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PHYTO-PHARMACOLOGICAL POTENTIAL OF *BUCHANANIA ARBORESCENS* (ANACARDIACEAE), ON WOUND HEALING AND CNS DEPRESSENT ACTIVITIES IN ALBINO WISTAR RATS

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ABSTRACT

To evaluate the wound healing and CNS depressenat activity of methanolic extract of leaves of *Buchanania Arborescens* (Anacardiaceae) in albino wistar rats. The (MEBA) have been proven experimentally to possess wound healing and CNS depressant activities in invivo methods. Acute toxicity studies of *Buchanania arborescens* extract can be used safety in the animals up to the dose of 2000 mg/kg body weight. Administration of methanolic extract 200mg ($P<0.05$) methanalic extract MEBA 400mg ($P<0.001$) and standard soframycin ($P<0.001$) significantly inhibited the wound compared to standard group. From this investigation it can be concluded that the MEBA 400mg showed significant healing the wound and comparable to standard sofromycin ($P<0.001$). Among the extracts the methanolic extract of *Buchanania arborescens* leaves (MEBA 400 mg/kg) dose showed more prominent depressant activity than the 200 mg/kg dose. The results of the investigations justify the folkoric use of *Buchanania arborescens* leaves in the treatment of wound and CNS depressant.

KEYWORDS: Wound healing, CNS depressenat, *B. arborescens*, Soframycin etc.

INTRODUCTION

Over three-quarters of the world population relies mainly on plants and plant extracts for health care (VP Kambol, 2000). More than 30% of the entire plant species, at one time or other, were used for medicinal purposes.[1] India is perhaps the largest producer of medicinal herbs and is rightly called the *"Botanical garden of the world"* There are very few medicinal herbs of commercial importance, which are collected or cultivated in this country. Medicinal herbs have been in use for thousands of years in one form or another, under the indigenous system of medicine like Ayurveda, Siddha and Unani. India has made tremendous progress in agrotechnology, process technology, standardization, quality control, research and development.[2]

It is estimated that world market for plant derived drugs may account for about Rs.2,00,000 crores. Presently, Indian contribution is less than Rs.2000 crores. Indian export of raw drugs has steadily grown at 26% to Rs.165 crores in 1994-'95 from Rs.130 crores in 1991-'92.[3] The *Ayurveda* system of medicine uses about 700 species, *Unani* 700, *Siddha* 600, Amchi 600 and modern edicine around 30 species. The drugs are derived either from the whole plant or from different organs, like leaves, stem, bark, root, flower, seed, etc. Some drugs are prepared from excretory plant product such as gum, resins and latex.[4] Even the Allopathic system of medicine has adopted a number of plant-derived drugs which form an important segment of the modern pharmacopoeia. Some important chemical intermediates needed for manufacturing the modern drugs are also obtained from plants (Eg. diosgenin, solasodine, -ionone). Not only, that plant-derived drug offers a stable market worldwide, but also plants continue to be an mportant source for new drugs.[5,6]

Wound infection is one of the most common diseases in developing countries because of poor hygienic conditions.[7] Wounds are the physical injuries that result in an opening or breaking of the skin and appropriate method for healing of wounds is essential for the restoration of disrupted anatomical continuity and disturbed functional status of the skin. In other words wound is a break in the epithelial integrity of the skin and may be accompanied by disruption of the structure and function of underlying normal tissue and may also result from a contusion, haematoma, laceration or an abrasion.[8,9] Healing of wounds starts from the moment of injury and can continue for varying periods of time depending on the extent of wounding and the process can

be broadly categorized into three stages; inflammatory phase, proliferate phase, and finally the remodeling phase which ultimately determines the strength and appearance of the healed tissue.[10]

CNS depressants slow normal brain function. In higher doses, some CNS depressants can become general anesthetics. Tranquilizers and sedatives are examples of CNS depressants. CNS depressants can be divided into two groups, based on their chemistry and pharmacology: 1. Barbiturates, 2. Benzodiazepines. There are many CNS depressants, and most act on the brain similarly they affect the neurotransmitter gamma-amino butyric acid (GABA). Neurotransmitters are brain chemicals that facilitate communication between brain cells.[11,12] GABA works by decreasing brain activity. Although different classes of CNS depressants work in unique ways, ultimately it is their ability to increase GABA activity that produces a drowsy or calming effect. Despite these beneficial effects for people suffering from anxiety or sleep disorders, barbiturates and benzodiazepines can be addictive and should be used only as prescribed.[13] CNS depressants should not be combined with any medication or substance that causes sleepiness, including prescription pain medicines, certain over-the-counter cold and allergy medications, or alcohol. If combined, they can slow breathing, or slow both the heart and respiration, which can be fatal.[14,15]

Based on literature survey we would like to several tone pharmacological action of *Buchanania arborescens* leaves natural product have less side effect when compared to the synthetic drug.[16] The plant is available in very less rain fed. This is grown locally. The genus was distributed all over country and also some American countries. The genus is native to India. The species contain more than 40 with this genus. It belongs to family anancardiaceae. It contains glycosides, carbohydrates, flavonoids, steroids. It cost is less. In view of above favourable points the examination was under taken.[17]

MATERIALS AND METHODS

Plant Collection and Identification

The fresh leaves of *Buchanania arborescens* leaves were collected in the month of July, 2020 from Tirumala Hills, Tirupathi, and Andhra Pradesh and authenticated by the Prof.P.Jayaram, Director of National Institution of Herbal Science, Chennai.

Preparation of plant extract

Around 400 gm of leaves were separated and dried under shade and extracted by not percolation method using sublet, extraction with methanol for about 48 hr, then concentrated and dried under reduce pressure. The percentage yield of methanol extract of *Buchanania arborescens* was found to be 18.5%w/w.

Ethics

Before starting the experiment on animals, the experimental protocol was subjected to the scrutiny of the institutional animal ethical committee. Clearance was procured before starting the experiments.

Animals used

Male Wistar rats (150-200g) were obtained from the animal house in Krishna Teja Pharmacy College, Tirupathi, and Andhra Pradesh. The animals were maintained in a well-ventilated room with 12:12 hour light/dark cycle in polypropylene cages. The animals were fed with standard pellet feed (Hindustan Lever Limited., Bangalore) and water was given *ad libitum*. Ethical committee clearance was obtained from IAEC (Institutional Animal Ethics Committee) of CPCSEA (Ref. No. IAEC / XIII / 05 / SVCP / 2008-2009).

Preparation of ointment

Simple ointment was prepared as per BP. **5% and 10% w/w** alcoholic extract of *Buchanania arborescens* ointment was prepared as per B.P.

Wound Creation

The hair on the back area of each rat was shaved using an electrical clipper. The area was disinfected using 70% alcohol. Rats were anaesthetized with diethyl ether in a closed chamber and wound was made on each rat. The outline of wound was marked using 6mm diameter biopsy punch and skin removed from the marked area using surgical scissors.[18,19] The lesions were traced onto a transparent paper using a black permanent marker. The area of the circular lesions was calculated as follows:

$$A = \frac{\Pi D_1 D_2}{4}$$

Where A is the Area of lesion, D_1D_2 were the Vertical and horizontal diameter of the lesion respectively, and $\pi = 3.143$

I. Excision wound model

For the excision wound studies, twenty four healthy Wister rats (200-250 g) was taken, divided in four groups of six each. Rats depilated by removing hair at the dorsal thoracic region before wounding and anaesthetized by diethyl ether.[20] The area of wound was made (1 cm away from the vertebral column 5 cm away from the ear with methylene blue) on the dorsal interscapular region,[21] of each animal by excising the skin with surgical sessiors, under aseptic conditions. Haemostasis was achieved by using wound with swab soaked in normal saline.[22] The wound areas were measured (in sq. mm) immediately playing the transparent polythene group paper over the wound and then tracing the area of the wound on it. This was taken as initial wound area.[23]

All the Samples control (PEG ointment base), standard (soframycin), Test- I (*Buchanania arborescens* leaves extract 200mg), Test-II (*Buchanania arborescens* leaves extract 400mg), were applied once daily for 16 days starting from the day of wounding.[24,25] Contractions,

which contribute for wound closure in the first two weeks and then an impression was taken on a millimeter scale graph paper after complete epithelization and time for compete epithalization in days was evaluated to calculate the degree of wound healing (Wermas S *et al*.,1994). The observation of the percentage wound closure was recorded on 4^{th}, 8^{th}, 12^{th}, 16^{th} post wounding day. The wound area each animal was measured at intervals of 24-228 hrs using tracing paper method.[26]

II. Dead Space Wound Model: In dead space wound model the animals were divided into four groups each group contain six rats, rats is anaesthetized by using diethyl ether. Deaf space wound were inflicted by implanting sterile cotton pellets (5 mg each), one on either side of the groin and axilla on ventral surface of each rat by the technique of D^1 Aray *et al*.[27] All the samples e.g. control (drinking water), standard (indomethacin) Test- I (*Buchanania arborescens* leaves extract 200mg), Test-II (*Buchanania arborescens* leaves extract400mg) were given orally. On the 10^{th} post-wounding day, the granulation tissue formed on the implanted cotton pellets was carefully removes under anaesthesia. The wet weight of granulation tissue collected was noted. These tissue samples were dried at 60°C for 12 hr and weight to determine the dry granulation tissue weight.

The dried tissue was added with 5 ml 6 N HCL and kept at 110° for 24 hr. the neutralized acid hydrolysate of the dry tissue was used for the determination of hydroxyl praline.[28] The additional pieces of wet granulation tissue was preserved in 10%, formation for historical studies, while the other part of granuloma tissue was used for determination of tensile strength.[29,30]

Grouping of animals

The selected healthy animals were divided into four groups of six animals in each group, where.

Group 1	Control	Drinking water
Group 2	Standard	Indomethcin
Group 3	Test 1	MEBA 200mg/kg
Group 4	Test 2	MEBA 400mg/kg

Wound Healing Evaluation Parameters

Measurement of Wound Area

An excision wound margin was traced after wound creation by using transparent paper. The measurement of wound area on graph paper was calculated in each 2 days interval. The period of epithelization of the wound was expressed as the number of days taken for complete epithelization.[31]

Histopathological Studies

Histopathological studies of the tissue (10 days) obtained from the extract group showed significant increase and well organized bends of collagen, fibroblast, macrophages, and tissue edema inflammatory cells. Granulation tissue selection obtained from control rats revealed more inflammatory cells, less collagen fibers and fibroblasts. The amount of collagen was quantified using Vangeison Stain (VG) and Toluidone Blue (TB) stains.[32]

Statistical analysis

The data were expressed as mean ± standard error mean (S.E.M).The Significance of differences among the group was assessed using one way and multiple way analyses of variance (ANOVA). The test followed by Turkey-Kramer multiple comparison tests, the p values less than 0.05 were considered as significance.

Evaluation of CNS depressant activity

I.Pheno barbitone sodium induced sleeping

The method used is an described by Leite et al., 1982. Twenty four mice were divided into four groups, each groupe containing six mice. The animals of group I treated orally with vehicle (10ml/kg) each serving as a control group.

Group II treated chlorpromazine (10mg/kg i.p) a standard sedative drug. Group III and IV treated with MEBA at a 200 and 400 mg/kg respectively. Thirty minutes later, each animal in all groups received Phenobarbitone at the dose of 47 mg/kg i.p. The sleeping time was noted by recording the interval betveenthe loss and regaining of right reflex.[33]

II.Rotarod method

Mice were divided into four groups, each group containing six mice. Before treatment, all the animals placed one by one on rotating rod. Notedown the fall of the tome when mice fall from the rotating rod. Group I animals were treated orally with 1% sodium hydroxy methyl cellulose 10ml/kg p.o and group II were treated with phenobarbitone sodium 47mg/kg i.p. group III and IV animals were treated with MEBA 200 and 400 mg/kg respectively per p.o. After administration of drug 1 hour later the all animals plassed individual on rotating rod. Compare the fall of the time of control animals and drugs treated animals.

RESULTS

A. Acute Toxicity Studies

The LD_{50} of aqueous and methanol bark extracts were found to be 25-30 mg/kg, b.w. One tenth of the dose was selected* for the evaluation of wound-healing activity i.e., 30 mg/kg, b.w. Significant promotion of wound-healing activity was observed in both aqueous and methanol leaves extracts in all the two wound models such as excision, and dead space wound.

Table 13: Acute Toxicity test studies of extraction of Buchanania Angustifolia Leaves.

S. No	Groups	Dose/kg b.w	Weight of animals		Signs of Toxicity	Onset of Toxicity	Duration of Study
			Before Test	After Test			
1	**MEBA**	2000 mg	160 g	160 g	No signs of Toxicity	Nil	14 days
2	**MEBA**	2000 mg	180 g	180 g	No signs of Toxicity	Nil	14 days
3	**MEBA**	2000 mg	200 g	200 g	No signs of Toxicity	Nil	14 days
4	**MEBA**	2000 mg	190 g	195 g	No signs of Toxicity	Nil	14 days
5	**MEBA**	2000 mg	195 g	195 g	No signs of Toxicity	Nil	14 days
6	**MEBA**	2000 mg	200 g	200 g	No signs of Toxicity	Nil	14 days

The acute toxicity tests for the MEBA were performed and it was found that was safe in 2000 mg/kg body weight

Fig No. 22 : 0 Day (Control)

Fig No. 23 : 16th Day (200 mg)

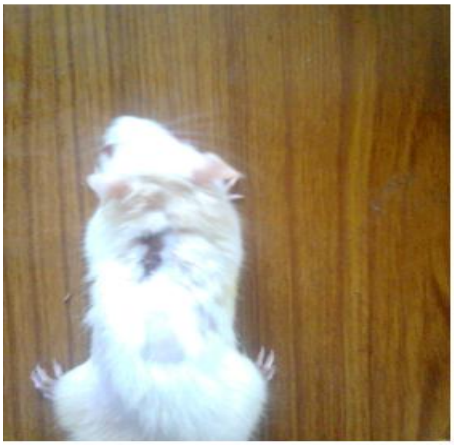


Fig No. 24 : 16th Day (400 mg)

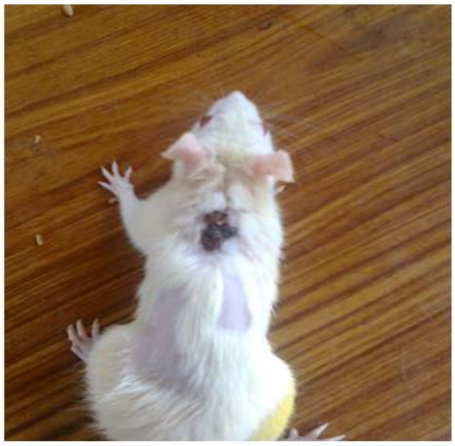


Fig No. 25 : 16th Day (Standard)

II. Dead space wound model

In dead space wound model, the mean breaking strength of granulation tissue in the was 217.6± 2.728g. Marked increases in breaking strength observed (311.8± 03.13 and 339.33±5.41 respectively) in the (200 and 400 mg) treated group when compared to control group. The breaking strength in the standard ointment treated group was 447.13±06.06. The mean dry weight of granulation tissue in control group was 35.4 ±1.056 mg increased to (43.31± and 62.15± 02.32 respectively) in treated groups with standard ointment.

Table 15: Effect of the leaves Extract of *Buchanania arborescens* on dead space wound model.

Group	Granulation dry weight	Breaking strength
Control (drinking water)	35.43±1.05	217.6±2.728
Stand (Indomethacin)	73.45±01.19	447.13±06.06
Test- I (extract200 mg)	43.31± 01.24	311.8±03.13
Test- II (extract 400 mg)	62.15±02.32	339.33± 5.41

All the values are expressed as mean ± SEM, n = 6, p<0.05, significant compared to control.

Histopathological Studies

The histopathological studies of the granulation tissue treated group showed significantly increases in collagen deposition, macrophages and fibroblasts. The histopathological studies of the granulation tissue of control group of animals showed more aggregation of macrophages with less collagen fiber.

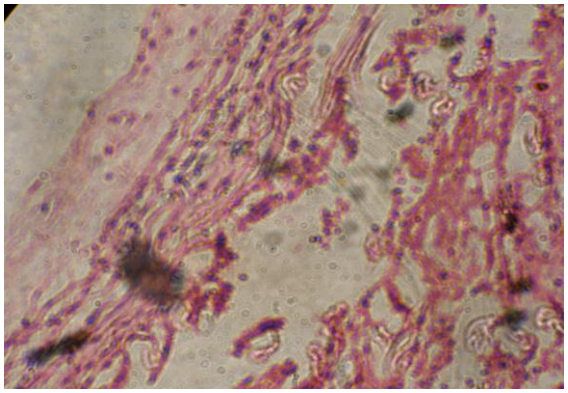


Fig. 26: Granulation tissue of standard ointment treated group animal showing moderate deposition collagen.

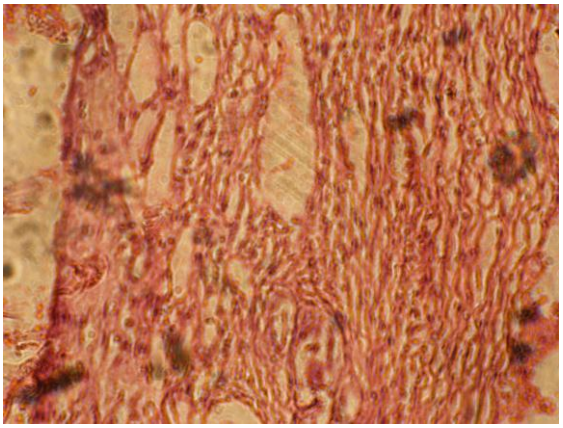


Fig. 27: Granulation tissue of extract (400 mg/kg) treated animal Showing with more collagen and less macrophages.

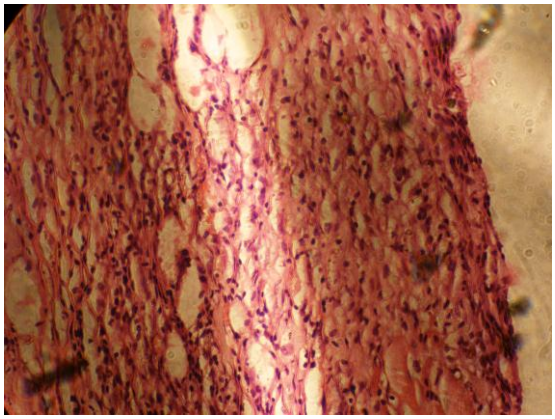


Fig. 28: Control group animal showing with less collagen and more macrophages.

Histogram No. 1

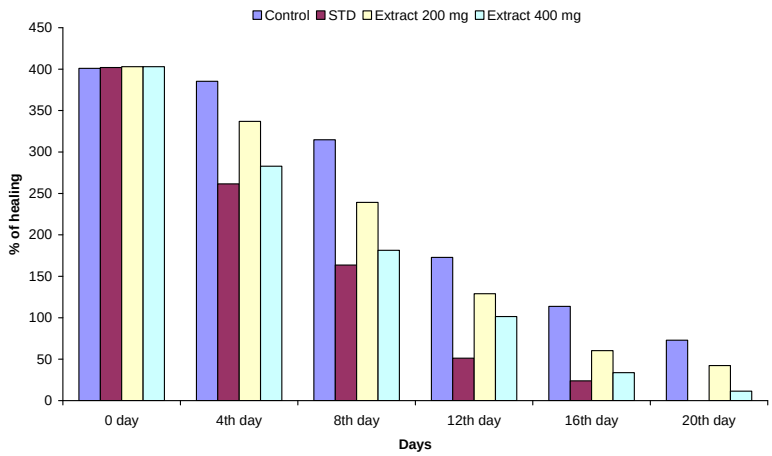


FIG. 1 : Post wound healing of excision wound model

Effect of MEBA on excision wound model in rates

n = 6, P<0.001, compared with control group (One-way ANOVA followed by Dunnatts text).

C. CNS Depressant activity

I. Phentobarbitone induced sleeping

In the pentobarbitone induced hypnosis test, the Leaves extract at the doses of 200 and 400 mg/kg significantly induced the sleep at an earlier stage and also prolonged the duration of sleeping time in test animals as compared to control (Table 16).

Table 16: Effect of *Buchanania arborescens* leaves of petroleum ether Extract on pentobarbitone induced sleeping time in mice.

S. No.	Treatment	Dose (mg/kg)	Route of administration	Onset of sleep (min)	Duration of sleep (min)
1.	Control	10 ml/kg	p.o	24.5±0.4282	34.166±0.3070
2.	Standard	47 ml/kg	i.p	10.166±0.3073	58.833±0.5426
3.	Test-I	400 ml/kg	p.o	12.833±0.4014	43.833±0.9098
4.	Test-II	200 ml/kg	p.o	19.166±0.3073	37.50±0.5627

Values of the mean ± SEM from 6 animals in each group. Statistical anaysis done by ANOVA, P<0.001 compared with control group.

Rotarod Method

MEBA at 200 and 400 mg/kg administered orally exhibites significant reductionof activity compared with control group of animals. The standared chlorpromazine hydrochloride also exhibited significant reduction CNS activity compared with control group animals.

Table 17: Effect of MEBA by Rota rod method.

S. No.	Treatment	Dose (mg/kg)	Route of administration	Before Treatment	After treatment
1.	Control	10 ml/kg	p.o	81.166±1.302	73.83±0,6540
2.	Standard	47 ml/kg	i.p	84,833±0.3073	9.33±0.333
3.	Test-I	400 ml/kg	p.o	83.166±0.3.73	16±0.6325
4.	Test-II	200 ml/kg	p.o	82.333±0.5578	344.66±0.8028

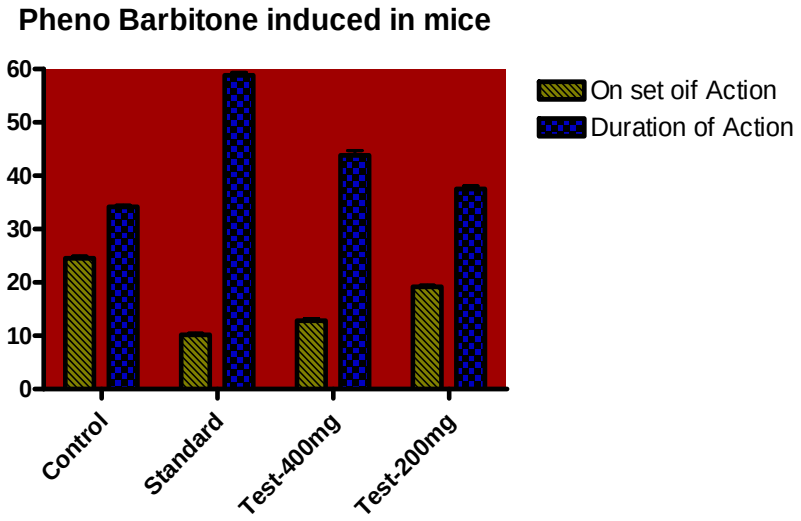
All the values are expressed as mean ± SEM, n = 6, P<0.05 significant compared to control.

Actophotometer: MEBA at 200 and 400 mg/kg administered orally exhibites significant reductionof activity compared with control group of animals. The standared chlorpromazine hydrochloride also exhibited significant reduction CNS activity compared with control group animals.

Table 18: CNS activity by Actophotometer method.

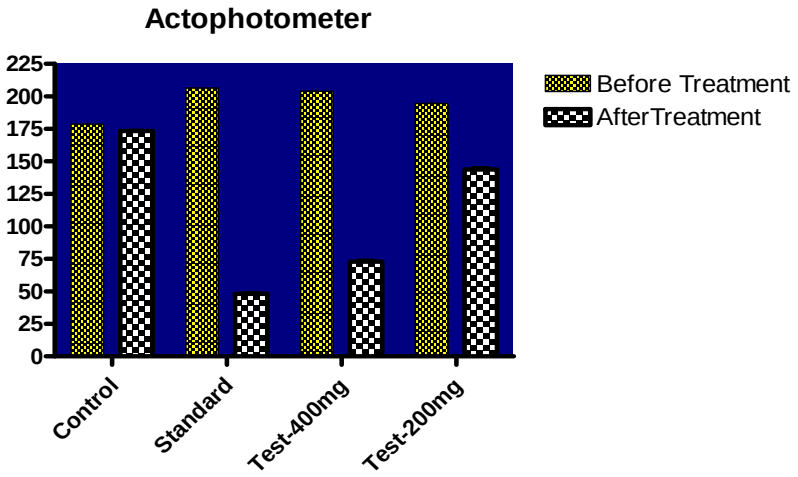
S. No.	Treatment	Dose (mg/kg)	Route of administration	Before Treatment	After treatment
1.	Control	10 ml/kg	p.o	178.33± 0.8433	173.33±0.6146
2.	Standard	47 ml/kg	i.p	206.33±0.5578	48±0.7746
3.	Test-I 400mg	400 ml/kg	p.o	203.83±0.8333	72.83±1.046
4.	Test-II 200mg	200 ml/kg	p.o	194.5±2.975	143.66±1.430

Histogram-2: Effect of MEBA on phenobarbitone-sleeping time in mice.



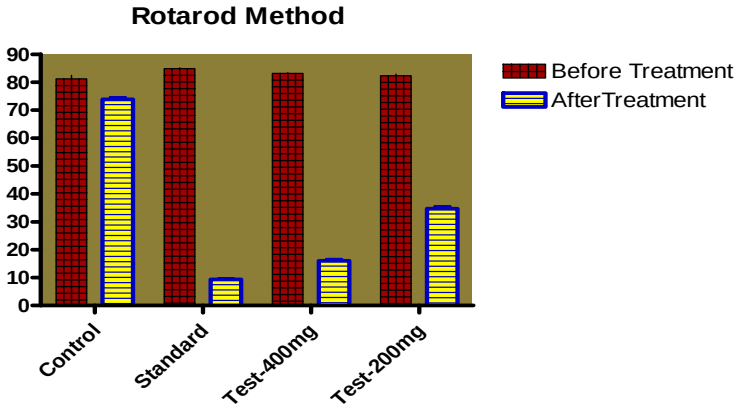
n= 6: P<0.001 different from control (one-way ANOVA followed by Dunnett's test)

Histogram-3: Effect of MEBA on Actophotometer activity in mice



Each value represents the mean p<0.05; p<0.001 compared with the control

Histogram-4: Effect of MEBA on rota rod activity in mice



Each value represents the mean p<0.05; p<0.001 compared with the control

DISCUSSION

Present study includes Pharmacognostic and Phytochemical investigation of the leaf of *Buchanania arborescens* and evaluation of the same for wound healing and CNS Depressant activities. Acute toxicity studies indicate that *Buchanania arborescens* used extract can be used safety in the animals up to the dose of 2000 mg/kg body weight. In the present investigation, BA ointment promotes wound contraction and increased rate of epithelialisation in excision wound which may be due to presence of phytochemical constituents like flavonoid, steroids. These constituents are known to promote wound contraction and increased rate of epithelialisation due to their astringent and anti-microbial property.

In the dead space wound model, the granulation tissue of the wound is primarily composed of fibroblast, collagen edema and small new blood vessels. The collagen is the major component of extracellular matrix cellular tissue, which give support and strength and is composed of amino acid hydroxyproline. Hydroxy praline content of granulation tissue of animals treated with BA ointment was significantly increased when compared to the control group indicating increased collagen turnover. Histopathological studies in granuloma tissue further indicate strengthen in tissue integrity by BA ointment by enhancing keratinization, epithelization, fibrosis, collagenation and angiogenesis.

The above observations suggest that BA ointment promotes wound healing through mainly by collagen formation and enhancing tissue integrity. Further, phytochemical studies are in progress where the methanol extract will be subjected to isolate the achieve compound responsible for these pharmacological activities. The present findings provide scientific evidence that methanol extract of really or BA as potential wound healer. In loco-motor activity assessed by actophotometer and the decrease in grip by rota-rod, which was found to the dose dependent. Decrease on locomotion reveals depression effect on CNS. The CNS depressant activity may be due to the increase in the concentration of GABA in brains. In the present study; the extract of *Buchanania arborescens* significant

decreased the spontaneous loco-motor activity in mice indicating central depressant effect.

CONCLUSION

From the phytochemical investigation of the *Buchanania arborescens* and evaluation of the same for wound heating and CNS depressant activities the following conclusions can be made. Our data demonstrate that the methonolic extract of *Buchanania arborescens* may be capable of promoting wound healing activity. The results of the present study of leaves extract of *Buchanania arborescens* passing significantly CNS activity which may be due to sedative property. The results of the investigations justify the folkoric use of *Buchanania arborescens* leaves in the treatment of wound CNS depressant and plant is worth for further chemical and pharmacological investigations.

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