EVALUATION THE BORIC ACID EFFECT TOWARD THE IMMUNE RESPONSE IN MICE INJECTED WITH SOMATIC VIBRIO ALGINOLYTICUS ANTIGEN

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I. SUMMERY

In this present study the immunological influence of the boric acid on immune responses was investigated and confirmed by measuring the phagocytic activity of non-specific immunity and proliferations of lymphocytes and antibody production against Vibrio alginolyticus in mice, the study consisted of four groups: group(I) treated with normal saline, group II treated with V. alginolyticus antigens only, group(III): injected subcutaneously with dose of (400 ug /Kg) and V. alginolyticus antigen, group IV treated with (300 ug /Kg) boric acid and V. alginolyticus , on day 8, the phagocytic activity index was estimated, the transformation of the lymphocyte was estimated on day I4. The results showed substantial differences in the NBT index and MTT tests between group III and group I, in other words, the results showed significant differences between group IV and group I, group II, while the differences increased between group III and group IV. More than this, the results of an indirect Immunofluorescence test, the results of the anti-V alginolyticus antibodies in the group III titration were shown to be increasingly significant after 21 days and after 28 days. The beneficial effect of boric acid on the immune response in treated mouse with V alginolyticus antigen has been demonstrated.

II. INTRODUCTION

Boric acid (BA) is a colorless, water-soluble boron chemical in the form of a salt-like white powder (1). Boric acid is used for a variety of medical applications, including antiseptic qualities against microbiological microorganisms such as bacteria and fungi, as well as insecticidal, fungicidal, and herbicidal properties. Its toxicity is mild (2). BA is an important chemical in the body that is easily absorbed and excreted through the urine since it does not accumulate in soft tissues unless in high levels of 2000ppm (3, 4).

The effects of boric acid on cells via cell membrane function and the hormone metabolic process because of its capacity to compound with hydroxy I groups, boric acid is involved in the manufacture of several hormones, including steroid hormones and vitamins such as vitamin D. (5 ) The animals have the ability to maintain the level of boron in their bodies (6. ) It has been established that boron is nutritionally important and impacts many immunological mechanisms and processes such as inflammatory responses, cytokine generation, and lymphocyte proliferation (T cell, B cell ) (7, 8, 9).

Vibrio alginolyticus is Gram negative Causes numerous illnesses like injury and otitis Tetrodotoxin V alginolyticus was initially identified as a human disease in 1973. It is also available in animals like fish and responsible for producing the strong neurotoxin (10).
III. MATERIAL AND METHODS

All investigations were conducted on male or female albino mice (Blab-c), which were averaged at 22-25 grams in the laboratories of College of Medical Technologies. Sawa University,

1-Isolation and identification V. alginolyticus
The bacterial isolate was obtained from Central Health Laboratory in Al-Muthanna province then activated for antigen preparation .

2-Preparation Somatic V. alginolyticus antigen
Somatic V.aliginolyticus antigen produced by Ultra soncater system according to a process described in (11). As following V.aliginolyticus was centrifuged (3000 rpm) for 30 minutes with PBS after harvesting from nutrient agar then discharged the supernatant and washed three times the precipitated cells in PBs placed in a Ultra soncater system (15 KHZ/Sec ( for a total of 5 minutes )with intervals (one minute) in an ice bath for 30 minutes at 4°C.

3-Preparation of boric Acid and Determine LD50
The boric acid powder (1 grams) was dissolved in 100 ml of PBS at room temperature (25ºC), then prepared 5 doses (100 ug /Kg, 200 ug /Kg and 300 ug /Kg, 400 ug /Kg ) to determine the LD50 by using 4 groups of mice each group included 5 animals.

4- Vaccination program
This experiment consisted four concentration to evaluate the level of the immunoglobulin IgG, group I injected with normal saline (0.2ml) as control, group II: injected with 45 ug / ml, group III: injected with 70 ug / ml ,group IV: injected with 88 ug / ml V.alginolyticus antigen.

5-Studied groups
Group I:(negative group ) injected with normal saline, group II : injected with V. alginolyticus antigen, group III was administrated by alginolyticus antigen+ (300 µg / kg) of boric acid, group IV: injected with V. alginolyticus antigen +400 µg / kg) of boric acid. After one month the serum was collected and kept at -20ºC until use for laboratory assessments.

Immunological parameters :
1- Nitro blue Tetrazolium (NBT) Index
The assay was done depending to a method presented by (12).

2- Lymphocyte Transformation Test (MTT)
The MTT assay was done depending on (12).

3- Indirect Fluorescent Antibody Test (IFAT)
The procedure of WHO (1997) was adopted to determine done to assess anti- V. alginolyticus antibody titrations.

Statistical Analysis
The Statistical Analysis achieved by (ANOVA), (LSD) test probability ≤ 0.05.

IV. THE RESULTS
The bacterial isolate was received from the Central Health Laboratory in Al-Muthanna province and identified with the Vitek 2 index. The nutrient broth and nutrient agar used to grow the bacteria c in order to enhance bacteria activities to prepared the whole antigen, respectively (14). The immunoglobulin level IgG was shown to increase considerably (P =0.05) in the animals group III 4321.4±6.28 compared to group I, II, IV (97±1.8, 252.2±3.8, 421.4±6.28) respectively.

The findings NBT have found significant variations between group III (2.39±0.2)and group I (0.190±0.33),groupII(0.295±0.03) as well as significant differences between group IV(2.49± 0.29), group I
(0.29±0.33) and group II(0.295±0.03) as well as significant differences between group IV(2.48±0.29) and group III (2.39±0.2), although they have been increased by no significant difference between Groups IV(2.39±0.03) and III(2.39±0.2), as in figure (1).

Figure (1): showed results of phagocytic activity in studied groups,

*H.S: highly significant. N.S: Non significant

On the other side the results of MTT showed group III (1.08±0.08 ) and group IV ( 1.26±0.107) significant differences (p≤0.05) comparison with other studied groups in same time group II showed a significant difference with other groups as showed in figure (2).

Figure (2): showed results of lymphocyte transformations in studied groups

*H.S: highly significant. N.S: Non significant

While the results of the indirect fluorescent antibody were found, certain variations were found for the group as the group I, which did not show anti- V alginolyticus antibodies after 21, days, but the other groups II, III, IV showed a positive response. Group III at title 1:256 recorded the highest positive reaction value. During 28 days, Anti V.alginolyticus exhibited at 1:32 in group II . Moreover, in group III, IV in titer 1:256, and 1: 128 correspondingly (Figure3 and 4), the greatest anti-V. alginolyticus antibody titer was obtained.
Figure (3): Anti *V. alginolyticus* antibodies at titer I: 256

Figure (4): Anti *V. alginolyticus* antibodies I: 128.

### V. DISCUSSION

The current study was focused to confirm the influence of boric acid on the immune response (non specific immunity and adaptive immunity) by measure the phagocyte activity index and for adaptive immunity, the lymphocyte proliferation by transformation lymphocyte index and the humoral immunity by Indirect Fluorescent Antibody test were achieved, previous studies were used the boron as dairy to study its potency on the immune system and other systems, this study used the boric acid to investigate its effect to the immune system process such as cell proliferations and antibodies production and phagocytosis activity as the boric acid is one of the boron compounds.

The results of vaccination program was revealed the immunoglobulin concentration IgG was a significant increasing (P ≤ 0.05) animals group II (70 ug / ml) compares with other group, because the group II with moderate does induce and not showed immune tolerance as in group II and Group IV (15).

Moreover the result of the boric acid LD50 showed no mortality Rates in studied groups, and this agreed (16, 17) that the boron maybe have essential effect in animal by influence processes such as hormonal and metabolic and nutritional processes, also it potential effects in immunity system process Furthermore, it is important to bone growth and others (17, 18, 19, 20).

The ability of animals to control boron levels inside their body at homeostatic levels resulted in doses that were not shown to have a major effect or mortality rate (6). The objective of this study is to study and confirm boric acid's ability to effect the immune system and to consider as immunodulatory agents that this is the reason *V. alginolyticus* antigen is injected, the immunomodulators are used to mediate the mechanism of immune stimulation in the immune system (21).
The NBT index is used to assess the phagocytic activity of innate immunity cells. According to the NBT results of this study, groups III and IV showed stronger phagocytic activity than group II and these findings agreed with (22 , 23 , 24 ), the boric acid has been linked to an increase in phagocytic cells, as well as an increase in cytokine production, such as tumor necrosis factor alpha. The main role of tumor necrosis factor alpha was to induce the production of anti-inflammatory cytokines. Boric acid also stimulates the production of plasma proteins which are stimulated TNF−α, IL-6, and IL1 (22).

Boron was found to cause an increase in NO, as well as the expression of iNOS (which helps to promote inflammation and immune responses) and an increase in cytokines production by the Lipopolysaccharides-primed macrophage (which was possibly to act through the Toll-like receptors, the boron as the boron can stimulate the LPS-induced responses of the macrophage (9 ).

The other probable action of boric acid is on the cellular level, which regulates the respiratory burst as the boron may operate positively as a non-specific immunity and activated lymphocyte proliferation and antibody class switching and antibody production, group III , IV reported the highest titer for Anti_ V.alginolyticus was after 21 and 28 days, respectively, while the Anti V. alginolyticus titer in group II was lower, this result agreed with ( 22 ) who reported that dietary boron supply may increase humoral immune response and reduces inflammatory response , Bai (27 ) revealed the rats that consumed diets containing a low concentration of boron gave a lower concentration of anti typhoid IgG and IgM attributed to the boric acid was acted to increased the B Cell receptors also increased in CD4 cells population that have important role to activate the B cell to produce Ab producing cells (34).

Furthermore, boric acid helped to enhance IL 6 production, which has the power to convert B cells into antibody (Ab)-producing cells (35) Finally the boric acid may act on CD19 to modulate signaling to B cell proliferation and differentiationand receptors (33 , 35, 36 ).

VI. CONCLUSION

According to the findings of the current study, boric acid has a favorable influence on phagocytic activity even at low concentrations, as demonstrated in results of the NBT index a, as well as a beneficial influence on lymphocyte proliferation as shown by lymphocyte transformation, and aid in increasing antibody production, the boron acid operated positively as a non-specific immunity and activated lymphocyte proliferation and antibody production.

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