this species, and may be regarded as responsible for the antioxidant properties of the extracts since they are widely acknowledged as potent radical scavengers (Hatano, 1995; Nanjo et al., 1996; Cai et al., 2006).

Polyphenolic compounds comprise a large class of secondary metabolites featuring more than one phenolic ring in their core structure, which is derived from shikimate or polyketide metabolic pathway (Quideau et al., 2011). Their antioxidant mechanisms have been known for some time and relate to the presence of the phenolic ring, which provides the ability to scavenge free radicals and form itself stable free radicals, by hydrogen-

atom transfer, or cation radicals, via single-electron transfer (Leopodini et al., 2011; Aquino et al., 2017). The stability of the radical formed is explained by the delocalization of the unpaired electron through the framework of phenolic rings existent in the molecular structure of these secondary metabolites (Quideau et al., 2011; Leopodini et al., 2010).

Wound healing is a complex and stepwise process that comprises different but complementary biochemical and cellular events in order to provide anatomical reconstruction and physiological regeneration of damaged tissue in most cases (Broughton et al., 2006). We believe in the view point that describes the wound healing as a four-stage process: coagulation, inflammation, proliferation and remodeling. The first stage, coagulation, takes place immediately after injury and involves the formation of blood clot to avoid excessively bleeding. Secondly, recruitment of inflammatory cells ensues due to the release of chemoattractant substances so that bacterial dissemination is prevented and removal of damaged tissue is carried out. At the same time, proliferation occurs and includes re-epithelialization and angiogenesis which altogether leads to wound contraction. Finally, this is followed by remodelling of the extracellular matrix and deposition of collagen to restore tensile strength of the newly formed tissue (Velnar et al., 2009).

Wound contraction involves a reduction in the size of the wound from the day of operation until complete epithelialization occurs. The qualitative histological criteria analysed in order to assay the degree of wound healing comprise the presence of inflammatory cells and necrotic tissue, the extent of epithelialization and angiogenesis, and the organization of connective tissue (Thakur et al., 2011). The results in Table 3 confirm increased rate of wound contraction. These observations are further supported by histopathological evidences that confirmed enhanced epithelialization by ethanolic and propylene glycol extracts, as well as the powdered stem bark, when compared to the control.

The inflammatory stage features the infiltration of mononuclear and polynuclear leukocytes at the skin continuity solution in order to provide defense against micro-organisms and to promote debridement of necrotic tissue (Koh et al., 2011). Even though inflammation is indispensable to wound healing, the migration of neutrophils to the wound site has marked influence in increasing the levels of Reactive Oxygen Species (ROS) locally. This can induce tissue damage to healthy cells by destroying their membranes through lipid peroxidation or by causing damage to essential macromolecules such as proteins and nucleic acids. This ultimately might delay or impair wound healing (Schäfer and Werner, 2008).

There are reports of the use of *A. cochliacarpus* to alleviate inflammatory diseases, through studies using experimental models in vitro and in vivo (Sánchez-

Fidalgo et al., 2013). Furthermore, the antiulcer effect of the aqueous extract of stem bark of *A. cochliacarpus* was tested in experimental alcohol gastric ulcers and had efficacy in promoting the healing of colonic lesions and reducing neutrophil infiltration, which may be related to the decrease of proinflammatory cytokines and down-regulation of inflammatory COX-2 (cyclooxygenase-2) and iNOS proteins (inducible Nitric Oxide Synthase), as well as JNK activation (c-Jun N-terminal Kinase) (Da Silva et al., 2010).

In addition, *A. cochliacarpus* has pharmacological potential against Gram-positive bacteria, mainly of the genus *Staphylococcus*, and can be exploited in the future to obtain bioactive compounds with antibacterial action (Tenório et al., 2016). The hydroethanolic extract from stem barks of *A. cochliacarpus* for the treatment of rats with induced burns and infected with strains of *S. aureus* reported that the animals showed complete healing in 21 days (Soares et al., 2013).

The relevance of the antioxidant activity in wound healing is well described (Süntar et al., 2012). Since phenolic compounds such as flavonoids and tannins are present in *A. cochliacarpus* extracts, and as these agents influence one or more phases of healing process, the wound healing activity might be related to these compounds. Several plant species from the Brazilian Caatinga ecoregion are known for their high total phenolic content, and hence are used for medicinal purposes by traditional communities to treat inflammatory conditions and for healing wounds (Oliveira et al., 2013). The phytochemistry of *A. cochliacarpus* was investigated in more detail by nuclear magnetic resonance studies that pointed to the presence of proanthocyanidins, mainly catechins (Da Silva et al., 2010).

Polyphenols are able to prevent lipid peroxidation directly by scavenging free radicals or by delaying the onset of cell necrosis and improving vascularity, which contributes to increase collagen viability, reduce cell damage and promote DNA synthesis (Getie et al., 2002). Moreover, tannins are known for promoting capillary vasoconstriction, which decrease vascular permeability and cause a local anti-inflammatory effect in addition to their action as scavengers of reactive species, which greatly contributes to their antioxidant potential and hence favours the healing (Lopes et al., 2005). As radicals can damage cell structures including membrane lipids, proteins, enzymes, and nucleic acids, the antioxidant role of tannins might be one of the most important components of wound healing (Edwin et al., 2008).

The solubility of polyphenols is determined by the chemical nature of the plant sample, as well as the polarity of the solvents used for extraction procedure. In addition, other factors such as the ratio between solid and solvent, and the particle size of the sample may influence the content of polyphenols (Dai and Mumper,

2010). Ethanol extracts of barks usually present a higher content of polyphenols in their composition when compared to aqueous extracts of the same plant parts, as shown by several investigations (Igbinsola et al., 2011; Olajuyigbe and Afolayan, 2011; Iloki-Assanga et al., 2015; Pawar and Dasgupta, 2016). Since the wound healing activity of *A. cochliacarpus* might be connected to the antioxidant activity of its chemical constituents, this may partially explain the lower efficacy of the aqueous extract.

## Conclusion

The crude ethanolic extract and the ethyl acetate fractions of the *A. cochliacarpus* showed antioxidant action in quantitative and qualitative DPPH and TBA assays. After partition, the ethyl acetate fraction was particularly distinctive, with 95.45% of antioxidant activity occurring in a concentration of 50 µg/ml for DPPH. These results are likely due to the presence of flavonoids, as well as hydrolysable and condensed tannins, indicated by the chromatographic profile and phytochemical screening. The ethyl acetate fraction also presented the highest content of phenolic compounds: 0.010 mg of Gallic acid/g of the sample.

This study confirmed microscopically and macroscopically that the healing process in horses was mediated by the use of *A. cochliacarpus* ethanolic and propylene glycol extracts, as well as the powdered stem bark, being the latter the best in terms of decrease in diameter of wounds and wound contraction. Thus the enhanced ability of wound healing in equines with the extracts could be explained on the basis of antioxidant effects of the chemical constituents of *A. cochliacarpus* and the in vivo model in equines showed enhanced rate of wound contraction and drastic reduction in healing time than control, which might be due to enhanced re-epithelialization.

## CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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*Full Length Research Paper*

## **Acute toxicity evaluation of ethanolic extract of the air parts of *Sida rhombifolia* L., in wistar rats**

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***Sida rhombifolia* L.**, popularly known in Brazil as "SIDA" or "mata-pasto", is considered a weed; a plant of the American continent and widely distributed in North Africa, belongs to the Malvaceae family. In Brazil, *S. rhombifolia* L. is scattered throughout the national territory, infesting agricultural crops. Certain species of the genus *Sida*, including *S. rhombifolia*, are widely used in Indian, Chinese, African and American medicine. The present study was carried out with the objective of evaluating the non-clinical acute toxicity of crude ethanolic extract (CEE) obtained from *S. rhombifolia* L. In treated males, there was a statistically significant reduction in water and feed intake. Biochemical analyzes showed statistically significant changes in the parameters of aspartate aminotransferase, alanine aminotransferase and creatinine; hematological parameters showed altered erythrocytes, mean corpuscular volume, mean corpuscular hemoglobin and eosinophil parameters; observed only in treated male animals. The animals' organs showed no significant changes. The results suggest that the ethanolic extract obtained from *S. rhombifolia* L. presents low acute dose toxicity. However, chronic toxicological studies should be performed to demonstrate the safety of long-term use of the drug.

**Key words:** *Sida rhombifolia* L., acute non-clinical toxicity, hematological parameters, biochemical analyses, histopathological parameters.

### **INTRODUCTION**

From antiquity, medicinal plants have been the most important and best known therapeutic resource; their

usage represents a characteristic link with the human species (Almeida et al., 2008). At present, due to the

immense biological diversity of flora on the planet and the apparent shortage of new drugs proceeding from this same diversity, there is a growing interest in natural product research, which could uncover new treatments for various diseases. Thus, phytotherapy in popular medicine, the seeking of new products with therapeutic properties based on ethno-pharmacological studies has grown (Elisabetsky, 2001; Maciel et al., 2002; Butler, 2004; Militão et al., 2012).

According to the World Health Organization (2011), from 70 to 95% of underdeveloped country populations depend on medicinal plants as their only form of disease treatment. This is because of the high cost of synthetic drugs. Most natural products come from popular culture, in the form of infusions, decoctions, tinctures and alcoholic solutions obtained from artisanal techniques, without having proven pharmacological properties, at least through non-clinical studies. This confirms the need to carry out toxicological and pharmacological studies aiming to transform such natural products into safe, effective and quality drugs (Veiga and Pinto, 2005; Franca et al., 2008; WHO, 2011).

*Sida rhombifolia* is a botanical genus inserted in the *Malvaceae* family, belonging to the order *Malvales* which contains 243 genera and 4225 species (Stevens, 2003), which present as sub-shrubs, shrubs and rarely as trees (Baracho, 1998). Species of this family are greatly distributed around the world, being found predominantly in tropical regions, and especially in South America (Heywood, 1993). In Brazil, it is scattered throughout the national territory, infesting agricultural crops. According to Fleck et al. (2003) *S. rhombifolia* L. is the most widespread species of *Sida* in the country. *S. rhombifolia* L. is popularly known in Brazil as “matapasto”, “guanxuma”, and “relógio”. Certain species of the genus *Sida*, including *S. rhombifolia* are widely used in Indian, Chinese, African and American medicines. Differing types of extracts and components isolated from these plants have demonstrated antimicrobial, anti-inflammatory, analgesic, anti-ulcerogenic, hypotensive, antioxidant and anti-diabetic activities, confirming the folk lore and beliefs about the species (Ajithabai et al., 2012; Pradhan et al., 2013; Galal et al., 2015).

Studies have reported isolated and identified phytochemicals from aerial parts of *S. rhombifolia* L. using chromatographic and spectroscopic methods. The study led to the isolation of the scopoletin, escoporone, ethoxy-ferulate, kaempferol, kaempferol-3-O-D-glycosyl-60- $\alpha$  D-rhamnose, quindolinone, 11-methoxy- quindoline, quindoline and the salt of cryptolepine.

In addition, quindolinone and the salt of cryptolepin

induced vasorelaxation dependent on the vascular endothelium, justifying the use of the species in folk medicine in India (Chaves., et al 2017). Based on the search for new pharmacologically active and safe agents, having several phytochemical constituents isolated from the aerial parts of *S. rhombifolia* L. (Chaves., Et al. 2017), this study evaluated the toxicity of the crude extract of *S. rhombifolia* L. which used non-clinical tests following the recommendations of the National Agency of Sanitary Surveillance (ANVISA).

## MATERIALS AND METHODS

### Plant collection

Aerial parts of *S. rhombifolia* L. (*Malvaceae*) were collected in the municipality of Santa Rita-Paraíba and botanical identification was performed by Dr Maria de Fátima Agra of Federal University of Paraíba. The exsiccate material is filed at the Prof. Lauro Pires Xavier Herbarium of Federal University of Paraíba under No. Agra 7045.

### Preparation of *S. rhombifolia* L. crude ethanolic extract

The crude ethanolic extract was prepared by the staff at the Phytochemical Laboratory of Professor Dr. Maria de Fátima Vanderlei. Aerial parts of *S. rhombifolia* L. were dehydrated in an oven with circulating air at an average temperature of 40°C for 96 h. They were then ground in a mechanical mill, obtaining approximately 5.5 kg of powder. It was macerated in 95% ethanol (EtOH) for 72 h for extraction of the organic constituents. The extractive solution was concentrated in an evaporator at 40°C, providing approximately 570.0 g of crude ethanolic extract (CEE).

### Experimental animals

24 *Wistar* rats were used, albinos, adults, male and female (nulliparous and non-pregnant), weighing between 180 and 220 g, as provided by the Prof. Thomas George bioterium of Research Institute for Drugs and Medicines of Federal University of Paraíba (IPeFarM/UFPB). The experimental protocol was approved by the Ethics in Animal Experimentation (CEUA) of Federal University of Paraíba (UFPB) , (process No. 029/2015). All were grouped in polyethylene cages, containing six animals each, and maintained under controlled conditions at a temperature of 21  $\pm$  2°C, without any medications, and having free access to food (*pellets*) and water.

### Acute toxicological testing

The parameters evaluated for acute toxicological tests were based on ANVISA Resolution RE 90/2004 (Brazil, 2004), using *Wistar* rats of both sexes. The rats were divided into two groups: control and treated. Each group consisted of 12 animals, 6 males and 6 females. The control group was distributed in two boxes that

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separated the animals by sex and the animals received water by gavage. The treated groups were equally distributed and received the dose of crude ethanolic extract (EEC) at 2000 mg / kg body weight (bW). After the administration of EEC, the observation of behavioral parameters with pharmacological screening was performed at intervals of: 30, 60, 120, 180 and 240 min, according to the experimental protocol developed, as previously described (Almeida et al., 1999). After 14 days of experimentation, the animals were by sacrificed administration of excess anesthetic (anesthesia of 80 mg / kg of xylazine and 5 mg / kg of ketamine), following the recommendations of the scientific community. Blood was withdrawn for laboratory analysis of hematological and biochemical parameters.

#### Laboratory analysis of the blood

Collection of the samples was carried out by bleeding the brachial plexus. The blood was collected in tubes with the anticoagulant ethylenediamine tetraacetic acid (EDTA) for determination of hematological parameters, and in tubes with separator gel - MicrotainerBectonDickson® - which were centrifuged for 10 min at 2026 g of force, to obtain serum for determination of biochemical parameters. The hematological analyses consisted in the study of the red cell series (erythrogram), white cell (WBC), and the platelet count. The erythrogram included the erythrocyte count, hematocrit, mean corpuscular volume (MCV), hemoglobin, mean corpuscular hemoglobin (MCH) and mean corpuscular hemoglobin concentration (MCHC). The WBC included a global leukocytes and cell differentiation counts. The biochemical analyses were performed for the serum samples. The total cholesterol, urea, glucose, triglycerides, alkaline phosphatase (ALP), albumin, globulin, and transaminases: Aspartate Amino Transferase (AST) and Alanine Amino Transferase (ALT), uric acid, creatinine, total protein, calcium ions and magnesium were analyzed in an automated biochemical apparatus ChemWell-T®.

#### Anatomy-pathological examination

The organs of the animals (livers and kidneys) were sectioned and immersed in a fixative solution. After 12 h of fixation, samples for histopathological processing were obtained by inclusion in paraffin and stained with hematoxylin and eosin.

#### Statistical analysis

For statistical analysis of the results, we used the Mann-Whitney and test "t" un-paired, using the software GraphPadPrism® 6.0. The results were considered significant for p values < 0.05.

## RESULTS

#### Behavioral evaluation and lethality

In the evaluation of behavioral changes after administration of the oral dose of the crude ethanolic extract (*S. rhombifolia* L.) at the dose of 2000 mg/kg body weight (bW), no motor and / or sensorial deficiencies were observed, nor did the dose tested cause no deaths in the animals within 14 days.

#### Weight evolution

Compared to their respective control groups, there was no statistically significant change in weight evolution of the male or female rats treated with *S. rhombifolia* L. of crude ethanolic extract (CEE) at an oral dose of 2000 mg/kg body weight (bW). The results are seen in Table 1.

#### Water and food consumption

The ingestion of water and feed was measured daily during the acute treatment with the substance. In the treated males, a statistically significant decrease in the consumption of water and ration was observed. On the other hand, the females did not show any changes in either parameter. The results are presented in Table 2.

#### Biochemical parameters

Biochemical findings were obtained from animal serum analyzes after the 14-day experimental period (Table 3). The animals treated (male) presented higher levels of alanine aminotransferase (ALT), aspartate aminotransferase (AST) and creatinine.

#### Hematological parameters

The hematological alterations obtained from the plasma analyses of the animals after the 14-day experimental period are described in Table 4. For the males treated with *S. rhombifolia* L. of crude ethanolic extract CEE at an oral dose of 2000 mg/kg body weight (bW), there were significant differences between the control and treated groups for erythrocytes, mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH) and eosinophils at 2000 mg/kg bw.

#### Anatomy pathological study

Macroscopically, the organs did not present significant anatomical changes (Figure 1).

## DISCUSSION

During the study, and after the administration of *S. rhombifolia* L. CEE at an oral dose of 2000 mg/kg body weight (bW), no sign of severe toxicity or death of animals was detected during the 14 days of evaluation, which corroborates previous studies conducted by Sireeratawong et al. (2008). In the behavioral screening assessment, the first four hours after administration of the

**Table 1.** Weight evolution of Wistar rats, male and female, after 14 days of administration of *S. rhombifolia*.

Weeks	Control	Treated (2000 mg/kg)
Males 1 <sup>o</sup> Week	43.92± 29.09	22.38± 6.80
Males 2 <sup>o</sup> Week	22.45± 11.23	11.42± 3.10
Females 1 <sup>o</sup> Week	6.28±6.70	4.27±6.19
Females 2 <sup>o</sup> Week	2.30± 4.10	5.25 ± 2.43

Values are expressed as mean± S.D. (n=6). "t" test Mann-Whitney.\*p< 0.05.

**Table 2.** Water consumption and ration of male and female Wistar rats after administration of *S. rhombifolia* L.

Variable	Control	Treated (2000 mg/kg)
<b>Males</b>		
Water consumption (ml)	259.8±15.10	221.0±27.68***
Ration consumption (g)	151.3± 3.80	131.5±13.05***
<b>Females</b>		
Water consumption (ml)	192.8±27.94	184.9± 23.08
Ration consumption (g)	100.7± 10.43	107.8± 7.01

Values are expressed as mean ± S.D. (n=6). "t" test Mann-Whitney.\*p< 0.05,\*\*p< 0.01, \*\*\*\*p<0.001.

**Table 3.** Biochemical parameters obtained from the serum of rats treated with *S. rhombifolia* L.

Variable	Male		Female	
	Control	Treated (2000 mg/kg)	Control	Treated (2000 mg/kg )
Total protein (g/l)	6.20± 0.83	6.83± 1.25	6.49±0.68	7.60± 0.72
ALT (U/L)	59.40± 14.19	124.70± 50.64*	65.80±23.04	63.50± 13.43
AST (U/L)	162.80± 62.15	337.7± 192.80*	185.60± 84.50	178.30± 42.04
ALP (U/L)	339.2± 114.1	346.0± 19.30	176.0± 58.15	189.20±31.45
Globulin (g/dl)	3.47± 0.91	4.10± 1.34	3.48± 0.76	4.22± 0.41
Cholesterol (mg/dl)	66.00± 8.28	74.83± 15.69	66.40± 9.55	80.50± 15.54
Triglycerides (mg/dl)	125.125.00±33.59	100.30±36.15	98.25± 26.83	155.30± 49.83
Calcium (mg/dl)	12.89± 2.91	11.89±1.38	10.15± 1.14	11.14± 1.78
Uric acid (mg/dl)	0.99± 0.33	0.97± 0.10	1.19± 0.48	1.24± 0.27
Urea (mg/dl)	60.00± 1.87	72.00± 13.37	59.33± 8.08	70.00± 9.50
Serum-creatinine (µmol/L)	0.30± 0.06	0.43 ± 0.04*	0.42±0.09	0.42± 0.03
Albumin (g/L)	2.73± 0.28	2.74±0.15	3.04± 0.17	3.37±0.36
Glucose (mg/dl)	102.6± 12.48	112.5±15.68	89.20± 18.74	107.8±12.91

ALT= Alanine Amino Transferase , AST: Aspartate Amino Transferase , ALP: Alkaline phosphatase. Values are expressed as mean ± S.D. (n=6). "t" test Mann-Whitney.\*p<0.05.

CEE no changes at the level of the Central Nervous System (CNS) or (Autonomic Nervous System (ANS) were detected, indicating that the plant has no activity on these systems.

Analyzing any possible toxic effects, the body weight gains of the animals and consumption of water and ration

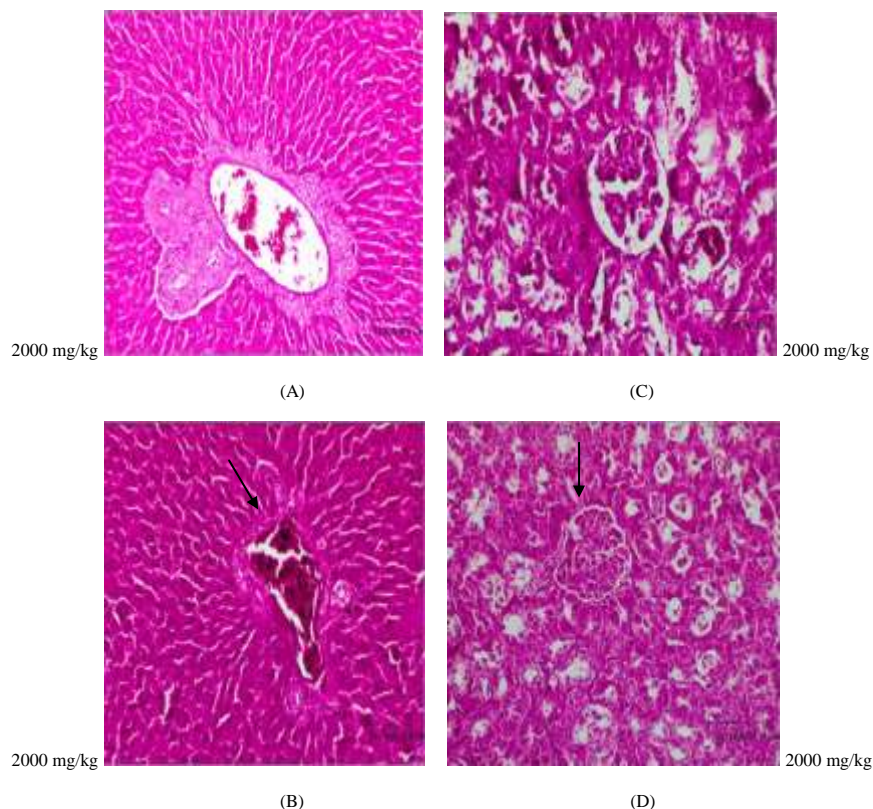
were observed and are shown in Tables 1 and 2, respectively. A significant decrease in the consumption of water and rations of treated males was detected, which may be associated with general discomfort, leading to a decrease in feeding of the treated rats, as suggested previously by Adeneye and Agbaje (2008), or it may be



**Table 4.** Hematological parameters obtained from the plasma of rats treated with *S. rhombifolia* L.

Variable	Male		Female	
	Control	Treated (2000 mg/kg)	Control	Treated (2000 mg/kg)
Hemoglobin (g/dl)	16.32 ± 0.62	16.33 ± 0.71	16.20 ± 0.25	16.30 ± 0.54
Hematocrit (%)	39.58 ± 1.17	40.05 ± 1.63	38.76 ± 1.28	39.38 ± 1.43
MCV ( $\mu\text{m}^3$ )	51.04 ± 3.17	43.28 ± 0.71**	50.80 ± 2.02	50.75 ± 2.45
MCH (pg)	21.06 ± 1.38	17.65 ± 0.34**	21.24 ± 0.78	21.03 ± 1.084
MCHC (g/dl)	41.24 ± 0.72	40.78 ± 0.44	41.82 ± 0.83	41.40 ± 0.90
Leukocytes ( $10^3/\text{mm}^3$ )	8.62 ± 3.43	5.15 ± 2.17	5.40 ± 2.07	6.08 ± 1.69
Neutrophils (%)	26.40 ± 4.40	21.20 ± 4.55	24.20 ± 5.54	28.33 ± 3.77
Eosinophils (%)	1.00 ± 0.00	0.00 ± 0.00**	2.00 ± 2.34	1.50 ± 0.84
Lymphocytes (%)	66.80 ± 7.80	76.17 ± 8.28	69.00 ± 6.28	67.67 ± 4.08
Monocytes (%)	4.40 ± 3.21	4.83 ± 2.56	4.00 ± 0.70	2.50 ± 1.22
Platelets ( $10^3/\text{mm}^3$ )	900.80 ± 143.10	671.50 ± 173.96	674.60 ± 193.46	769.83 ± 217.62

MCV: mean corpuscular volume, MCH : mean corpuscular hemoglobin, MCHC: Mean corpuscular hemoglobin concentration. Values are expressed as mean ± S.D. (n=6). "t" test Mann-Whitney \*p < 0.05 \*\*p < 0.01.



**Figure 1.** Histopathology of liver (A) and kidney (B) organs of male and female rats treated with the crude ethanolic extract of *S. rhombifolia* L. at an oral dose of 2000 mg / kg . None of the animal organs presented histological peculiarities (liver and kidneys). Hepatic tissue and space-portal tract (black arrow) without peculiarities (Female liver - A). Liver tissue and space-portal tract (black arrow) without peculiarities (Male liver - B). Renal tubules and glomeruli (black arrow) without peculiarities (female kidneys- C). Renal tubules and glomerulus (black arrow) without peculiarities (male kidney- D). H & E 200x.

that *S. rhombifolia* L., interferes directly in the lipid metabolism of treated animals, which leads to a decrease in the body weight of these animals. However, the decrease was not statistically significant, indicating that CEE has low toxicity; since in general changes behavior and weight gain are critical parameters for assessment of effects of a compound on animals; such changes are often the first signs of toxicity and indicative of adverse drug effects (Auletta, 1995; Teo et al., 2002; El-Sanusi and El-Adam, 2007).

During biochemical parameter analyses we observed an increase in the levels of ALT and AST for the treated males, whereas in females, there were no statistically significant changes. The liver is one of the most important organs in the body, being responsible for the metabolism and detoxification of all toxins that enter the body. Liver function may be evaluated through blood tests to provide information about the status of the liver and cellular integrity. Certain enzymes and proteins can be used as indicators of liver problems, such as ALT, AST, gamma-glutamyl transferase and bilirubin (Brandt et al., 2009). Certain drugs and medications are known to induce lipid peroxidation, causing swelling and necrosis of the liver cells, which results in the release of cytosolic enzymes, such as ALT, AST and ALP (Agbor et al., 2005). Thus, increases of ALT and AST in plasma may be indicative of hepatic lesions.

ALT is considered the most sensitive parameter for the liver, in cases of liver damage this enzyme leaks into the bloodstream. As an example of drugs that have high hepatotoxicity and cause changes in the levels of ALT and AST, stanozolol and acetaminophen are highlighted, yet they are routinely used (Basu et al., 2009; Mosallanejad et al., 2011). The increase in AST and ALT caused by the administration of CEE for *S. rhombifolia* L. indicates that the plant presents some signs of hepatotoxicity; as support of Ouédraogo et al. (2013), with similar results. When we observed the values obtained, there was a significant increase in creatinine levels for treated rats compared to the control group. However, this result has no clinical significance, since it is within the reference values (Giknis and Clifford, 2006; Castello Branco et al., 2011). Regarding the values obtained from the treated females, we did not obtain significant alterations of this group.

Blood parameter analysis is important for risk assessments of certain substances when administered to humans; the hematological system has great value to predict the first signs of toxicity. The hematopoietic system is very susceptible to toxic substances; an important system for analyzing physical health, and to evidence pathology in humans and animals (Olson et al., 2000; Li et al., 2010). Few statistically significant differences were found among the majority of hematological parameters between the control and treated groups. However, a significant decrease between

the controls and the treated groups for the parameters of erythrocytes, MCV, MCH, and eosinophils for the males treated was observed. In the females, no parameter suffered statistically significant alteration, indicating that *S. rhombifolia* L. CEE presented low toxicity relative to the hematological system (Konaté et al., 2012)

The increase in the erythrocyte values of the male rats may be related to sex, because the erythrocyte number varied and males obtain higher values than those of women. Another factor that controls the emission of erythrocytes in the blood is the level of oxygenation of the tissues, in conditions of low oxygen pressure, during oxygen depletion erythropoiesis stimulation occurs (Lorenzi, 2006). The mean corpuscular volume (MCV) is considered one of the main criteria for the classification of anemic disorders, however, the decline in the MCV of the males cannot be considered as indicative of anemia, since the value was still close to reference and the other parameters that may indicate an anemia were not significantly altered (Bessman et al., 1983). The changes in the values of MCH and for eosinophils, despite being statistically significant do not have clinical relevance, since the values were close to reference. Such differences can be explained by biological variability among rats (Lewis et al., 2002; Giknis and Clifford, 2006; Castello Branco et al., 2011).

Qualitative macroscopic analyzes revealed that the dose tested did not produce changes in the vital organs of the treated animals and no changes suggestive of toxic effects were observed in the histopathological analyzes. These results are in agreement with the data obtained in the biochemical analyzes.

## Conclusion

After acute treatment at oral dose of 2000 mg / kg body weight (bW) in male and female rats with crude ethanolic extract of *S. rhombifolia* L. (CEE), it was observed that the plant had no activity at the levels of the CNS or ANS, and has little influence on animal feeding, leading to only small weight losses. Regarding the toxicity, evaluated by biochemical and hematological parameters, it was observed that CEE has low toxicity. Few parameters showed significant changes. This justifies the extensive popular use found in the Brazilian northeast and allows a more comprehensive evaluation being necessary to evaluate the potential toxicity of this plant species when used chronically.

## CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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*Full Length Research Paper*

# **Efficacy of transurethral resection of prostate in patients of benign prostatic hyperplasia with lower urinary tract symptoms**

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Transurethral resection of the prostate (TURP) has been the prime procedure for decenniums to treat benign prostatic hyperplasia (BPH), but is mainly predicated on historic data that lacks utilization of validated measures. It is noteworthy that TURP done nowadays significantly differs from that performed 30 years ago. Men who had undergone TURP between 2013 and 2018 were reviewed. 50 patients presented in the Department of Urology, Lahore General Hospital, Pakistan, with lower urinary tract symptoms (LUTS) due to BPH (without retention) were recruited in the study. Related/required investigations were done and patients were operated for TURP after noting certain parameters. They were advised for follow up at 3 intervals (1st, 4th and 12th week postoperatively) and post-operative parameters were compared with pre-operative ones. International Prostate Symptom Score (IPSS), Quality of Life (QOL) and peak urinary flow rate ( $Q_{max}$ ), and Post-Void Residual (PVR) were recorded. Operative details and postoperative complications were documented. Patients were then invited to attend for repeat assessment. Out of 50, 43(86%) patients had severe symptom score and 07 (14%) had moderate symptoms score (pre-operative). Out of total 50, 36 (72%) patients had  $Q_{max}$  less than 10 ml/s and 14 (28%) patients had  $Q_{max}$  between 10 and 15 ml/s as well as Post Micturition Residual Urine (PMRU) more than 50 ml pre-operatively. After 3 months of successful TURP, 49 (98%) patients' conditions improved and they developed a normal flow rate of more than 20 to 25 ml/s. All the 50 patients remained with a PMRU of less than 50 ml. There was an improvement in 47 patients and only 3 patients did not improve to their satisfaction. TURP is very effective and still considered a gold standard treatment for BPH with LUTS without retention of urine.

**Key words:** TURP, BPH, LUTS, PMRU

## **INTRODUCTION**

Benign Prostatic Hyperplasia (BPH), a chronic progressive disorder causing Lower Urinary Tract

Symptoms (LUTS) is a prevalent diagnosis among ageing males (Chughtai et al., 2016). One third of men above

60 years are most liable to develop obstructive symptoms due to BPH, and approximately 25% of them will eventually require surgical intervention (Patel and Parsons, 2014; Malhotra, 2000). TURP, by far, has been the standard surgical procedure for LUTS associated with BPH (Marszalek et al., 2009). Traditional diathermy endoscopic prostatectomy is still a favored surgical intervention more often than not for BPH, despite introduction of newer procedures because a good number of surgeons still believe that the outcomes are more durable (Kallenberg et al., 2011). Although TURP has been around for many years, the present operation differs considerably from that performed 30 years ago. There have been amendments in operative technique, instrument technology, and anesthetic methods. TURP is now safer, with a much lower mortality rate reported, but the effect these changes have had on long-term outcomes is largely unknown (Mebust et al., 2002; Rassweiler et al., 2006). The literature available on diathermy endoscopic prostatectomy either focuses on technical changes or associated limitations are compared. Long-term studies, that is, more than 3 years, which include TURP use, the procedure as the control arm (Rajeev et al., 2018). In these type of studies, the sample size is very small and strict inclusion criteria are employed, that is, prostate size restrictions that may render the study sample unrepresentative of the general TURP patient cohort (Ahyai et al., 2007; Tuhkanen et al., 2003; Kaya et al., 2007). Therefore, many studies on this issue have different or even contradictory results. Another limitation of older studies is that many were performed in the 1980's and early 90's (Meyhoff and Nordling, 1986; Nielsen et al., 1989; Roos et al., 1989). These studies typically use retrospective registry data and non-standardized survey instruments, while International Prostate Symptom Score (IPSS) and International Index Of Erectile Function (IIEF) emerged in the late 1990's which makes comparison with current data difficult (Kallenberg et al., 2011).

Nonetheless, TURP has its own complications, for instance about 80% of patients develop retrograde ejaculation, and 13% may need blood transfusions, while 15% complain of erectile dysfunction. Long term complications may necessitate a repeat procedure in 10% of the patients within 5 year while 5% may develop bladder neck stenosis or urethral stricture (Kallenberg et al., 2011; Yang et al., 1999). Fresh studies are therefore needed to update and validate the previously available literature. Hence, the aim of this study is to assess the long-term clinical outcomes following TURP in elderly Pakistani males (60+). The UN agreed cutoff is 60+ years

to refer to the older or elderly persons.

## MATERIALS AND METHODS

### Patients

The study included 50 patients with BPH who were diagnosed at the Department of Urology, Lahore General Hospital, Pakistan between 2013 and 2018, with LUTS due to BPH (without retention) were included in the study. Minimum age of the patient was 60 years and maximum 86 years old. Related/Required investigations were done and patients were operated for TURP after noting certain parameters. They were advised for follow up at 3 intervals (1st, 4th and 12th week postoperatively) and post-operative parameters were compared with pre-operative ones. Elderly patients with high surgical risk were defined as patients aged  $\geq 60$  years who experienced at least one internal comorbidity, e.g., hypertension or diabetes. Patients with BPH were included in the study if (1) they were aged  $\geq 60$  years, (2) they regularly used  $\alpha$ -receptor blockers, 5 $\alpha$ -reductase inhibitors, and/or muscarinic-receptor blockers for more than six months but did not achieve a satisfactory International Prostate Symptom Score (IPSS); and (3) they were willing to undergo TURP and provided written informed consent. All persons gave their informed consent prior to their inclusion in the study. Patients were assigned centrally at the Department of Urology, Lahore General Hospital, Pakistan, and each eligible patient was interviewed. The details of the surgical methods, such as benefits and drawbacks, risk, and expected expenses, were fully explained to the eligible patients. Next, the patients were given suggestions based on their specific condition. However, the surgical method, that is, TURP, was eventually decided by the patients. The type of surgical method selected was known to both the patient and his surgeon.

The trial was approved by the Ethical Committee of Lahore General Hospital, Pakistan and the methods were carried out in accordance with the approved guidelines.

### Preoperative preparation

In addition to routine examinations, following measures were taken for patients with different internal comorbidities before they underwent TURP, (1) controlling blood pressure within 140/90 mmHg in hypertensive patients; (2) maintaining fasting blood glucose at 6 to 8 mmol and 2-h postprandial blood glucose below 11 mmol/L; (3) improving and maintaining pulmonary or cardiac function in patients with chronic bronchitis, emphysema, or cardiac dysfunction; and (4) controlling blood pressure or treating anemias with active symptomatic treatment in patients with chronic renal insufficiency. Patients with deteriorating or unstable comorbidities were transferred to other departments for further treatment before being reconsidered for inclusion in the study.

### Surgical procedure

A TURP system (Olympus, Tokyo, Japan) was used for treating patients in the TURP group (280 W for cutting and 100 W for coagulation; 0.9% NaCl as irrigation fluid). All the patients were

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placed in the lithotomy position and were given general anesthesia with Propofol (Yang et al., 2016). Bladder irrigation was initiated immediately after the patient was transferred to a ward or intensive care unit (ICU).

### Outcome measures and follow-up

The following data were collected before the surgery and at one year after the surgery: (1) IPSS, (2) maximum flow rate (Qmax), (3) post-void residual (PVR), and (4) QoL. Preoperative prostate volume (PV) was measured by performing B-ultrasonic examination. All the patients were asked to visit our department at any time if they experienced any discomfort.

### Statistical analysis

SPSS software version 13.0 was used for data analysis. Discrete and continuous variables were compared using Chi-square test and t test, respectively. P values less than 0.05 were considered statistically significant.

## RESULTS

A total of 50 patients of benign prostate hyperplasia with lower urinary tract symptoms presenting without retention of urine were included in the study. IPSS, QOL, PMRU, and Q max were analyzed at pre-operatively and 1st, 4th and 12th week post-operative, as shown in Figure 1. 43 out of 50 patients (86%) had severe symptom score and 07 (14%) patients had moderate symptom score (pre operatively). According to American Urological Association Designed International Prostate Symptom Scoring System (IPSS), mild score ranges from 0-7, moderate score ranges from 8-19, and severe score ranges from 20-35.

### Pre and postoperative symptom score

Selection criteria for surgery was IPSS > 16 and Q max <10 ml/s. transurethral resection of prostate was done in the patients; the control visit was performed at 3 months. Treatment success was defined as Q max above 15 ml/s, residual urine of less than 100 ml, a 50% reduction in IPSS and absence of urinary retention. Preoperative Mean QOL (score) in the present study was 4.80. Our patients' QOL (score) also improved within first week postoperatively from 4.80 to 1.48. At 2nd follow up, maximum number of patients reported a very satisfying QOL (score) shown in Table 1.

The mean PMRU preoperatively was 210 ml and postoperatively mean PMRU at 1st follow up at 1st week was 49.4 ml. Mean PMRU at 2nd follow up at 4th week was 8.86 ml and at 3rd follow up at 12th week it was 4.60 ml. Results indicate that a high proportion of patients successfully operated (71.1%) had a combination of

IPSS > 16 and Q max < 10 ml/s (Table 1).

### Post-operative comparison

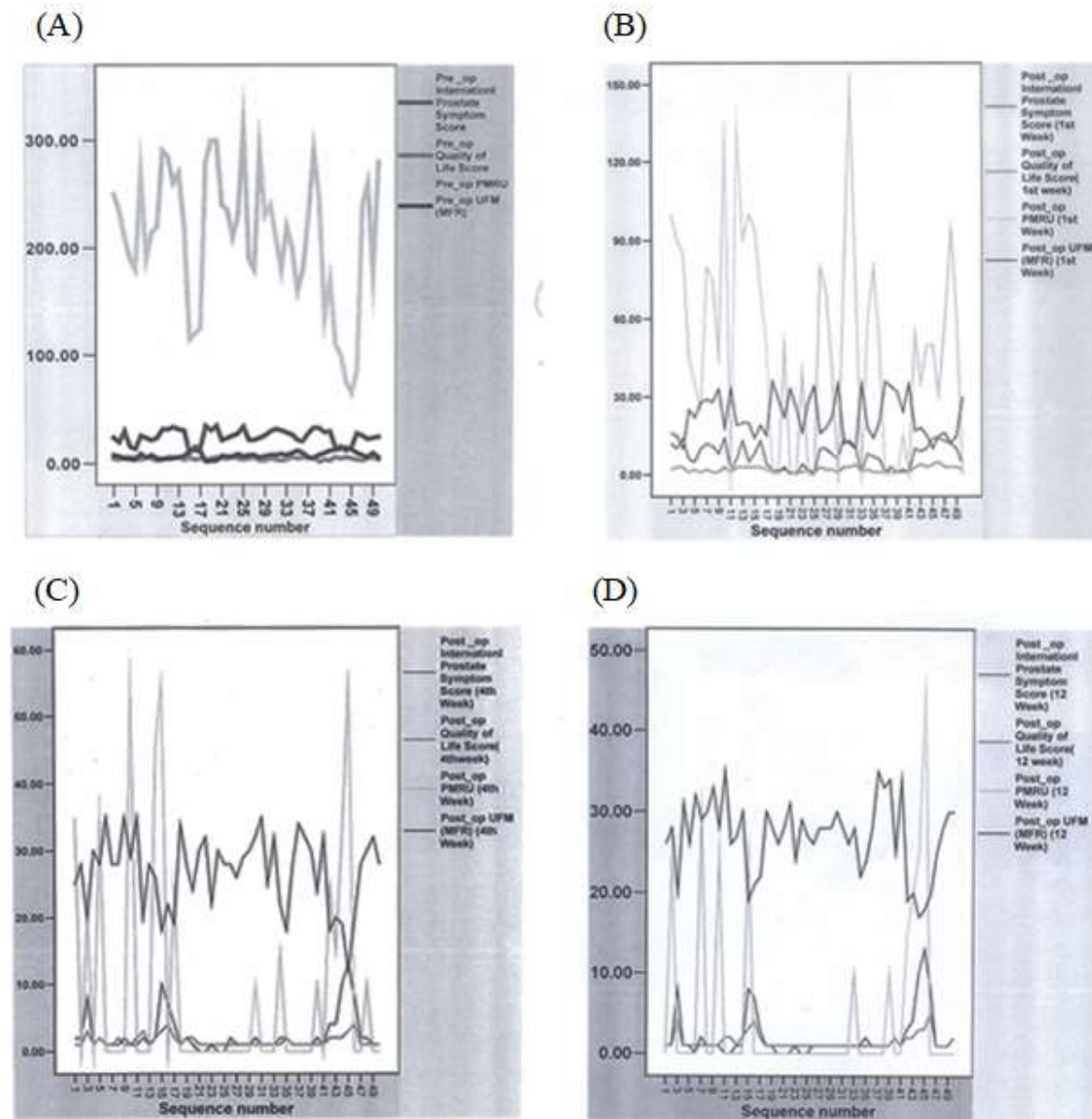
Post-operative follow-up of IPSS in Table 2 shows that after remarkable improvement in IPSS score between 1st and 4th week with a p value <0.0001, there was only minor change between 4th week and 12th week, with the p value <0.510, which shows that no significant change occurred during this period. Post-operative follow-up of QOL score in Table 3 shows that after remarkable improvement in QOL score, between 1st and 4th week with a p value between < 0.0015 and <0.0018, there was only minor change between 4th week and 12th week with the p value <0.900, which shows that no significant change occurred during this period.

Post-operative follow-up of PMRU in Table 4 shows that after remarkable improvement in PMRU, between 1st and 4th week with a p value was <0.0001, there was only minor change between 4th and 12th week, the p value < 0.1200, shows no significant change during this period. Post-operative follow-up of maximum urinary flow rate (Qmax) in Table 5 shows that after remarkable improvement in Qmax between 1st and 4th week with a p value < 0.0010 and < 0.0005, there was only minor change between 4 and 12th week, the p value < 0.940, shows that no significant change occurred during this period. The mean IPSS was 25.20 (preoperatively) and postoperatively mean IPSS at 1st follow up at 1st week was 7.22. Mean IPSS at 2nd follow up at 4th week was 2.38 and at 3rd follow up at 12th week it was 2.02.

The mean QOL (score) preoperatively was 4.80 and postoperatively mean QOL (score) at 1st follow up at 1st week was 2.12. Mean QOL (score) at 2nd follow up at 4th week was 1.50 and at 3rd follow up at 12th week it was 1.48. The mean PMRU preoperatively was 210 ml and postoperatively mean PMRU at 1st follow up at 1st week was 49.4 ml. Mean PMRU at 2nd follow up at 4th week was 8.86 ml and at 3rd follow up at 12th week it was 4.60 ml. The mean Qmax preoperatively was 7.94 ml/s and postoperatively mean Qmax at 1st follow up at 1st week was 7.22 ml/s. Mean Qmax at 2nd follow up at 4th week was 22.2 ml/s and at 3rd follow up at 12th week it was 26.9 ml/s.

## DISCUSSION

Transurethral resection of prostate remains the standard tool for the urologists to manage BPH (Marszalek et al., 2009; Rajeev et al., 2018). The new techniques may be more facile to utilize and are less traumatic for the patients but they require long term evaluation and are more extravagant. The quandary of dealing with large prostate (more than 100 g) still remains, for which open



**Figure 1.** (A) Pre-operative (IPSS, QOL, PMRU, Q max), (B) Post-operative (IPSS, QOL, PMRU, Q max) at 1st week, (C) Post-operative (IPSS, QOL, PMRU, Q max) at 4th week, (D) Post-operative (IPSS, QOL, PMRU, Q max) at 12th week.

prostatectomy or double sitting surgery is advised in most cases, that is, resection of one lobe in one surgery and other lobe in second surgery. This greatly reduces the chances of TURP syndrome (Porter and McCormick, 2003).

The morbidity/complications rate with TURP was lower in our patients as compared to the international studies (Cornu et al., 2015). The low morbidity/complications in this study is probably due to extra care regarding sterilization, associated risk factors such as medical diseases preoperatively, proper catheter care, good

patient's compliance and evaluation and management of the patients (Saint et al., 2016).

When evaluating surgical modalities for BPH, it is important to assess the impact of each treatment on both the direct (peak urinary flow rate and residual urine) and indirect outcomes. The latter being outcomes that are of greater relevance to the patient as they directly affect either the extent or quality of life (Eckhardt et al., 2001b). The discussion of the outcome is expanded from the analysis carried out for the Agency for Health Care Policy and Research (AHCPR) Guidelines (Eckhardt et al.,

**Table 1.** Pre and postoperative symptom score.

Variable	Pre-operative data	Post-operative data week1	Post-operative data week2	Post-operative data week3
IPSS	25.20	15.9	22.7	23.1
QOL	4.80	12.4	17.0	16.2
PMRU	210	15.0	21.6	22.5
Q max	7.94	7.22	22.2	26.9

**Table 2.** Post-operative follow-up of IPSS comparison between 1st, 4th and 12th week.

Week	t value	Standard deviation	Df	P Value
1st and 4th week	6.33	3.83	98	<0.0001
1st and 12th Week	6.81	3.82	98	<0.0001
4 and 12th week	1.64	2.81	98	<0.510

**Table 3.** Post-operative follow-up of QOL comparison between 1<sup>st</sup> week, 4th week and 12<sup>th</sup> week

Week	t value	Standard deviation	Df	P Value
1st and 4th week	3.28	0.94	98	<0.0015
1st and 12th Week	3.20	0.99	98	<0.0018
4 and 12th week	1.11	0.87	98	<0.900

**Table 4.** Post-operative follow-up of PMRU comparison between 1st, 4th and 12th week

Week	t value	Standard deviation	Df	P value
1st and 4th week	6.54	30.9	98	<0.0001
1st and 12th Week	7.56	29.6	98	<0.0001
4 and 12th week	1.56	13.7	98	<0.1200

**Table 5.** Post-operative follow-up of MFR comparison between 1st, 4th and 12th week.

Week	t value	Standard deviation	Df	P Value
1st and 4th week	-3.38	6.97	98	<0.0010
1st and 12th Week	-3.62	6.62	98	<0.0005
4 and 12th week	-0.001	5.02	98	<0.940

2001a). In this series, the selection criteria for TURP were subjective clinical presentation as assessed by American Urological Association (AUA) scoring method which was taken as median. However, we carried out Digital Rectal Examination (DRE) of prostate and abdominal ultrasound on every patient. Prostate size estimation by digital rectal examination is a reliable procedure; it is a subjective sign dependent on the skill and experience of the examiner (Liu et al., 2004).

Conventionally, patients were reviewed in out-patients' department for 03 months after TURP.

In the present study, the age ranged from 60 to 86 years. Mean age of patients in 63% cases was similar to other studies where an average age of 69 years was reported (Speakman, 1999). No relationship is found between the size of prostate and the degree of bladder outlet obstruction (Stage and Hairston, 2005). Immediate complications are related to the size of the adenoma,



resection time, technique and age of patients and the presence of severity of pre-TURP symptoms. Off and on stress incontinence was reported in 3 patients that relieved later on. Total incontinence was not found in the present study although it has been reported in many other studies which could be due to sphincter damage or edema of sphincter tissues. In the present study, substantial numbers of patients were satisfied with the results of TURP which is comparable with other studies (Donovan et al., 1996). In our study we tried to determine the different parameters that affect the outcome of TURP in patients of BPH with LUTS presenting without retention of urine. The four parameters were IPSS, QOL (score), PMRU, and urine flow rate. We also tried to assess any correlation of certain factors such as age, prostate size and severity of symptoms with the outcome of TURP. In this study, all the four parameters improved after TURP. Most of the patients improved at first follow up at 1st week post operatively and almost all the patients improved to their maximum at 2nd follow up at 4th week post operatively. There was a minor improvement at 3rd follow up at 12th week post operatively.

Three patients who did not improve to their level of satisfaction were among those 08 patients whose preoperative PMRU was between 50 and 100 ml. These patients reported persistent irritative symptoms after TURP. There was no marked improvement in their IPSS and QOL (score). This low PMRU could be one reason that leads to less or no improvement in symptoms after TURP. Although 05 patients whose PMRU was between 50 and 100 ml improved satisfactorily. Considering these results, we can say that sometimes irritative symptoms due to BPH, do not improve much as compared to the improvement in obstructive symptoms. In a study, van Venrooij et al. (2008) compared the outcomes after TURP in urodynamically obstructed versus urodynamically unobstructed, or selected equivocal men and concluded that TURP could be a good treatment alternative for selected equivocal or unobstructed men who opt for resection, did not benefit from medical therapy, and as a requirement for treatment discontinuation. They also added that TUR-P can result in a significant reduction in urethral resistance, even in unobstructed man (van Venrooij et al., 2008).

None of our patients were with decompensated urinary bladder. Every patient underwent cystometry to rule out any neurogenic bladder. It has been proven in many studies that relieving the urethral resistance also relieve the patient from lower urinary tract symptoms, if they are due to BPH. Prostatic resection decreases the amount of power required by the bladder per ml urine expelled (Dmochowski, 2005). Preoperative IPSS range (mean) in the present study was 25.20. In the present study, IPSS improved from 25.20 to 2.02 within one week postoperatively. Maximum improvement was observed at

second follow up at 4th week postoperatively. After that there was only minimal change in the IPSS at our 3rd follow up at 12th week postoperatively. Same has been proven by van Venrooij et al. (2018). Symptoms and well-being were quantified by American Urological Association symptom index (SI), quality-of-life score (QOL). They studied the improvements of Qmax, and nocturia, after TURP and the improvements of IPSS, QOL, and PMRU (van Venrooij et al., 2008).

It was concluded that voiding data should have a prominent role in the initial evaluation of men with LUTS suggestive of BPH. Preoperative Mean QOL (score) in the present study was 4.80. Our patients QOL (score) also improved within first week postoperatively from 4.80 to 1.48. At 2nd follow up, maximum number of patients reported a very satisfying QOL (score). Seki et al. (2006), conducted a study to determine preoperative predictive variables regarding treatment outcomes following transurethral resection of the prostate (TURP) of patients with symptomatic benign prostatic enlargement (BPE) (Seki et al., 2006). All the patients had completed the evaluation of International Prostate Symptom Score (I-PSS), and quality of life (QOL) index, and had undergone full urodynamics before the surgery. Outcomes were assessed at 12 months after surgery. The association between those variables obtained by preoperatively performed urodynamic analysis and the degree of improvement in lower urinary tract symptom and QOL following TURP was statistically elucidated in a large number of patients with symptomatic BPE. A higher degree of baseline BOO positively predicts the postoperative improvement in I-PSS and QOL, while the baselines DO negatively. Post void residual urine, a very accurate and non-invasive method to see any bladder outlet obstruction was also a part of our parameters.

The preoperative mean PMRU in this study was 210 ml. The PMRU improved from 210 to 4.60 ml. Maximum improvement was noted at 2nd follow up at 4th week post operatively. This has also been proved in a study conducted by Porru et al. (2002) in Italy to evaluate the predictive value of a combination of IPSS, uroflowmetry and ultrasound determination of residual urine volume in the determination of bladder outflow obstruction and in predicting treatment outcome. Forty-five out of a group of 60 BPH symptomatic patients were included. Pre-operative evaluation: IPSS, QOL score, uroflowmetry and residual urine along with other required tests was done. Selection criteria for surgery was IPSS > 16 and Q max <10 ml/s. transurethral resection of prostate was done in these patients; the control visit was performed at 3 months. Treatment success was defined as Q max above 15 ml/s, residual urine of less than 100 ml, a 50% reduction in IPSS and absence of urinary retention (Porru et al., 2002).

The overall success rate was 86% when measured by the IPSS. Its preoperative value was 16.9, and dropped

significantly to 4 ( $P = 0.005$ ). The score improved significantly after surgery only in the obstructed group compared to the non-obstructed group ( $P = 0.001$ ), however, preoperative IPSS did not correlate with objective treatment results. Patients with no or mild infra vesical obstruction had only minimal improvement of IPSS and Uroflowmetry after surgery proved that Qmax improved and overall patient was satisfied. Normal urinary flow rate in men is 20 to 25 ml/s. If flow rate decreases to 15 ml/s, there is a suspicion of obstruction, but if the flow rate decreases below 10 ml/s then it is stated that a definite obstruction is there. This can be labeled as a case of obstruction distal to bladder neck only if the detrusor power has been assessed urodynamically by cytometry. It is not possible to confirm obstruction only on the basis of decreased urine flow rate. Improvement in flow rate above 15 ml/s was considered success. Urinary flow rate, a very important parameter in diagnosis and comparison with post-operative results, also improved significantly from 7.94 to 26.9 ml/s. A study was conducted by Hakenberg et al. (2003), to assess the value of preoperative symptom score assessment and pressure-flow measurement in men undergoing transurethral prostatectomy (TURP). There were significant improvements in mean IPSS (-10.87 points) and peak flow rate (+ 7.06 mL/s) 3 months after TURP. Clinical decision-making remains a valid instrument for selecting patients for TURP. Both the IPSS and pressure-flow assessment are useful to exclude patients who are unlikely to benefit from TURP. The present results of the preoperative evaluation [Qmax, IPSS, QOL (score), and PMRU], post-operative variables [Qmax, IPSS, QOL(score), and PMRU], and the improvement in these scores were similar to those reported in other published studies (Thomas et al., 2004). In the present study, there was no effect of age, prostate size or severity of symptoms on the outcome of TURP. Also, there was no major complication either preoperatively or in the early post-operative period. Considering the results of the present study, we can say that there is no need to wait until the patient goes into retention of urine and then operate him. If there is indication, the patient fulfills the inclusion criteria and he is willing, then we can operate him for TURP as early as possible. By doing this, he can be saved from psychological trauma of being catheterized and any undue urinary tract infection due to catheterization, and above all of these, he can be saved from deterioration of kidneys (leading to renal failure) due to back pressure of PMRU.

## Conclusion

With the present observations, it can be concluded that TURP is very effective even in those patients who

present with lower urinary tract symptoms due to BPH without retention of urine. Urologists should not wait if the patient is not responding to the medical treatment. TURP should be done as early as possible to save the patient from undue catheterization and its complications. In the present set up where the socioeconomic situation is not that stable and health insurance is not provided to majority of our patients, early TURP can save the patient from financial burden and psychological trauma.

## CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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*Full Length Research Paper*

# **Antibacterial activities of three medicinal plant extracts and their synergistic effect on *Staphylococcus aureus* isolated from burn wounds**

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**This study was undertaken to investigate the antibacterial activities of three medicinal plant extracts and their synergistic effect on *Staphylococcus aureus* isolated from burn wounds. A total of 50 swab samples of burn wounds were collected from burn wound patients attending Federal Teaching Hospital Abakaliki and screened for *S. aureus* using standard microbiological techniques. Three plant materials (*Cucurbita pepo* leaf, *Alchornea cordifolia* leaf, and *Terminalia ivorensis* bark root) were dried under room temperature and ground into powdered form. Twenty grams of each plant materials was soaked in 100 ml of solvents (cold, warm water, ethanol, and methanol) for 24 h and filtered with muslin cloth. The crude extracts were mixed with dimethyl sulphoxide and subjected to 2 folds serial dilution. The results show that out of the 50 burn wounds swab samples collected, 32(64%) were positive for *S. aureus*. The susceptibility test results revealed that *Terminalia ivorensis* was the most active against the *S. aureus* isolates with an inhibition zone diameter (IZD) of 20 mm in warm water solvent at 100 mg/ml and 10 mm at 50 mg/ml. Results also revealed that a combination of ethanol extracts of *A. cordifolia* and root bark of *T. ivorensis* yielded 29 mm IZD. The combination of these two extracts exhibited a higher IZD against the *S. aureus* isolates. *C. pepo*, *A. cordifolia*, and *T. ivorensis* extracts could serve as putative agents for the development of novel drugs for the treatment of wound infections caused by *S. aureus*.**

**Key words:** Medicinal plant, sensitivity, synergistic, bacteriological, bacteria and *Staphylococcus aureus*.

## **INTRODUCTION**

Plants are very important for the health of humans and may serve as food source, medicinal, in environmental

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protection and beautification (Joshi et al., 2011). Plants are by far the richest source of drugs of traditional Systems of medicine, modern medicine, pharmaceutical intermediates and chemical entities for synthetic drugs (Joshi et al., 2011). The concept of finding healing powers in plants is an ancient practice that is as old as humanity (Cowan, 1999). Therefore, medicinal plants are used as antimicrobials that addresses the problems of drug resistance of microbial pathogens. According to Merriam Webster, an antimicrobial agent is defined as an agent that destroys or kills microorganism or reduce their metabolic activity leading to retardation of their growth. The development of antibiotic resistance is multifactorial, including the specific nature of the relationship of bacteria to antibiotic, use of antibacterial agents, host characteristics and environmental factors. This condition has forced scientists to carry out research for new antimicrobial substances from various sources as novel antimicrobial chemotherapeutic agents.

The invention of antibiotics has completely transformed the face of medicine in the 21<sup>st</sup> century coupled with the introduction of vaccination which lead to eradication of disease such as polio and small pox in the developed world (Nathisuwan et al., 2001). Due to the fact that these drugs are easily accessible and equally effective it leads one to over use them in raising livestock prompting bacteria to develop resistance. The problems of bacterial resistance is happening every day in our different environments. That is why scientists are seriously making research or other alternative means of controlling pathogenic bacteria with herbal plants (Cowan, 1999). Herbal medicine is a practice that makes use of natural plant substances to treat and prevent disease (Tyler and Foster, 1999). Herbal medicine is the use of plants or plant parts, their water or solvent extracts, essential oil, gums whole resins, therapeutically to provide prevention and cure of disease (Blumenthal, 2008). Most herbal plants (leaf, bark, oil and seeds) which have indicated antimicrobial potentials are yet to be validated of their claimed effects and possibly drug production. Example of plants with antimicrobial potential are *Fluted pumpkin* (*Cucurbita pepo*) *Terminalia ivorensis* (Black afara) and *Alchonia cordifolia* (Dove wood). *Terminalia ivorensis*, *Fluted pumpkin* and *Alchonia cordifolia* plants were used to treat burns and skin infections. Most wounds infections are contaminated by the individual's own endogenous flora which is present on the skin, mucous membrane, or hollow viscera. Usual pathogens on the skin and mucous surface are gram-positive cocci, mainly *Staphylococcus aureus* (Rosenbluth et al., 2004). In other words, Gram-negative aerobic and anaerobic bacteria can contaminate skin wounds of the groin and perinea areas. Therefore, the aim for this study was to investigate the antibacterial activities of three medicinal plant extracts and their synergistic effect on *S. aureus* isolated from burn wounds.

## MATERIALS AND METHODS

### Study area

The sample collection of this study was carried out at Federal Teaching Hospital (FETHA II) Abakaliki, Ebonyi State Nigeria. The sample was analyzed at Ebonyi State university Microbiology Laboratory complex. Ebonyi state has a population of 2,173,501 people (NPC, 2006). It is situated between latitude 6° 03'11.38N and longitude 8°09'46.22"E. The occupation of the people within the area of study is majorly farming and the season is rainy (April - September) and dry (October - March).

### Ethical clearance

Ethical clearance was obtained at Federal Teaching Hospital Abakaliki (FETHA 11) Ebonyi State, Nigeria.

### Collection, Identification and preparation of plant materials

The plant materials used in this study include fresh leaves of *C. pepo* (fluted pumpkin), *A. cordifolia* (Ubu plant) and bark root of *T. ivorensis* (Black afara). The plant materials were collected from Obeagu Item forest in Ikwo Local Government Area of Ebonyi State, Nigeria and were identified by Prof. J. C. Okafor and Prof. S. C. Onyekwelu (Taxonomists) in the Department of Biological Sciences of Ebonyi State University, Abakaliki. The leaves of the plant materials were carefully washed with clean tap water and rinsed with distilled water. The plant leaves were air dried at room temperature of 25-28°C and then grinded into powdered form with manual grinding machine and stored in air tight containers (Kudi et al., 1999).

The extraction of active constituents of plants was carried out according to the method of Parekh and Chanda (2006). Briefly, the leaves and bark root of the plant materials were dried under room temperature and grinded into powdered form using manual grinder. Each of the grinded herbal sample was respectively weighed and 20 g of each of the sample was soaked in 100 ml of the solvents (cold water, warm water, methanol and ethanol) used for extraction. The cold water preparation was allowed to stand for 24 h only with interval of 30 min shaking. Warm water, methanol and ethanol preparations were allowed to stand for 48 h. After this, the preparations, were filtered using Muslin filter cloth. The filtrate was poured into flat plate and air dried at room temperature to recover the extracts. The crude extracts recovered was weighed accordingly and recorded.

### Collection of wound swab samples

A total of fifty wound samples were collected by a nurse using sterile swab sticks from wound burn patients at Federal Teaching Hospital II Abakaliki, Ebonyi State (FETHA II). After collection, they were transported to Applied Microbiology Laboratory Complex, Ebonyi State University, Abakaliki for microbiological analysis.

### Bacteriological analysis clinical samples

The following media, Nutrient agar, mannitol salt agar and (Oxoid, USA) Nutrient broth were prepared according to manufacturer's instructions. Each swabbed sample was inoculated on nutrient

**Table 1.** Antibacterial Activities of *C. pepo* extract against *S. aureus* at different solvent concentrations.

Solvent	Concentration (µg/ml)	Inhibition zone diameter (mm)	Control IPM (10 µg)
Cold water	100	10	10
	50	NI	
	25	NI	
Warm water	100	5	15
	50	NI	
	25	NI	
Methanol	100	12	20
	50	10	
	25	NI	
Ethanol	100	10	20
	50	NI	
	25	NI	

**Key:** IPM = imipenem, NI – No inhibition.

broth and was incubated for 18-24 h aerobically and tubes which showed turbidity were re-inoculated onto nutrient agar and mannitol salt agar plates and incubated at 37°C for 18-24 h. After incubation, plates with growths were further characterized using standard microbiological techniques and biochemical tests including Gram staining, catalase, and coagulase tests.

#### Determination of antibacterial activities of plant extracts

This was determined by the method of Esimone et al. (2010) using agar well diffusion method. A 15-20 ml of molten Mueller-Hinton agar was aseptically poured into sterile petri dishes of equal sizes and was allowed to gel or solidify. The surface of the Mueller Hinton-agar was swabbed with the test organism (adjusted to 0.5 McFarland turbidity standards). Thereafter, a sterilized 6 mm cork borer was used to bore three holes on the Mueller-Hinton agar plates and the three holes were filled with 0.5 ml equal volume of the respective plant extracts at different concentrations of 100, 50 and 25 µg/ml. The aqueous extracts (cold water and warm water), methanol and ethanol extracts of *C. pepo*, *A. cordifolia* leaves and bark root of *T. ivorensis* were diluted with 4 ml of Dimethyl sulphoxide (DMSO) Imipenem 10 µg and ciprofloxacin 5 µg (Oxiod U.K.) paper disk which were used as positive control.

#### Determination of minimum inhibitory concentration of plant extracts on *S. aureus* isolated from burn wounds

The test organisms that were susceptible to the stock concentration of the herbal extract was further subjected to minimum inhibition concentration (MIC) using different concentration of 100, 50 and 25 µg/ml. The pure culture of the organisms were inoculated into nutrient broth and incubated at 37°C for 4-6 h. Then 0.5 ml of the broth culture of the bacteria were seeded on the surface of Mueller-

Hinton agar plates and spread evenly. Three wells of 6 mm in diameter was cut on the seeded agar plates using a sterile cork borer, and the bored wells were each filled with the different herbal extracts concentration that is 100, 50 and 25 µg/ml in a separate agar plates. These were repeated for different plants *C. pepo*, *A. cordifolia* and *T. ivorensis* (cold and warm water, methanol and ethanol) and incubated at 37°C for 18-24 h. After which, the inhibition zone diameter (IZD) were measured to the nearest milliliter and the lowest concentration of that inhibited bacterial growth was taken as MIC.

#### Synergistic activities of herbal plant extracts against *S. aureus*

To determine the interaction of herbal extract combined, one gram each of the extracts was weighed and mixed together in the proportion of 1:1 ratio and was dissolved using 90% Dimethyl sulphoxide (DMSO) concentration. The mixture were used to fill the holes bored in the Mueller-Hinton agar seeded with 0.5 MacFarland turbidity standard of the isolates and were incubated at 37°C for 18-24 h. After which the zone of inhibition were measured and recorded.

## RESULTS

Out of the 50 burn wounds samples collected from patients visiting FETHA II, 32 (64%) strains of *S. aureus* were isolated. The inhibition zone diameter of the plant extracts of *C. pepo* against *S. aureus* in different solvent and concentrations were shown in Table 1 The highest concentration of the *C. pepo* extracts was at 100 and 50 µg/ml in methanol (12 and 10 mm) and in cold water (10

**Table 2:** Antibacterial activities of *A. cordifolia* extract against *S. aureus* in different solvent concentrations.

Solvent	Concentration (µg/ml)	Inhibition zone diameter (mm)	Control IPM (10 µg)
Cold water	100	20	15
	50	15	
	25	5	
Warm water	100	10	15
	50	10	
	25	NI	
Methanol	100	10	14
	50	7	
	25	NI	
Ethanol	100	15	15
	50	15	
	25	5	

**Key:** IPM = imipenem, NI = No inhibition.

mm). No inhibitory zone diameter observed at 25 µg/ml as shown in Table 1.

Table 2 revealed that the inhibition zone diameter of plant extracts of *A. cordifolia* against *S. aureus* in different solvents concentrations. The highest inhibition zone diameter of cold water extracts of *A. cordifolia* yielded 20 mm at 100 µg/ml while at 50 µg/ml, 15 mm was recorded. In ethanol extracts it yielded 15 mm at 100 µg/ml while at 50 µg/ml 15 mm also recorded. In warm water extracts, it yielded 10 mm at 100 µg/ml while at 50 µg/ml, 10 mm also recorded and in methanol extracts, it yielded 10 mm at 100 µg/ml while at 50 µg/ml, 7 mm was also recorded. The lowest inhibition zone diameter was observed in cold water and ethanol extract at 25 µg/ml, which yielded 5 mm.

The highest synergistic effect was observed in ethanolic extract of *A. cordifolia* and root bark of *T. ivorensis* which gave 29 mm while in warm water extract of root bark of *T. ivorensis* and ethanolic extract *A. cordifolia* yielded 26 and 25 mm respectively. The inhibition zone diameter of plant extracts *T. ivorensis* against *S. aureus* in different solvent concentrations. The highest inhibition zone diameter of warm water of *T. ivorensis* extracts yielded 20 mm at 100 µg/ml while at 50 µg/ml 10 mm was recorded. Ethanolic and cold water extracts gave 15 mm at 100 µg/ml while at 50 µg/ml 12 and 13 mm was recorded respectively. At 25 µg/ml, methanolic extracts yielded 11 mm while cold, warm water and ethanolic extracts gave 8 and 5 mm. It was observed that among the three plant extracts. *T. ivorensis* had highest inhibition

zone diameter against *S. aureus*. It have inhibition zone diameter in all the solvents in comparing to the control drug (Table 3).

Table 4 showed the minimum inhibition concentration of all the three plant extracts (*C. pepo*, *A. cordifolia* and *T. ivorensis*). The lowest minimum inhibition concentrations among the three plants were observed in *T. ivorensis* and *A. cordifolia* extracts, in cold water and ethanol. Extracts gave 5 mm respectively. This showed that *T. ivorensis* has the highest healing component followed by *A. cordifolia* in treatment of infection caused by *S. aureus*. Table 5 demonstrated the synergistic activities of herbal plantextracts against *S. aureus*. The highest inhibition zone diameter at 100 µg/ml was observed in ethanolic leaf extract of *A. cordifolia* and ethanolic bark root extract of *T. ivorensis* (29 mm), followed by warm water extract of *T. ivorensis* and ethanolic leaf extract of *A. cordifolia*, (26 mm) and warm water extract of *T. ivorensis* and *A. cordifolia* (25 mm).

## DISCUSSION

Antibiotic resistance is a great global concern. There have been an increasing incidence of multiple drug resistance in human pathogenic microorganisms in recent years, largely due to indiscriminate use of commercial antimicrobial agents commonly used in the treatment of infectious diseases (Marjorie, 1999). This has forced scientists to search for new antimicrobial

**Table 3.** Antibacterial activities of *T. ivorensis* extracts against *S. aureus* at different solvent concentrations.

Solvent	Concentration ( $\mu\text{g/ml}$ )	Inhibition zone diameter (mm)	Control drug IPM (10 $\mu\text{g}$ )
Cold water	100	15	15
	50	12	
	25	8	
Warm water	100	20	10
	50	10	
	25	5	
Methanol	100	12	15
	50	12	
	25	11	
Ethanol	100	15	25
	50	13	
	25	5	

**Table 4.** The minimum inhibition concentration of all the three herbal plant extracts against *S. aureus*.

Solvents	Plant extracts Conc. ( $\mu\text{g/ml}$ )	Inhibitory zone diameter (mm)		
		<i>C. pepo</i>	<i>A. cordifolia</i>	<i>T. ivorensis</i> (5 mm)
Cold water	100	Nil	25	25
Warm water	50	Nil	25	25
Methanol	25	Nil	Nil	25
Ethanol	100	Nil	25	25

**Key:** NI = No Inhibition, MIC = Minimum Inhibition Concentration.

substances from various sources like the medicinal plants. A total of 50 clinical samples were collected from burn wound patients, 32 were isolated and identified as *Staphylococcus* species based on microbiological and biochemical tests. The result showed Gram positive cocci and clustered in arrangement with purple colour. The result is in line with Sule et al. (2002) and Thanni et al. (2003) who reported that *S. aureus* is a normal flora of the skin and a major cause of both surgical and accidental wound infections. In this study, result obtained showed that the three plants used for the study possess bioactive compound against *S. aureus* isolated from wound burns. This is in agreement with Dweck (2001) who reported that medicinal plants possesses antimicrobial activity. In this work, *C. pepo* showed antibacterial activity against *S. aureus* in different solvents. *C. pepo* also revealed that different extracts in solvent have different compounds with antibacterial activity. This could be compounds with antibacterial activity. This could be due to the different classes of

compounds or solvent used. This is in line with work of Marjorie (1999) who reported that different extracts in solvent have different compounds with antibacterial activity. Again, *C. pepo* extracts in methanol was observed to have highest inhibition zone diameter of 10 mm. This could mean that active ingredient of *C. pepo* extracts were not equally soluble in ethanol, methanol and water. This result is in agreement with the findings of who reported that alcohol/ ethanol as the best solvent for extraction of plant active substances of medical importance. Methanolic extract of *C. pepo* was also found to be active against *S. aureus*. *A. cordifolia* also revealed that almost the plant extracts in different solvents was equally able to inhibit the microbial growth of the isolate, *S. aureus*. It was observed that cold water and ethanolic extracts of *A. cordifolia* have the highest inhibition zone diameter (29 mm). This could be due to the infusion of dried and crushed leaves of the plant. *A. cordifolia* is soluble in cold water and ethanol moves faster in dried and crushed leaves of plant materials. This is in



**Table 5.** The synergistic activities of herbal plant extracts against the *S. aureus*.

Herbs	Inhibition zone diameter (mm) 100 (µg/ml)
A	14
B	9
C	11
D	14
E	19
F	18
G	19
H	19
I	29
J	19
K	26
L	25

Key: A = Cold water extract of *C. pepo* and *A. cordifolia*, B = Cold water extract of *C. Pepo* and root bark of *T. ivorensis*, C = warm water extract of *C. pepo* and *A. cordifolia*, D = warm water extract of *C. pepo* and root bark of *T. ivorensis*, E = Methanolic extract of *C. pepo* and root bark of *T. ivorensis*, F = Methanolic extract of *C. pepo* and *A. cordifolia*, G = Ethanolic extract of *C. pepo* and root bark of *T. ivorensis*, H = Cold water extract of *A. cordifolia* and root bark of *T. ivorensis*, I = Ethanolic extract of *A. cordifolia* and root bark of *T. ivorensis*, J = methanolic extract of *A. cordifolia* and root bark of *T. ivorensis*, K = warm water extract of root bark of *T. ivorensis* and ethanolic of *A. cordifolia*, L = warm water extract of root bark of *T. ivorensis* and *A. cordifolia*.

agreement with the work of Niemann et al. (2005) who reported that cold infusion of dried and crushed leaves of *A. cordifolia* acts as a durescic cicatrisant and antibacterial activities to wounds infections.

Among the three plant extracts used, *T. ivorensis* bark root have the highest inhibition zone diameter against *S. aureus*. This could be as a result of substance or constituent contained in the plant. This is in line with the work of Lawal et al. (2014) who reported that they contain substances like tannins, saponins, phenols, alkaloids and cyanogenic glucoside. Again alkaloids have been reported as the active ingredient in medicinal plants exhibiting potency as antibiotic, antidiabetic, and insecticidal agent (Abreu and Pereiru, 2001).

The comparison of MIC of the ethanol, methanol and aqueous extracts of the plant leaves and bark root was observed that ethanol extract showed greater antibacterial activity of 5 mm compared to its corresponding extracts in methanol and aqueous extracts with 5 mm. The combination of the plant extracts were very significant especially with *C. pepo* which was resistant on single

testing in different solvent concentrations but on combination was able to inhibit the isolate. This is in agreement with the work of Yang et al. (2009) who reported that effectiveness of the herbs when used in pairs than when used individually in treatment of various ailments like malaria, HIV, even cancer promotes the advantage of combination therapy in treatment of such ailments.

## CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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*Full Length Research Paper*

## **Sciatica: Medical treatment or Physiotherapy?**

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**Low back pain is usually self-limiting. It has a sizeable impact on patients, and is associated with high healthcare and societal costs as much as \$16 billion each year. Patients are commonly treated in primary care but a small proportion is referred to secondary care and may eventually have surgery. There are numerous treatment approaches for sciatica. Many people receive only medical treatment and are unaware of the availability and efficacy of the physiotherapy treatment. Objective of this study is to find out best treatment by comparison between medical interventions versus physiotherapy treatment for Sciatica. This study is a quasi-experimental study which includes patients presenting with sciatica divided into two groups. One group received medical treatment from a general physician and other group received physical therapy treatment from physiotherapist along with medical treatment from general physician. The study was conducted at Fatima Memorial Hospital, Services Hospital, Mayo Hospital and Hamid Latif Hospital, Lahore. The study was completed within the time duration from February 2017 to July 2017. Non-probability purposive sampling technique was used to collect data. Pre-treatment evaluation was done with Visual Analogue Scale, Oswestry Disability Index and Straight Leg Raising Test. The physical inspection involved the straight leg raising test and active knee extension tests. The data was managed and analyzed using SPSS version 21. Physiotherapy along with medical treatment is observed to give better interventional outcomes.**

**Key words:** Sciatica, medical intervention, physiotherapy, oswestry disability index, straight leg raising test

### **INTRODUCTION**

Sciatica, known by a range of synonyms such as lumbosacral radicular syndrome, nerve root compromise, nerve root pain, and nerve root entrapment or is identified by radiating leg pain below the knee in one or more than

one lumbar or sacral dermatomes, it can also occur as an event of compression of nerve root or neurological insufficiencies (Smeele et al., 1996; Pinto et al., 2012). Disc prolapse is a typical cause of sciatica. Spinal

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or stenosis of lateral recess, cancers, radiculitis and also piriformis syndrome are also amongst the causes of this symptom (Luijsterburg et al., 2008; Stam, 1996). Individuals with overweight and obesity are likely to have lumbar radicular pain and sciatica (Shiri et al., 2014).

Sciatica is chiefly identified by taking previous history and physical examination. Regarding its management, during the first 6 to 8 weeks, there is congruity that management of sciatica ought to be conventional. Direction to be active as much as possible, physical therapy maneuvers or exercises, analgesics, non-steroidal anti-inflammatory drugs (NSAIDs), epidural corticosteroid inoculations and transforaminal periradicular injections of corticosteroid are advised (Vloka et al., 2001). Modifiable risk elements could be cigarette smoking, obesity, occupational elements and health conditioned. Non-alterable elements include age, gender and social status. A significant number of well-known risk elements that are identified in first time sciatica are alterable, providing the potential benefits of key avoidance. Moreover, those risk factors likewise contribute to unhealthy way of life (Cook et al., 2014). There is a unity about the management of sciatica, that it should be conservative in the first 6 to 8 of treatment. However, required content of the conservative treatment is not understood till now (Vroomen et al., 2000). Bed rest is no longer believed to be a management possibility for sciatica (Mens et al., 2009). For the conservative management of sciatica, the essential choice to lessen the pain is by painkillers or else by decreasing compression on the nerve root. It is believed that conservative managements do not evidently recover the usual pattern of sciatica in maximum patients or decrease symptoms (Koes et al., 2007). Giving the patients the awareness of the causes and anticipated outcome might be an essential part of the treatment protocol.

Pain killers or non-steroidal anti-inflammatory drugs, acupuncture, epidural steroid injections, spinal manipulation, traction therapy, physical therapy, behavioral treatment, multidisciplinary treatment have mysterious effectiveness (Koc et al., 2009). Surgical treatment for sciatica stresses on elimination of the herniated slice of the disc and ultimately the part of the disc or on foraminal stenosis, with the motive of eradicating the ambiguous source of the sciatica (Peul et al., 2009). Agreement is that a cauda equina disorder is a clear notion for immediate surgery. Elective surgery is the choice for one sided sciatica (Gardner et al., 2011). The surgical intervention of patients with lumbar disc prolapse is preferred over conservative treatment. However, it has been additionally determined that the long-lasting positive outcomes of surgical intervention are suspicious and the assurance about the ideal timing of surgery is also undetermined (Devillé et al., 2000). It has been observed that patients with sciatica reported a quick decrease in pain and disability in the first 3 months, but still had mild

to moderate symptoms 5 years after surgery. While no remarkable differentiation was found, micro discectomy revealed great improvements in comparison to other surgical interventions (Machado et al., 2016). Acupuncture, that has an extended past history in primitive China as a conservative method of management in Chinese medicine, has been broadly used in various pain relief application in the western world since the 1970s. About managing sciatica patients by means of acupuncture, studies have unveiled the benefits (Liu and Chen, 2017). A comparison was made between disc surgery and conservative treatment. In one experiment micro discectomy was compared with conservative management in patients of sciatica since 6 to 12 weeks. No remarkable contrasts were found for associated leg pain, backache and individual disability after follow-up of two years. Leg pain, nevertheless, appeared to be upgraded initially more rapidly in patients in the discectomy category (Weinstein et al., 2006). Sciatica can be debilitating, and verification regarding medical managements is restricted. Pregabalin is efficacious in the management of few types of neuropathic pain. It has been observed that treatment with pregabalin did not remarkably decrease the intensity of leg pain linked with sciatica and did not remarkably improve other outcomes, as compared with placebo, after the time period of 8 weeks. The occurrence of bad events was significantly greater in the pregabalin group than in the placebo group (Mathieson et al., 2017). However, physical therapy was considered to be of great value with patients participating in exercises to reduce pain and restlessness, improve gait and posture (Newsome et al., 2014). Jeong and Associates stated after their experiment that mobilization approaches for the sciatic nerves may improve nervous system compliance and lessen the sensitivity, that would help to relieve the symptoms (Jeong et al., 2016). The use of stimulated form of vitamin B12 Methylcobalamine has shown promising effect in patients with low back pain, especially in diabetic patients (Beliveau, 1971; Zhang et al., 2013). Where pharmacological management is frequently suggested, sometimes contrary effects takes place. Acupuncture is a remarkable alternate when medical intervention is barred. This therapy evades side effects of medications, however it should be taken under consideration as an additional therapy (Miladi et al., 2014). This study was conducted to validate the view that Physiotherapy along with medications is a better option to treat sciatica than physiotherapy or pharmacotherapy alone.

## MATERIALS AND METHODS

### Study design

Quasi experimental study.

**Table 1.** Descriptive statistics for the age, height, weight and BMI of the patients.

Study group		N	Minimum	Maximum	Mean	Std. deviation
Medical intervention	Age of the patient	25	22.00	64.00	49.0000	10.35213
	Height of the patient	25	140.00	185.00	164.5200	11.61579
	Weight of the patient	25	20.00	105.00	72.3600	18.29135
	BMI of the patient	25	21.00	31.00	26.5600	3.41663
	Valid N (list wise)	25	-	-	-	-
Physiotherapy along with medical intervention	Age of the patient	25	21.00	62.00	43.6800	11.94334
	Height of the patient	25	118.00	182.00	156.6000	13.54314
	Weight of the patient	25	48.00	90.00	68.2800	12.77211
	BMI of the patient	25	18.00	45.00	27.3080	6.89377
	Valid N (list wise)	25	-	-	-	-

### Study setting

The study was carried out at Fatima Memorial Hospital, Hamid Latif Hospital, Mayo Hospital and Services Hospital Lahore, Pakistan. The study was completed within the time duration from January 2017 to June 2017 at Fatima Memorial Hospital Lahore, Pakistan. 50 patients were taken, 25 in each group. Non-probability purposive sampling technique was used to collect the data.

### Data collection procedure

A total of 50 questionnaires were distributed and all of them were responded. All ethical issues were considered in this study, an informed consent was taken from the head of department for their approval to conduct the study and the patients.

### Treatment

All the subjects were allocated into 2 groups, of which one group got only medical treatment and other group was given physiotherapy treatment along with medicines. Treatment plan given to group 1 included; tablet Orphenadrine (35) + Paracetamol (450 mg) three times a day, tablet Tizanidine (2 mg) two times a day, tab Calcium + vitamin D once a day, tab Methycobalamine 500 mcg three times a day.

Group 2 treatment included stretches of hamstrings and piriformis muscle, hot pack and TENS (Transcutaneous Electrical Nerve Stimulation), sciatic nerve mobilization along with the same pharmacotherapy as Group 1.

### DATA ANALYSIS

The data was managed and analyzed using SPSS version 21.

### RESULTS

The distribution of age in medical intervention group (group 1) was  $49.0 \pm 10.3$ , while distribution of age in physiotherapy along with medical intervention (group 2)

was  $43 \pm 11.9$ . The distribution of weight (kg) in group 1 was  $72.3 \pm 18.2$  and in group 2 it was  $68 \pm 12.7$ . The distribution of height (cm) in group 1 was  $164.5 \pm 12$  and in group 2 it was  $156.6 \pm 14$ . The distribution of BMI in group 1 out was  $26.5 \pm 3.41$  and the distribution of BMI in group 2 was  $27 \pm 6.89$  (Table 1).

In group 1 male were 15 (60%) and female were 10 (40%) while in group 2 male were 3 (12%) and female were 22 (88%) (Table 2). In group 1 the office employees were 10 (40%), laborers were 6 (24%) and House wives were 9 (36%). In group 2 office employees were 8 (32%), house wives were 16 (64%) and Students were 1 (4%) (Table 3). In group 1, 5 (20%) presented with acute pain 20 (80%) with chronic pain and in group 2, 7 (28%) presented with acute pain and 18 (72%) with chronic pain (Table 4). In group 1, 5 (20%) patients had active life style and 20 (80%) had a sedentary life style. In group 2, 11 (44%) had an active life style while 14 (56%) had a sedentary life style (Table 5). In group 1, 3 (12%) had positive active knee extension test and 22 (88%) had negative knee extension test. In group 2, 13 (52%) had positive knee extension test and 12 (48%) had negative knee extension test (Table 6).

Independent sample t-test for visual analogue scale pre-treatment reading showed the non-significant p value 0.529 with Mean Difference (-1.72000) and DF (48). Week 1 reading showed the non-significant p value 0.161 with Mean Difference (4.76000) and DF (48). Week 2 reading showed the non-significant p value 0.002 with Mean Difference (10.24000) and DF (48). Week 3 reading showed the non-significant p value 0.00 with Mean Difference (16.00000) and DF (48) (Table 7).

Independent Sample t-test for Oswestry Disability Index Pre-treatment reading showed the non-significant p value 0.268 with Mean Difference (-2.68000) and DF (48). Week 1 reading showed the non-significant p value 0.480 with Mean Difference (1.48000) and DF (48). Week 2 reading showed the non-significant p value 0.00 with Mean Difference (6.56000) and DF (48). Week 3 reading

**Table 2.** Gender of the patient.

Study Group			Frequency	Percent	Valid percent	Cumulative percent
Medical intervention	Valid	Male	15	60.0	60.0	60.0
		Female	10	40.0	40.0	100.0
		Total	25	100.0	100.0	-
Physiotherapy along with medical intervention	Valid	Male	3	12.0	12.0	12.0
		Female	22	88.0	88.0	100.0
		Total	25	100.0	100.0	-

**Table 3.** Occupation of the patient.

Study group			Frequency	Percent	Valid percent	Cumulative percent
Medical intervention	Valid	Employee	10	40.0	40.0	40.0
		Laborers	6	24.0	24.0	64.0
		House Wife	9	36.0	36.0	100.0
		Total	25	100.0	100.0	-
Physiotherapy along with medical intervention	Valid	Employee	8	32.0	32.0	32.0
		House Wife	16	64.0	64.0	96.0
		Student	1	4.0	4.0	-
		Total	25	100	100	100.0

**Table 4.** Type of pain.

Study group			Frequency	Percent	Valid percent	Cumulative percent
Medical intervention	Valid	Acute	5	20.0	20.0	20.0
		Chronic	20	80.0	80.0	100.0
		Total	25	100.0	100.0	-
Physiotherapy along with medical intervention	Valid	Acute	7	28.0	28.0	28.0
		Chronic	18	72.0	72.0	100.0
		Total	25	100.0	100.0	-

showed the non-significant p value 0.00 with Mean Difference (11.56000) and DF (48) (Table 8).

Independent Sample t-test for Straight Leg Raising Test Pre-treatment reading showed the non-significant p value 0.489 with Mean Difference (2.00000) and DF (48). Week 1 reading showed the non-significant p value 0.78 with Mean Difference (-5.12000) and DF (48). Week 2 reading showed the non-significant p value 0.03 with Mean Difference (-8.00000) and DF (48). Week 3 reading showed the non-significant p value 0.00 with Mean Difference (-13.28000) and DF (48) (Table 9).

Pairwise Comparison of Visual Analogue Scale Pre-treatment and Post-Treatment for Medical intervention

Group Mean Difference Week 3 was (34.200) with significance (0.00) (Table 10).

Pairwise comparison of Visual Analogue Scale Pre-treatment and Post-Treatment for Physiotherapy along with Medical intervention Group Mean Difference Week 3 was (51.920) with significance (0.00) (Table 11).

Pairwise Comparison of Oswestery Disability Index Pre-treatment and Post-Treatment for Medical intervention Group Mean Difference Week 3 was (19.800) with significance (0.00) (Table 12).

Pairwise Comparison of Oswestery Disability Index Pre-treatment and Post-Treatment for Physiotherapy along with Medical intervention Group Mean Difference

**Table 5.** Life Style of the patient.

Study group			Frequency	Percent	Valid percent	Cumulative percent
Medical intervention	Valid	Active	5	20.0	20.0	20.0
		Sedentary	20	80.0	80.0	100.0
		Total	25	100.0	100.0	-
Physiotherapy along with medical intervention	Valid	Active	11	44.0	44.0	44.0
		Sedentary	14	56.0	56.0	100.0
		Total	25	100.0	100.0	-

**Table 6.** Active knee extension test.

Study group			Frequency	Percent	Valid percent	Cumulative percent
Medical intervention	Valid	Positive	3	12.0	12.0	12.0
		Negative	22	88.0	88.0	100.0
		Total	25	100.0	100.0	-
Physiotherapy along with medical intervention	Valid	Positive	13	52.0	52.0	52.0
		Negative	12	48.0	48.0	100.0
		Total	25	100.0	100.0	-

Week 3 was (34.040) with significance (0.00) (Table 13).

Pairwise Comparison of Straight Leg Raising Test Pre-treatment and Post-Treatment for Medical intervention Group Mean Difference Week 3 was (-26.080) with significance (0.00) (Table 14).

Pairwise Comparison of Straight Leg Raising Test Pre-treatment and Post-Treatment for Physiotherapy along with Medical intervention Group Mean Difference Week 3 was (-41.360) with significance (0.00) (Table 15).

## DISCUSSION

This study found that sciatica prevails more among those who have deskbound and sedentary life style, this is in keeping with Peter György Horváth and associate who stated that the body built and the posture in which a person sits has a substantial influence on the pressure distribution and peak pressures in the selected zones. Alongside a comfortable seat special attention should be paid also to a correct posture, because many of health complains can be linked to the latter (Horváth et al., 2017). Our study showed remarkable effects of muscle relaxants along with vitamin B12 in-keeping with most international procedures where pharmacological treatment has been recommended to decrease the pain in patients of sciatica, involving paracetamol, non-steroidal anti-inflammatory medication, opioid pain killers, anti-convulsants, and corticosteroids. But, generally there is

very restricted acknowledgement on the effectiveness, safety, and durability of these drugs alone in old age patients who have sciatica (Ferreira and McLachlan, 2016).

We observed that there is prevalence of sciatica more among females which agrees with Karjalainen and co-workers who reported that female gender complained about sciatic pain more than male gender (Karjalainen et al., 2013). There is small evidence that outcomes of exercise produces greater effects on leg pain as compared to the advice to stay active and energetic for the short period of time in patients experiencing sciatica (Fernandez et al., 2015).

In people with acute sciatica, muscle relaxants show clinically remarkable short-period pain relief (Abdel Shaheed et al., 2017). Pharmacotherapy with physiotherapy showed significant pain relief in our study. However, it was noticed in the whole process of the study that medical intervention relieved the symptoms of sciatica for short period of time while, physiotherapy along with medical intervention has remarkable effects in reducing the pain, gaining range and also improved the quality of everyday life in the long run. The age of the patients included in our study ranged from 18 to 65 years.

The results showed that 40 to 45 years range was more affected, that could be due to lumbar degeneration and inactivity, this agrees with professor Koes and co-workers who suggested that the peak age for onset of lumber pain is about the same reported in our study

**Table 7.** Independent samples test for visual analogue scale.

Variable		Levene's test for equality of variances		T-test for equality of means				
		F	Sig.	t	Df	Sig. (2-tailed)	Mean difference	Std. error difference
Visual analogue scale pre-treatment	Equal variances assumed	1.168	0.285	-0.635	48	0.529	-1.72000	2.70932
	Equal variances not assumed	-	-	-0.635	45.005	0.529	-1.72000	2.70932
Visual analogue scale post treatment week1	Equal variances assumed	3.913	0.054	1.423	48	0.161	4.76000	3.34568
	Equal variances not assumed	-	-	1.423	34.931	0.164	4.76000	3.34568
Visual analogue scale post treatment week 2	Equal variances assumed	0.199	0.657	3.316	48	0.002	10.24000	3.08796
	Equal variances not assumed	-	-	3.316	45.812	0.002	10.24000	3.08796
Visual analogue scale post treatment week3	Equal variances assumed	2.903	0.095	7.404	48	0.000	16.00000	2.16102
	Equal variances not assumed	-	-	7.404	42.863	0.000	16.00000	2.16102

**Table 4.** Independent samples test for Oswestry disability index.

Variable		Levene's test for equality of variances		t-test for equality of means				
		F	Sig	T	Df	Sig. (2-tailed)	Mean difference	Std. error difference
Oswestry disability index pre-treatment	Equal variances assumed	4.642	0.036	-1.120	48	0.268	-2.68000	2.39313
	Equal variances not assumed			-1.120	39.481	0.270	-2.68000	2.39313
Oswestry disability index post treatment week1	Equal variances assumed	3.547	0.066	.712	48	0.480	1.48000	2.07878
	Equal variances not assumed			.712	43.061	0.480	1.48000	2.07878
Oswestry disability index post treatment week2	Equal variances assumed	0.113	0.739	4.963	48	0.000	6.56000	1.32167
	Equal variances not assumed			4.963	47.999	0.000	6.56000	1.32167
Oswestry disability index post treatment week3	Equal variances assumed	1.648	0.205	12.275	48	0.000	11.56000	0.94177
	Equal variances not assumed			12.275	46.727	0.000	11.56000	0.94177

(Koes et al., 2007).

Females were found to be more prone to develop sciatica. People who have active life style are less likely to get affected by sciatica. Two tests were performed to confirm the presence of sciatica, one was straight leg raising test which

showed 100% positive result and the other was active knee extension test which showed mixed, that is, negative and positive results. Medications usually prescribed in sciatica are analgesics, vitamin B12 and muscle relaxants. In our study one group was being treated with the same

medical intervention and compared to the other group that was treated by physiotherapy along with medical intervention, results showed more relief in pain, better quality of life of the patient and the SLR test came out to be negative after three weeks of treatment in the latter group.



**Table 5.** Independent samples test for straight leg raising.

Variable		Levene's test for equality of variances		T-test for equality of means				
		F	Sig.	t	Df	Sig. (2-tailed)	Mean difference	Std. error difference
Straight leg raising test pre-treatment	Equal variances assumed	0.503	0.482	.697	48	0.489	2.00000	2.87080
	Equal variances not assumed			.697	42.483	0.490	2.00000	2.87080
Straight leg raising test post treatment week1	Equal variances assumed	0.742	0.393	-1.801	48	0.078	-5.12000	2.84296
	Equal variances not assumed			-1.801	43.796	0.079	-5.12000	2.84296
Straight leg raising teat post treatment week2	Equal variances assumed	0.527	0.471	-3.102	48	0.003	-8.00000	2.57925
	Equal variances not assumed			-3.102	45.678	0.003	-8.00000	2.57925
Straight leg raising test post treatment week3	Equal variances assumed	1.630	0.208	-5.284	48	0.000	-13.28000	2.51314
	Equal variances not assumed			-5.284	43.115	0.000	-13.28000	2.51314

**Table 6.** Pairwise comparison of medical intervention group for visual analogue scale.

Pairwise Comparisons							
Study group	(I) VAS	(J) VAS	Mean difference (I-J)	Std. error	Sig. <sup>a</sup>	95% Confidence interval for difference <sup>a</sup>	
						Lower bound	Upper bound
Medical intervention	1	2	13.600 <sup>*</sup>	1.370	0.000	9.661	17.539
		3	24.480 <sup>*</sup>	2.153	0.000	18.289	30.671
		4	34.200 <sup>*</sup>	2.249	0.000	27.735	40.665
	2	1	-13.600 <sup>*</sup>	1.370	0.000	-17.539	-9.661
		3	10.880 <sup>*</sup>	1.434	0.000	6.756	15.004
		4	20.600 <sup>*</sup>	2.068	0.000	14.654	26.546
	3	1	-24.480 <sup>*</sup>	2.153	0.000	-30.671	-18.289
		2	-10.880 <sup>*</sup>	1.434	0.000	-15.004	-6.756
		4	9.720 <sup>*</sup>	1.476	0.000	5.477	13.963
	4	1	-34.200 <sup>*</sup>	2.249	0.000	-40.665	-27.735
		2	-20.600 <sup>*</sup>	2.068	0.000	-26.546	-14.654
			3	-9.720 <sup>*</sup>	1.476	0.000	-13.963

Physiotherapy is emerging choice of treatment in recent times. Unlike international state of affairs people in Pakistan people are not so much aware

of the treatment choices they can have. In recent times people in Pakistan are becoming aware of effectiveness of physiotherapy treatment, at least

they are curious to know the outcomes of different treatments.

The Results of this study can broaden their

**Table 7.** Pairwise comparison of physiotherapy along with medical intervention group for visual analogue scale.

Study group	(I) VAS	(J) VAS	Mean difference (I-J)	Std. error	Sig. <sup>a</sup>	95% Confidence interval for difference <sup>a</sup>	
						Lower bound	Upper bound
Physiotherapy along with medical intervention	1	2	20.080*	2.572	0.000	12.685	27.475
		3	36.440*	2.501	0.000	29.248	43.632
		4	51.920*	1.854	0.000	46.590	57.250
	2	1	-20.080*	2.572	0.000	-27.475	-12.685
		3	16.360*	1.733	0.000	11.378	21.342
		4	31.840*	2.618	0.000	24.314	39.366
	3	1	-36.440*	2.501	0.000	-43.632	-29.248
		2	-16.360*	1.733	0.000	-21.342	-11.378
		4	15.480*	1.791	0.000	10.331	20.629
	4	1	-51.920*	1.854	0.000	-57.250	-46.590
		2	-31.840*	2.618	0.000	-39.366	-24.314
		3	-15.480*	1.791	0.000	-20.629	-10.331

Based on estimated marginal means

\*. The mean difference is significant at the 0.05 level.

a. Adjustment for multiple comparisons: Bonferroni.

**Table 8.** Pairwise comparison of medical intervention group for oswestry disability index.

Pairwise comparisons							
Study group	(I) ODI	(J) ODI	Mean difference (I-J)	Std. error	Sig. <sup>a</sup>	95% Confidence interval for difference <sup>a</sup>	
						Lower bound	Upper bound
Medical intervention	1	2	7.640*	0.700	0.000	5.628	9.652
		3	12.720*	0.863	0.000	10.238	15.202
		4	19.800*	1.106	0.000	16.620	22.980
	2	1	-7.640*	0.700	0.000	-9.652	-5.628
		3	5.080*	0.818	0.000	2.727	7.433
		4	12.160*	1.059	0.000	9.114	15.206
	3	1	-12.720*	0.863	0.000	-15.202	-10.238
		2	-5.080*	0.818	0.000	-7.433	-2.727
		4	7.080*	0.594	0.000	5.372	8.788
	4	1	-19.800*	1.106	0.000	-22.980	-16.620
		2	-12.160*	1.059	0.000	-15.206	-9.114
		3	-7.080*	0.594	0.000	-8.788	-5.372

**Table 9.** Pairwise comparison of physiotherapy along with medical intervention group for oswestry disability index.

Study group	(I) ODI	(J) ODI	Mean difference (I-J)	Std. error	Sig. <sup>a</sup>	95% Confidence interval for difference <sup>a</sup>	
						Lower bound	Upper bound
Physiotherapy along with medical intervention	1	2	11.800*	0.926	0.000	9.139	14.461
		3	21.960*	1.387	0.000	17.971	25.949
		4	34.040*	1.844	0.000	28.737	39.343
	2	1	-11.800*	0.926	0.000	-14.461	-9.139
		3	10.160*	1.112	0.000	6.964	13.356
		4	22.240*	1.541	0.000	17.810	26.670

**Table 10.** Contd.

	1	-21.960*	1.387	0.000	-25.949	-17.971
3	2	-10.160*	1.112	.000	-13.356	-6.964
	4	12.080*	.844	.000	9.652	14.508
	1	-34.040*	1.844	.000	-39.343	-28.737
4	2	-22.240*	1.541	.000	-26.670	-17.810
	3	-12.080*	.844	.000	-14.508	-9.652

Based on estimated marginal means

\*. The mean difference is significant at the 0.05 level.

a. Adjustment for multiple comparisons: Bonferroni.

**Table 11.** Pairwise comparison of medical intervention group for straight leg raising test.

Pairwise comparisons							
Study group	(I) SLR	(J) SLR	Mean difference (I-J)	Std. error	Sig. <sup>a</sup>	95% Confidence interval for difference <sup>a</sup>	
						Lower bound	Upper bound
Medical intervention	1	2	-7.520*	1.083	0.000	-10.635	-4.405
		3	-17.680*	1.550	0.000	-22.136	-13.224
		4	-26.080*	1.931	0.000	-31.633	-20.527
	2	1	7.520*	1.083	0.000	4.405	10.635
		3	-10.160*	.966	0.000	-12.936	-7.384
		4	-18.560*	1.490	0.000	-22.844	-14.276
	3	1	17.680*	1.550	0.000	13.224	22.136
		2	10.160*	0.966	0.000	7.384	12.936
		4	-8.400*	0.847	0.000	-10.834	-5.966
	4	1	26.080*	1.931	0.000	20.527	31.633
		2	18.560*	1.490	0.000	14.276	22.844
		3	8.400*	0.847	0.000	5.966	10.834

**Table 12.** Pairwise comparison of physiotherapy along with medical intervention group for straight leg raising test.

Study group	SLR	(J) SLR	Mean difference (I-J)	Std. Error	Sig. <sup>a</sup>	95% Confidence interval for difference <sup>a</sup>	
						Lower bound	Upper bound
Physiotherapy along with medical intervention	1	2	-14.640*	1.005	0.000	-17.529	-11.751
		3	-27.680*	1.289	0.000	-31.387	-23.973
		4	-41.360*	1.762	0.000	-46.425	-36.295
	2	1	14.640*	1.005	0.000	11.751	17.529
		3	-13.040*	1.012	0.000	-15.951	-10.129
		4	-26.720*	1.496	0.000	-31.022	-22.418
	3	1	27.680*	1.289	0.000	23.973	31.387
		2	13.040*	1.012	0.000	10.129	15.951
		4	-13.680*	1.084	0.000	-16.798	-10.562
	4	1	41.360*	1.762	0.000	36.295	46.425
		2	26.720*	1.496	0.000	22.418	31.022
		3	13.680*	1.084	0.000	10.562	16.798

Based on estimated marginal means

\*. The mean difference is significant at the 0.05 level.

a. Adjustment for multiple comparisons: Bonferroni.

options in terms that either they are focusing on short term treatment or long-term management. It provides awareness about impact of physiotherapy in improvement of quality of life of the patients of sciatica.

Patients who were participating in different exercises to reduce pain and discomfort found physiotherapy of great value. International medical guidelines recommend pharmacological intervention for the management of pain but it doesn't produce long term effects in elderly patients. So rationally Physiotherapy along with medical intervention is more effective and decent choice in terms of improving pain and making the quality of life better in patients of sciatica.

## CONCLUSION

It was concluded that Physiotherapy along with medical intervention is more beneficial as compared to medical intervention alone in terms of improving the overall quality of life of the patient.

## CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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