

## Short Communication

# Immunomodulating properties of Trino IB immunobooster

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**Trino IB mixture at a very minimal concentration (0.2 ml) was found not only to protect rats against lethal dose of pathogenic isolates of *Escherichia coli* and *Staphylococcus aureus* but it is also capable of ameliorating the deleterious effect of very high concentration of sodium arsenite (6 mg/kg body weight) in rat and bird.**

**Key words:** Immunobooster, *Escherichia coli*, *Staphylococcus aureus*, sodium arsenite.

## INTRODUCTION

Immunostimulators are drugs, which stimulate immune system by inducing activation or increasing activity of any of its components. Immunostimulators are of two categories: specific immunostimulator, which provides antigenic specificity in immune response, such as vaccines or any antigen; non-specific immunostimulators which act irrespective of antigenic specificity, to augment immune response of other antigen or stimulate component of the immune system without antigenic specificity.

The use of immune modulators either as prophylaxis and/or part of a treatment regimen following exposure, may present a broad-spectrum approach to protect the public when exposed to a pathogenic challenge (Babineau et al., 1994). Immune modulators can increase the non specific components of the immune system such as the macrophage/neutrophil innate immune response, and stimulate the maturation of myeloid progenitor cells. Macrophage/neutrophil activation is largely considered the first line of defense against bacterial, fungal and some viral infections. These cells are also necessary for the efficient presentation of antigens, and their level of activation determines the efficacy of the acquired immune system, the second line of defense leading to effective humoral (antibody) and cellular (T-cells) immune responses (Abbas et al., 1994).

Immunomodulators have a wide range of uses as evident in the result of several works e.g. as anti-inflammatory in bowel diseases, anti-infectivity against bacteria, antiviral, antitumour, burn infection therapy, antidermatitis, anti-allergy, modulators of heat and cold stresses (Bill et al., 2003; Christopher et al., 2005; Di Luzio et al., 1979). So, in assessing the immunomodulating property of a test agent one has a choice to monitor after administration either for raised immunoglobulin like IgG or to assess directly for ability of the test agent to reduce challenged microbial load along with prevention of cancer in the presence of cancerous agents.

Trino IB is a natural product which several people in Nigeria often use to bring under control different immunocompromised conditions. In this work the ability of Trino IB to prevent death in mice challenged with lethal dose of pathogenic *Staphylococcus aureus* and *Escherichia coli* along with its ability to ameliorate the cumulative effect of sodium arsenite challenge is used as a measure of its immunomodulating property. Intake of arsenic contaminated water has been associated with hypertension, cancer, diabetes mellitus, atherosclerosis etc. (Chen et al., 1995; Lai et al., 1994; Tseng et al., 1996; Chiou et al., 1995). In addition, it has been shown that if 6 mg/kg of sodium arsenite is administered to rat for 26 days it produces deleterious effect on the vital organs (Mahitosh et al., 2003).

## MATERIALS AND METHODS

### Experimental animals

Male albino rats of 10 – 12 weeks old were purchased from central Animal House, College of Medicine, University of Ibadan. The rats were allowed to acclimatize for one week before the commencement of the study. Ten rats were used for each treatment.

Nera black cocks of about 10 months having an average weight of approximately 2 kg were purchased from the Animal House of the Ladoko Akintola University of Technology, Ogbomoso. The birds were allowed to acclimatize for one week before use. Ten

**Table 1.** Survival rate and bacterial load of rats given Trino IB with lethal doses of *Escherichia coli* and *Staphylococcus aureus* after 26 days of administration.

Rat	Microorganism			
	<i>E. coli</i> and Trino IB	<i>E. coli</i> only	<i>S. aureus</i> and Trino IB	<i>S. aureus</i> only
Survival rate (%)	100	10	100	6
Bacterial load/g liver weight	0	$4.43 \times 10^7$	0	$7.17 \times 10^7$

**Table 2.** Amelioration by Trino IB of the effect of sodium arsenite on sperm count sperm motility and spermatids count.

Treatment	Rat		Neural black cock
	Sperm count ( $\times 10^6$ /ml)	Sperm motility (%)	mean spermatids count ( $\times 10^6$ /ml)
Normal saline only	72	86	45
Sodium arsenite only	10	6	23
Sodium arsenite and Vitamin E	71	40	20
Sodium arsenite and Trino IB	71	72	38

birds were used per treatment.

#### Bacteria

While *S. aureus* was isolated from a patient's pus, *E. coli* was isolated from urine of a hospitalized patient at Baptist Medical Centre, Ogbomoso.

#### Rats

Using cannula, 0.2 ml ( $4.0 \times 10^7$ /ml) bacterial suspension and/or 0.2 ml Trino IB were administered by gavage along with or without 0.2 ml of 6 mg/kg body weight of sodium arsenate to different groups of rats daily for 26 days. Vitamin E was used as positive control. Rats were given 0.2 ml of 0.94 mg/kg body weight of Vitamin E by gavage daily with or without sodium arsenate for 26 days. On the 27<sup>th</sup> day, six rats were randomly selected from each group and killed by cervical dislocation. Blood samples were collected by cardiac puncture. Heparinized plasma was prepared and taken for hormone radio-immunoo assay.

Rats' scrotal sacks were opened immediately and the testis weighed (result not shown). Epididymis was trimmed off the right testis; their cauda epididymis was cut off the epididymal length and placed in a beaker containing 1 ml of physiological saline solution. The cauda epididymis was then lacerated at many spots so that their spermatozoa would swim out into the solution within a few minutes. Epididymal sperm motility and concentration were determined using an adaptation of Brooks and Welbster (1975).

The heart, liver, testis and kidneys were dissected out, trimmed the attaching tissues and weighed (result not shown). The organs were fixed in formol-saline for histological study.

Microbial clearance was determined by homogenizing livers of surviving rats in 20 ml sterile nutrient broth. Diluents were plated on nutrient agar and incubated for 24 h at 37°C. Bacterial load per liver gram weight was computed.

#### Birds

Using cannula, 0.2 ml of 6 mg/kg body weight of sodium arsenite with or without 0.2 ml of Trino IB mixture was administered to each bird for 5 weeks consecutively i.e, day 7, 14, 21, 28 and 35.

Vitamin E served as positive control. Vitamin E was administered by gavage once daily. The dose given was 6.7 mg/bird.

The birds were bled using needles and syringes to draw the blood from the main vein in the armpit. The blood was used for hormonal assay. Organs collected for histopathology test from slaughtered bird include liver, kidney, heart, and spleen. Elongated spermatids count was carried out (Brooks and Welbster, 1975).

## RESULT AND DISCUSSION

All the rats given lethal doses of *E. coli* and *S. aureus* along with Trino IB survived throughout the 26 days of experiment while 10 and 6% respectively of those given *E. coli* and *S. aureus* only survived (Table 1). In addition, while there were no organism in the liver of those animals that were given Trino IB with the bacteria, while heavy bacterial load was detected in the liver of those animals given bacteria only (Table 1).

When sodium arsenite alone was administered to rats and neural black cocks, it consistently has a very deleterious effect on sperm count, sperm motility, mean spermatid count and on the different hormonal systems (Tables 2, 3). Trino IB mixture however, also consistently ameliorates the deleterious effect of sodium arsenite on sperm count, sperm motility and hormonal system of the animals. From the results obtained in this work, Trino IB ameliorates the effect of sodium arsenite better than vitamin E which is a known antioxidant.

The histopathology examinations show that the spleen of the group treated with sodium arsenite alone show moderate hyperplasia of the lymph nodes with haemosiderosis. In addition the liver of the animals treated with sodium arsenite alone also demonstrated significant haemosiderosis with a moderate testicular hypoplasia of some somniferous tubules of the testis. The groups treated with sodium arsenite along with either Trino IB or Vitamin E appeared normal.

**Table 3.** Amelioration by Trino IB of the effect of sodium arsenite on the hormonal status of rat and neural black cock.

Treatment	Rat			Neural Black cock
	Leutenizing hormone (mIU/ml)	Follicle stimulating hormone (mIU/ml)	Testosterone (ng/ml)	testosterone (ng/ml)
Normal saline only	3.75	2.25	0.85	2.5
Sodium arsenite only	2.15	1.5	0.40	0.6
Sodium arsenite and Vitamin E	2.75	1.35	0.65	1.4
Sodium arsenite and Trino IB	3.51	2.10	0.77	2.11

## Conclusion

Since Trino IB mixture not only protect rats against lethal dose of pathological isolates of *E. coli* and *S. aureus* and since it is also capable of ameliorating the deleterious effect of sodium arsenite in rat and bird, the mixture should be regarded as an excellent immune modulator.

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