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RESEARCH ARTICLE

In vivo Antibacterial Studies and Haematological Values of Tridax Procumbens L Extracts on Rabbits Orogastrically Dosed with Animal Pathogens

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ABSTRACT

Attempts were made to evaluate in vivo the anti-bacterial potential of whole plant extract of Tridax procumbens L and their effects on haematological values. This was tested on *Escherichia coli* and *Staphylococcus aureus* to know the degree of effectiveness of the extracts on Gram negative and Gram positive organisms. Sixteen (16) grower rabbits were divided into four (4) treatments of four (4) rabbits per treatment. The rabbits were orogastrically infected with *Escherichia coli* and *Staphylococcus aureus* and were certified to be ill. Treatment 1 was treated with tetracycline, while treatments 2, 3 and 4 were administered 2ml, 3ml and 5ml of the Tridax procumbens L extracts respectively with 10 mg/ml as the concentration of the extract. The blood samples were collected aseptically from the rabbits through the jugular vein for the determination of haematological values. Their antibacterial activities were evaluated in vivo against Gram positive bacterium (*Staphylococcus aureus*) and a Gram negative bacterium (*Escherichia coli*). The extracts at various levels exhibited a pronounced antibacterial activity against the two organisms. There was no significant difference ($P<0.05$) between T_1 with oxytetracycline and other treatment groups with Tridax procumbens L extracts in the antibacterial activities, haematological values and weight gain. Tridax procumbens whole plant extracts possess broad spectrum antibacterial activities and more effective in the clearing of the bacterial cells than the synthetic antibiotic drug (tetracycline). This result supports the traditional use of Tridax procumbens L. whole plant as an antibacterial agent and feed ingredient for rabbits.

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INTRODUCTION

Meat animals are surrounded by countless micro-organisms. There has been emergence of pathogenic microorganisms that are resistant to major class of antimicrobials due to indiscriminate use of synthetic antimicrobial drugs (Karaman *et al*., 2003). In addition, high cost and adverse side effects are commonly associated with popular synthetic antibiotics (such as hypersensitivity, allergic reactions, immunosuppresant etc) in treating infectious diseases (Schinor *et al*., 2007).

Scientists are always in search of new antimicrobial agents to control the ever increasing menace of the microbes, but resistance in microbes towards these drugs has increased (Nino *et al*., 2006). Medicinal plants are gifts of nature to cure limitless number of diseases among farm animals (Bushra and Ganga, 2003). The abundance of plants on the earth's surface has led to an increasing interest in the investigation of different extracts obtained from traditional medical plants as potential sources of new antimicrobial agents (Nwankwo and Amaechi, 2013).

Tridax procumbens L. commonly known in English as coat buttons/Mexico Daisy is an ethno-botanically important medicinal plant. It has been extensively used in Indian traditional medicine as anticoagulant, antifungal and insect repellant; in bronchial, catarrh, diarrhea and dysentery (Sreeramulu *et al*., 2013). It is also used to check hemorrhage from cuts, bruises and wounds (Ravikumar *et al*., 2005). The plant which is considered as a gregarious weed in the tropics is also used as forage for

rabbit feed in Nigeria and has yielded interesting compounds like luteolin, β-amyron, lupeol, fucosterol, flavones, arachidic acid, glycosides etc (Suseela *et al*., 2002).

There are limited reports from literature on the in vivo evaluation of antibacterial activity of crude extracts from Tridax procumbens, which could provide scientific evidence for it use in traditional medicine. Therefore, the present study was designed to study the antibacterial potential of Tridax procumbens against animal pathogens by in vivo assessment and their effects on haematological values and growth parameters.

MATERIALS AND METHODS

Plant material and extract preparation

Fresh green leafy whole plants of this weed (Tridax procumbens) were harvested within the University environment after due identification of the herbarium at the Department of Plant Health Management of Michael Okpara University of Agriculture, Umudike, Nigeria. The plant samples were washed thoroughly with distilled water, air dried and was further dried in a hot air oven at 55^{o}C till they become crispy. They were grinded into powder with a mechanical grinder and stored in an air tight container. The powdered whole plant was extracted successfully with hot water boiled to 100^{o}C (100mg/ml). The extracts were filtered and concentrated at 45^{o}C using rotator vacuum evaporator.

Preparation of Crude Extract for Antibacterial Assay

The methods of Akujobi *et al*. (2004) and Esimone *et al*. (1998) were adopted for the preparation of crude extracts. The crude extracts were dissolved in 30% dimethyl sulphoxide and further diluted to obtain 100 mg/ml concentration which was used for the determination of antimicrobial activity by in vivo assessment using rabbits.

Experimental Animals and Management

Sixteen grower rabbits were used for this study. The rabbits were randomly sorted into four groups of four animals per group. The animals were housed in metallic cages (hutches) in a well-ventilated room. The faecal and blood samples were collected and examined for the presence of any pathogen. The animals were fed commercial poultry growers mash forage. Feed and water were given *ad libitum* the animals were weighed before and after experiment.

Preparation of Inoculums

The test organisms *Escherichia coli* and *Staphylo-coccus aureus* were isolated using their selective media i.e. MacConkey agar and Parker's median respectively. They were further identified using the conventional biochemical tests. Their colonies on the selective media were collected together and diluted with 5ml of water to make a solution of bacteria colonies as inoculums. These pure cultures were sub cultured on nutrient agar slants. They were stored at 4^{o}C until required for the study.

Inoculation Process

The animals were certified clinically healthy by the National Veterinary Research Institute, Medical Laboratory, Umudike, Nigeria. After that, inoculums of *Escherichia coli* and *Staphylococcus aureus* were inoculated into the animal orally using oral cannula. A 5ml single dose was inoculated into the rabbit which brought them down with illness within a week. Thereafter, the administration of Tridax procumbens L. extracts began.

Methods of Administration of the Extracts

The solution of Tridax procumbens L extract was stirred to maintain a uniform suspension and administrated to the rabbits every morning. The drugs/extracts were administered thus: T_1-2mls of tetracycline, T_2-2mls of tridax extracts, T_3-3mls of tridax extracts, and T4-5mls of tridax extract. These drugs/extracts were administered in the morning for four days. The animals recovered within the 3rd and 6th day. After seventh day of treatment, the blood samples of the animals were collected and examined for their haematological values.

Haematological Examination

The blood samples of the rabbits were collected into sterile universal bottles containing ethylene deamine tetracetic acid (EDTA) powder as anti-coagulant. These were used for the determination of the Red blood cell count, white blood cell count, haemoglobin concentration, packed cell volume (PCV) as described by Decie and Lewis, (1991). The weights of the animals were also determined before infection, during infection and after treatment.

Bacteria Population Count

The faecal samples of the animals were used to determine the bacterial load. Serial dilutions of collected faecal samples from different treatments were made with sterile water (0.9g sodium chloride in 100ml distilled water) and bacterial loads of the different treatment were enumerated by pour plate method (Quinn *et al*., 1992). The bacterial loads were calculated as the total number of living bacteria cells and it was expressed in colony forming unit (cfu).

Statistical analysis

The experiment was carried out in a completely randomized design (CRD). The data collected was subjected to analysis of variance (ANOVA) as described by Steel and Torrie (1980).

RESULTS

The results from the antibacterial study using rabbits are presented below:

Table 1 showed the effect of Tridax procumbens L extracts on bacterial population count. A Gram negative organism, *Echerichia coli,* was used in this study. The average bacteria count was taken before the animals were inoculated with the test organism (*E. coli*), after the animals where inoculated with the test organism after treatment with the test ingredients. The table showed that there was an improved efficiency of clearance with tridax procumbens L extracts than with tetracycline.

Table 2 showed the effect of Tridax procumbens extract on *Staphylococcus aureus.* Treatment four (T_4)

Table 1: Effect of Tridax procumbens extract on bacteria (E.coli) population count

E.coli	T_1	T_2	T_3	T_4
Average bacteria count before bacterial inoculation cfu/mg	10.50	15.30	12.45	13.40
Average bacterial count after bacterial inoculation (cfu/mg)	66.00	69.00	68.20	70.50
Average bacterial count after treatment (cfu/mg)	21.50	18.15	16.23	14.00
% efficiency of clearance	67.42	73.70	76.20	80.14

Table 2: Effect of Tridax procumbens L. extracts on bacteria (*Staphylococcus aureus*) population count

S. aureus	T_1	T_2	T_3	T_4
Average bacteria count before bacteria inoculation cfu/mg	05.04	12.06	09.76	05.76
Average bacteria count after bacteria inoculation (cfu/mg)	50.90	76.03	25.00	66.13
Average bacteria count after treatment (cfu/mg)	34.41	31.00	08.27	20.01
% efficiency of clearance	32.40	59.23	66.92	69.74

recorded the highest percentage efficiency of clearance (69.74%), while tetracycline (T_1) has the least percentage clearance of 32.40%. *Staphylococcus aurus* was used here to study the effect of the plant extracts on Gram positive organisms when compared to the conventional antibiotic i.e. tetracycline.

Figure 1 above presents bar charts showing the percentage clearance of the bacteria use. The percentage clearance of the bacteria used was higher with *Escherichia coli*, a gram negative organism than with *Staphylococcus aureus*, and a gram positive organism. The antibiotic used as T_1 has better clearance efficiency on *E.coli* (67.42%) than with *S.aureus* (34.41%). Comparatively, tridax procumbens has better percentage clearance efficiency on *E.coli* than on *S.aureus*.

Table 3 showed the haematological values of rabbits used in the study. There was an increase in the number of white blood cell during infection. This number came down to normal after treatment in T_2, T_3, and T_4, but not with T_1. The increase in white blood cell number in T_1 after treatment indicates that the tetracycline was not able to reduce the bacterial load to normal. The presence and persistence of these bacteria in the body system resulted to the increase in the number of WBC to fight the pathogens.

Table 4 showed that there was a decrease in the weight of the rabbits during infections when the weights of the rabbits were taken one week after treatment, there was a gradual increase in weights of the rabbits in T_2, T_3 and T_4 slightly more than the average weight before infection. That is T_2 from 1.95 kg to 2.00 kg; T_3: 2.00 kg to 2.02 kg, T_4; 2.05 to 2.07 kg. In the case of T_1, the average weight of the rabbit dropped from 2.05kg before infection to 2.00kg one week after treatment.

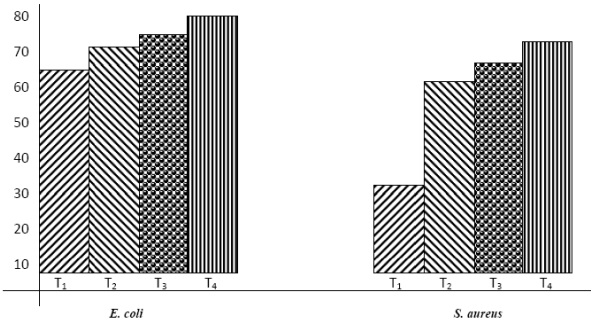

Fig. 1: Bar chart showing percentage (%) clearance of the bacteria used; Key: ▨ = T_1 ▩ = T_2 ▦ = T_3 ▥ = T_4

Table 3: Haematological values of rabbits used in the study

		PCV (%)	Hb (g/dL)	WBC ($x10^6mm^3$)	RBC ($x10^6mm^3$)
Before	T_1	40	11.0	0.070	6.65
Bacteria	T_2	39	09.0	0.050	5.29
Infection	T_3	42	11.0	0.126	6.45
	T_4	40	09.2	0.175	6.57
During	T_1	27	11.2	0.175	6.08
Bacteria	T_2	30	10.2	0.153	5.89
Infection	T_3	30	11.2	0.156	6.40
	T_4	28	9.0	0.192	6.50
After treatment	T_1	37	11.4	0.100	6.80
	T_2	36	10.2	0.040	5.89
	T_3	38	11.2	0.121	6.47
	T_4	34	09.6	0.165	6.54

Table 4: Effect of disease and tridax extract treatment on the weight of the rabbit

	Average weight before infection (kg)	Average weight during infection (kg)	Average weight one week after treatment (kg)
T_1	2.05	1.90	2.00
T_2	1.95	1.88	2.00
T_3	2.00	1.88	2.02
T_4	2.05	1.95	2.07

DISCUSSION

Plant derived products have been shown to have the potential to inhibit bacterial growth (Prestos *et al*., 2005) and there are certain reports on the utility of phytochemicals for antimicrobial actions (Sudjana *et al*., 2009). Tridax procumbens has been used as a phytomedicine by traditional healers and practinioers (Wani *et al*., 2010). This traditional usage stems from the fact that Tridax is associated with antibacterial activity (Mundada and Shivhare, 2010). We studied the efficiency of crude extract of Tridax as antibacterial agents against Gram-positive bacteria; *Staphylococcus aureus* and against Gram negative bacteria; *Escherichia coli* when compared with the conventionally used antibiotics; tetracycline. The crude extract of Tridax showed very good antibacterial activities on *S.aureus* and *E.coli* when compared to tetracycline (Yoga-latha *et al*., 2010; Sharma and Kumar, 2009). The antibacterial activity of Tridax is known to be due to alkaloids, flavonoids, tannins and saponins (Mundada and Shivhare, 2010). Gram positive bacteria like *E.coli* showed a reduction in their growth on treatment with crude extracts of Tridax procumbens. The degree of effectiveness as measured by in vivo assessment using rabbits, reported that crude extract was more effective on gram-negative bacteria than the gram-positive bacteria. This finding is in agreement to Sasikumar *et al*.,

(2003) who reported similar thing from different plant sources. The in vivo antibacterial activity of Tridax against gram negative organism in experimental animals has also been well established (Oladunmoye, 2006).

The white blood cells (WBC) increased during infection with the pathogenic organisms. This might have resulted from the production of more WBC to engulf the antigens. The value of the WBC dropped for the animals treated with the plant extract. The reason may be due to the antimicrobial activity of the Tridax extract on the organisms which consequently suppressed or modulated the immune system from producing antibody (Tzianabos, 2000). The packed cell volume (PCV) reduced in rabbits dosed with the organisms suggested that the infection is haemolytic. After treatment with Tridax procumbens extracts there was restoration of the haematological values. This showed that some of the structural components of the extracts may be haemopoetic, in agreement with report of Ogwumike (2002).

The increased in weight of rabbits one week after treatment with Tridax extracts suggested the growth potential of the weed. The weed can also be used as forage in the tropics especially Nigeria because it contains growth yielding compounds like luteolin, β-amyron, Lypeol, fucosterol, flavones, arachidic acid, glycosides etc (Suseela *et al*., 2002). In order to make more scientific formulations and for optimizing the clinical usage, further research will be needed in this direction. In the wake of development of antimicrobial resistant strains, this can have long term applications in the future use of phytochemicals in the treatment of bacterial infections.

Conclusion

The results suggested that the extract of Tridax procumbens L have various pharmacological effect, anti-microbial activity against both gram positive and gram negative bacteria and stimulates growth in rabbits. The best result was obtained when 5ml of 100mg/ml concentration of the extract was given.

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