eISSN: 09748369, www.biolmedonline.com

Preliminary assessment of antimicrobial properties of aqueous extract of plants against infectious diseases

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Abstract
The increasing rate of development of resistance to commonly use antibiotics have led to search for newer, more effective, affordable and easily available drugs. In this study, aqueous extracts of *mangifera indica, allium cepa* and *carica papaya* were used against infections of *E. coli, salmonella enteritis,* and *shigella flexneri* respectively. They were observed to be effective against these infectious diseases. From these observations, it may be suggested that the plants extract may possess effective antimicrobial activities which may be explored in the management of these infectious diseases.

Keywords: Antimicrobial, resistance, microbes, aqueous extracts, microorganisms.

Introduction

The general belief that the advent of antibiotics will bring end to the occurrence of infectious diseases was cut short with the occurrence of resistance to antimicrobial drug. The incidence and increasing frequency of microorganisms that are resistant to common and generally accepted effective first choice drugs is on the increase. The development of resistance to the newer antibiotics by the microbes causing most of the infectious diseases with debilitating effects made the case worse. Resistance to penicillin by *S. aureus* was first reported in 1942 and by 1960, more than 80% of both community – and hospital- acquired staphylococcal isolates were resistant to penicillin (Lowyi, 2003).

The rate of resistance to these drugs is higher in developing countries when compared with developed countries. This may be due to the indiscriminate use of antibiotics and also self medications without prescription by physician. Furthermore, the use of antibiotics in animal feeds may induce resistance. As this resistivity increases, the need for newer and/or alternative therapy becomes very necessary. This study was designed to assess potentials of aqueous extracts of *Carica papaya, Allium cepa* and *Mangifera indica* as alternative therapies in the management of infectious disease.

Materials and Methods

In this study, the antimicrobial efficacy of *Allium cepa* was tested against infection due to *Salmonella enteritis*; efficacy of *Mangifera indica* was tested against *E. coli* infection while that of *Carica papaya* was tested against *Shigella flexneri*. 15 rabbits were used and categorized into 3 equal groups as follows:

Group 1: consisted of 5 rabbits infected with *E. coli* and later treated with aqueous extract of *mangifera indica*.

Group 2: consisted of 5 rabbits infected with *Salmonella enteritis* and later treated with aqueous extract of *allium cepa*.

Group 3: consisted of 5 rabbits infected with *Shigella flexneri* and later treated with *carica papaya*.

Bacteria culture and inoculation of animal

The organisms were cultured on different media. A pure colony was picked for each organism and mixed with 9ml of sterile distilled water. This gave a concentration of 10^{-1}. Sterile pipette was used to remove 1ml of the dilution and 9ml of sterile distilled water was added and to give 10^{-2}. From 10^{--2} dilutions, 2ml was orally given to the rabbits as the inoculum size i.e. group 1 was infected with *E.coli*; group 2 was infected with *Salmonela enteritis*; group 3 was infected with *shigella flexneri*. Also, their feeds were mixed with organism to ensure infection. After 24 hours post challenge, specimen such as stool was collected from the animals and cultured to ascertain that they have been infected with the organism. After infection has been confirmed, parameters such as weight, temperature, packed cell volume and stool sample were examined again. The essence was to determine any alteration during infection.

Blood samples were collected for 24 hours post challenge analysis of biochemical parameters (infected state).

Administration of aqueous extracts of plants
2ml of aqueous extracts of *mangifera indica, allium cepa* and *carica papaya* were administered to infected rabbits in groups 1, 2, and 3 respectively. The administration was twice daily i.e. morning and evening for three (3) consecutive days. By 72 hours, effectiveness of the water extract was determined by culturing stool sample for the presence of the organism. The organism was observed to be absent from stool culture. After confirmation of absence of micro-organism, post treatment examination of the stools, packed cell volume, weight, temperature and biochemical parameters, were again carried out (post-treatment data).

Determination of packed cell volume
Blood at various stages of study i.e. at baseline, after infection and post-infection was taken from the animals by bleeding the rabbits. The Packed cell volume (PCV) was measured using whole blood directly from animals, and was carried out using the Heamatocrit method as described by Schalm et al. (1975).

Determination of biochemical parameters
The serum total protein, albumin and globulin were determined spectrophotometrically.

Statistical analysis
Analysis of variance (ANOVA) was used for general comparison while student's t-test was used to determine the significance of differences in the selected physical, biochemical and hematological parameters before infection when compared with the status during infection (i.e. effect of infection). The differences were considered significant when the p-value was less than 0.05.

Results
The results were presented using tables and histograms. These showed the status of evaluated selected physical, hematological and biochemical parameters at pre-infection state (baseline), infection state, and post-infection state (post treatment). The changes in the status of all parameter assessed during infection when compared with baseline values reached level of statistical significance ($P<0.05$; 95% confidence interval).

Table 1: Showing the changes in the status of physical, hematological and biochemical parameters assessed during *E. coli* infection and post- infection (after *M. indica* treatment) states.

Before infection (baseline)	Infection state	Post-infection state (After treatment)	Parameters
1.25 ± 0.31	1.05 ± 0.32$^{\times}$	1.18 ± 0.27	Body weight (kg)
36.25 ± 6.52	32.25 ± 6.50$^{\times}$	37.00 ± 9.00	PCV
39.07 ± 1.20	42.25 ± 3.52$^{\times}$	38.25 ± 0.77	Temperature (^{0}c)
7.23 ± 1.95	7.33 ± 1.05$^{\times}$	6.28 ± 0.35	Total proteins (g/l)
3.35 ± 0.55	3.63 ± 0.35$^{\times}$	3.53 ± 0.35	Albumin (g/l)
3.88 ± 1.65	3.70 ± 1.30$^{\times}$	2.75 ± 0.55	Globulin (g/l)

$^{\times}$ Significantly different from baseline ($p<0.05$)

Table 2: Showing the changes in the status of physical, hematological and biochemical parameters assessed during *Salmonella enteritis* infection, post infection (i.e. after treatment with aqueous extract of *Allium cepa*).

Parameters	Pre- *S.enteritis* infection (baseline)	During *S. enteritis* infection	Post *allium cepa* treatment
Body weight (kg)	1.48 ± 0.13	1.29 ± 0.15[×]	1.37 ± 0.08
Body Temperature (^{0}c)	39.5 ± 0.35	41.2 ± 1.16[×]	38.8 ± 0.49
PCV	35.6 ± 4.16	33.00 ± 2.65[×]	36.3 ± 3.05
Total proteins (g/l)	5.80 ± 1.29	6.90 ± 0.85[×]	7.95 ± 0.30
Albumin (g/l)	2.97 ± 0.85	3.70 ± 0.24[×]	3.23 ± 0.21
Globulin (g/l)	2.87 ± 0.64	3.23 ± 0.21[×]	4.03 ± 0.25

[×]Significantly different from baseline ($p<0.05$)

Table 3: Showing the changes in the status of physical, hematological and biochemical parameters assessed during *shigella flaxneri* infection, post infection (i.e. after treatment with aqueous extract of *carica papaya*).

Parameters	Pre-*Shigella flexneri* infection	During *shigella flexneri* infection	Post *carica papaya* treatment
Body weight (kg)	1.63	1.49[×]	1.60
Body Temperature (^{0l})	38.67	39.35[×]	38.37
PCV	38.67	35.00[×]	40.33
Total proteins (g/l)	6.55	8.20[×]	6.93
Albumin (g/l)	3.63	3.73[×]	3.80
Globulin (g/l)	2.92	4.47[×]	3.13

[×]Significantly different from baseline ($p<0.05$)

It was observed that during infection, the packed cell volume (PCV) and the weights of the rabbits were significantly reduced ($P<0.05$) while the body temperature, total proteins, albumin and globulin were significantly raised ($P<0.05$). However, after treatment of the different infections with the different aqueous extracts, the observed alterations become normalized.

Discussion

The increased frequency of resistance to commonly used antibiotics led to search for newer, effective, cheap and easily affordable drugs in the management of infectious diseases. In this study, the induced infectious diseases caused significant alterations in all the parameters assessed. For instance, the body temperature of experimental rabbits was significantly raised by the infections. The increased body temperature may be due to activation of cytokines which act as endogenous pyrogens and these are interleukin-1, interleukin -6 and tumor necrosis factor (TNF) Samuel and Thomas, 1990). Their activation leads to a general increase in body temperature which significantly depicts an infection state (Karel, 2001). Raised body temperature may also be due to impaired homeostatic function of the kidney due to the infection. Impaired homeostasis may likely cause raised body temperature.

In this study, packed cell volumes (PCV) decreased significantly during infection states when compared with baseline values. This may be due to degradation of red blood cells during infection. This may also be due to infection affecting some major organs like liver, spleen, etc, whose infection may reduce their activities causing reduced red cells production. The loss of body weight may be due to excessive loss of body water i.e. diarrhea, which contribute substantially to body weight. Ingested bacteria may pass through the stomach and adhere to the epithelial cells lining the terminal small intestine, caecum and colon. They enter the epithelial cells and penetrate into the underlying lamina propria, causing inflammation. It is the inflammatory response that mediates the release of prostaglandins which stimulate active fluid secretion, contributing to diarrhea. The increased total proteins observed during infection in this study may be due to excessive loss of water via watery stool & vomiting culminating in diarrhea. Plasma proteins may be high in patients with severe dehydration due to changes in plasma volume. The increased serum globulins may also be due to secretion of immunoglobulin fractions to defend the body (Ranjna, 1990). However, treatment with the aqueous extracts cured the infections and normalizes the altered physical, biochemical and haematological parameters assessed. For instance, the temperature was brought down to normal, the pcv was increased, the loss in body weight was reversed and the frequent passage of watery and bloody stool was stopped. These suggest the efficacy of these plant extracts in the management of the infections.

Although chemical drugs are popular, however, herbal medicine continued to be practised due to richness of certain plants in varieties of secondary metabolites such as alkaloids, flavonoids, tannins, terpenoids which have been reported to have antibacterial activities (Lewis and Ausubel, 2006, Cowan, 1999). The phytochemical analysis of aqueous extract of *Mangifera indica* has been reported to contain tannins, phlobatanins, Cardiac glycosides, Saponin and Polyphenol (Madunagu et al., 1990; Ross and Brain, 1977). Nwinuka et al., (2008) reported positive effects of *mangifera indica* on haemopoietic system.

Plants have traditionally provided a source of hope for novel drug compounds, as plant herbal mixtures have made large contributions to human health and well-being (Iwu et al., 1999). Because plant herbs have been used to treat infectious diseases, frequent researches for those that have antibacterial properties are being made. Stapleton et al, (2004) reported that aqueous extracts of tea (*Camellia sinensis*) reverse methicillin resistabce MRSA and also to some extent reduces penicillin resistance in beta-lactamase-producing *staphylococcus aureus*. Also, Betoni et al., (2006) reported synergistic interactions between extracts of guaco (*mikania glomerata*), guava (*Psidium guajava*), clove (*syzyguim aromaticum*), garlic (*allium Satirum*) lemon grass (*cymbopogon citratus*) ginger (*Zingiber officinale*) cargueja (*baccharis trimera*), and mint (*Mentha Pieria*) and some antibiotics against *S. aureus*. However, this is a preliminary work and more works are needed to actually determine the active ingredients in these plants extracts and this may help in improving management of the different infectious diseases that are developing resistance to commonly use antibiotics. Furthermore, toxicological studies can also be carried out to determine the reliance on these herbs without many side effects.

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