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Anti-Inflammatory Effects of Boron Alone or as Adjuvant with Dexamethasone in Animal Models of Chronic and Granulomatous Inflammation

A Thesis

Submitted to the Department of Pharmacology and the Committee of Post Graduate Studies of Faculty of Medical Sciences/School of Medicine at University of Sulaimani in Partial Fulfillment of the Requirements for the Master of Science in Pharmacology

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Dedicated To……

my husband,
my mother,
and my bird

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Abstract

Background:

The side effects of currently available anti-inflammatory agents are considered as a major problem during their clinical use. Therefore, developing a newer, effective, and safe anti-inflammatory agent is important to be taken into account. Recently, significant progress has been made through the utilization of Boron-containing compounds as anti-inflammatory agents, which are effective, relatively free of side effects, and can be used effectively as a supplement. The present study was designed to evaluate the dose-response relationship of the anti-inflammatory activity of Boron in rat models of induced chronic inflammation compared to that produced by the standard drug Dexamethasone, and to evaluate the anti-inflammatory activity of its adjuvant use with Dexamethasone.

Methods:

Sixty-six Wistar rats were used in the present study, divided into 5 groups; the first group: 6 rats treated with vehicle only without induction of inflammation as a negative control. Second group: 12 rats divided into two sub-groups, each containing 6 rats, and treated with vehicle only with the induction of chronic and granulomatous inflammation, as a positive control. Third group: 24 rats divided into four groups, each containing 6 rats, for the study of the anti-inflammatory activity of different doses of Boron (3 and 6 mg/kg BW) in both models of inflammation. Fourth group: 12 rats used to study the anti-inflammatory activity of Dexamethasone (1 mg/kg BW) in the same models. Fifth group: 12 rats used to study the anti-inflammatory activity of Boron (3 mg/kg Bw) when used as adjuvant with Dexamethasone (1 mg/kg BW) in the same models.

Results:

The result of the present study indicated that Boron in a dose-dependent pattern (3 and 6 mg/kg BW) significantly suppresses inflammation in rat models of formaldehyde induced chronic inflammation and cotton pellet-induced granuloma. Boron (3 mg/kg BW) in adjuvant with Dexamethasone (1 mg/kg BW) significantly suppresses inflammation in rat models of formaldehyde induced chronic inflammation and cotton pellet-induced granuloma, which is significantly higher than all of the effects produced by other approaches of treatment when Boron is used alone.

Conclusion:

Boron, in a dose dependent pattern, is effective in suppressing formaldehyde-induced chronic inflammation and cotton pellet-induced granuloma in rats. Therefore, it may be considered as a treatment for chronic inflammatory conditions in human. Boron, as an adjuvant with the standard anti-inflammatory agent, Dexamethasone, improves the anti-inflammatory activity of the latter, with a chance to reduce its dose.

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List of Abbreviations

°C……. Celsius
5-HT ……..……..……5-hydroxytryptamine
ADP……..…….. Adenosine diphosphate
ANOVA…….……Analysis of variance
AP1…….……..……..Activator protein1
BW……..……..…….. Body weight
CBC……..……..…. Complete blood count
CD…….…….. Cluster of differentiation
CF…..……..…. Calcium frucoborate
CRP……..……. C - reactive protein
CSFs…..…….……Colony-stimulating factors
CVD…….…….Cardiovascular disease
D.W……..…….. Distilled water
DNA……...Deoxyribonucleic Acid
EDTA……..…Ethylene diamine tetraacetic acid
ELISA……. Enzyme-linked immune sorbent assay
ESR……..…Erythrocyte sedimentation rate
GC……..…. Glucocorticoid
GSH…….……..……Glutathione
HDL……High density Lipoprotein
HRP…….…….. Horseradish peroxidase
hs-CRP……..... High sensitive C-reactive protein
Ib……..……Pound
IL-1β…… Interleukin 1 beta
ILs. ……..……...Interleukins

INFs……Interferons

LDL……Low-density Lipoprotein

mRNA……Messanger Ribonucleic acid

NAD^+……Nicotinamide adenine dinucleotide

NADPH……Nicotinamide Adenine Dinucleotide Phosphate Hydrogen

NC……Negative control

NCEP……National Cholesterol Education Program

NF-κB…… Nuclear factor kappa B

NSAIDs…… Non-steroidal anti-inflammatory drugs

OA……Osteoarthritis

OH^-……Hydroxide ion

PC…….. Positive control

PDE4……Phosphodiesterase 4

PGs…… Prostaglandins

PPM……Part per million

RA…….. Rheumatoid arthritis

rpm……Round per minute

S.c……. Subcutaneous

SD……Standard deviation

SEM…….. Standard error of mean

TC……....Total cholesterol

TGF……Transforming growth factor

TNF-α…….. Tumor necrosis factor-alpha

WBC……White blood cell

WHO…… World Health Organization

Chapter one

Introduction and literature review

Chapter One

Introduction and Literature Review

1.1 Inflammation

Inflammation is a protective response involving host cells, blood vessels, and proteins and other mediators that is intended to eliminate the initial cause of cell injury [1], as well as the necrotic cells and tissues resulting from the original insult, and to initiate the process of repair [2].

The cardinal signs of inflammation are pain (*dolor*), heat (*calor*), redness (*rubor*), swelling (*tumor*), and inhibited or loss of function (*functio laesa*) [3]. All these signs may be observed in certain instances, but none is necessarily always present [2,4]; they occur due to changes in blood flow caused by changes in smooth muscle cell function which lead to vasodilatation, also, alterations in vascular permeability engendered by cytoskeletal contraction in endothelial cells, and migration of phagocytic leukocytes to the site of inflammation, and phagocytosis [5].

Inflammation is normally controlled and self-limited. In response to the injurious stimulus, the mediators and cells are activated, but are short-lived and are degraded or inactivated as the injurious agent is eliminated. In addition, various anti-inflammatory mechanisms become active. If the injurious agent cannot be quickly eliminated, the result may be chronic inflammation, which can have serious pathologic consequences [2].

1.2 The inflammatory Response

The inflammatory response is a series of local cellular and vascular responses which are triggered when the body is injured, or invaded by antigen, resulting in the production of a variety of mediators that act both locally and systemically [6]. The primary physical effect of the inflammatory response is for blood circulation. In particular, the blood

vessels around the site of inflammation dilate, permitting increased blood flow to the area and allowing the larger cells of the blood, i.e. the immune cells, to pass, through the gaps that appear in the cell walls surrounding the infected area [7].

Chemical mediators of inflammatory response include: vasoactive amines that cause vasodilatation and increase vascular permeability when released such as, Histamine, produced by circulating basophils, platelets and mast cells, and Serotonin that is produced by platelets [4,8]. Also include cytokines (interleukins ILs, colony-stimulating factors CSFs, interferons INFs, transforming growth factor TGF, chemokine's and tumor necrosis factor TNF), plasma proteases, arachidonic acid products (prostaglandins, leukotrienes, and lipoxins), platelet-activating factors, and nitric oxide [8,9]. The inflammatory response is not only an important component in the defense against pathogens, but it is also an important contributor to pathophysiologic processes such as atherosclerosis, autoimmune diseases, and endotoxic shock [10].

1.3 Types of Inflammation

Inflammation can be acute or chronic. Acute inflammation is rapid in onset and of short duration, lasting from a few minutes to as long as a few days, and is characterized by fluid and plasma protein exudation and a predominantly neutrophilic leukocyte accumulation. Chronic inflammation may be more insidious (Table 1-1), is of longer duration (days to years), and is typified by influx of lymphocytes and macrophages with associated vascular proliferation and fibrosis [2].

1.3.1 Acute Inflammation

Acute inflammation constitutes the body's principal mode of defense against infection and other harmful agents, and neutrophils are the

Table 1-1 Fundamental Features of Acute and Chronic Inflammation [2]

Feature	Acute	Chronic
Onset	Fast: minutes or hours	Slow: days
Cellular infiltrate	Mainly neutrophils	Monocytes/macrophages and lymphocytes
Tissue injury, fibrosis	Usually mild and self-limited	Often severe and progressive
Local and systemic signs	Prominent	Less prominent; may be subtle

primary effector cells in this process [11]. Acute inflammation has three major components [12]:

1. Changes in Vascular Flow and Caliber: These are of primary importance in the development of the acute inflammatory reaction, because they determine (to a large extent) the amount of exudate. If local blood flow is decreased, or temporarily stopped, exudate will be reduced or abolished.
2. Changes in Vascular Permeability: vascular leakage, after local injury, that can occur by at least two distinct mechanisms: a) directly, as an effect on the injurious agent itself (heat, mechanical trauma, etc.), or b) indirectly, as an effect of chemical substances that appear in and around the site of injury, permit plasma proteins and leukocytes to leave the circulation.
3. Emigration of the leukocytes from the microcirculation, their accumulation in the focus of injury, and their activation to eliminate the offending agent (Figure 1-1) [2,12].

A lot of insults including mechanical injury, infectious pathogen, chemical injury, burn, radiation, tissue injury, and shock can induce acute inflammation [13].

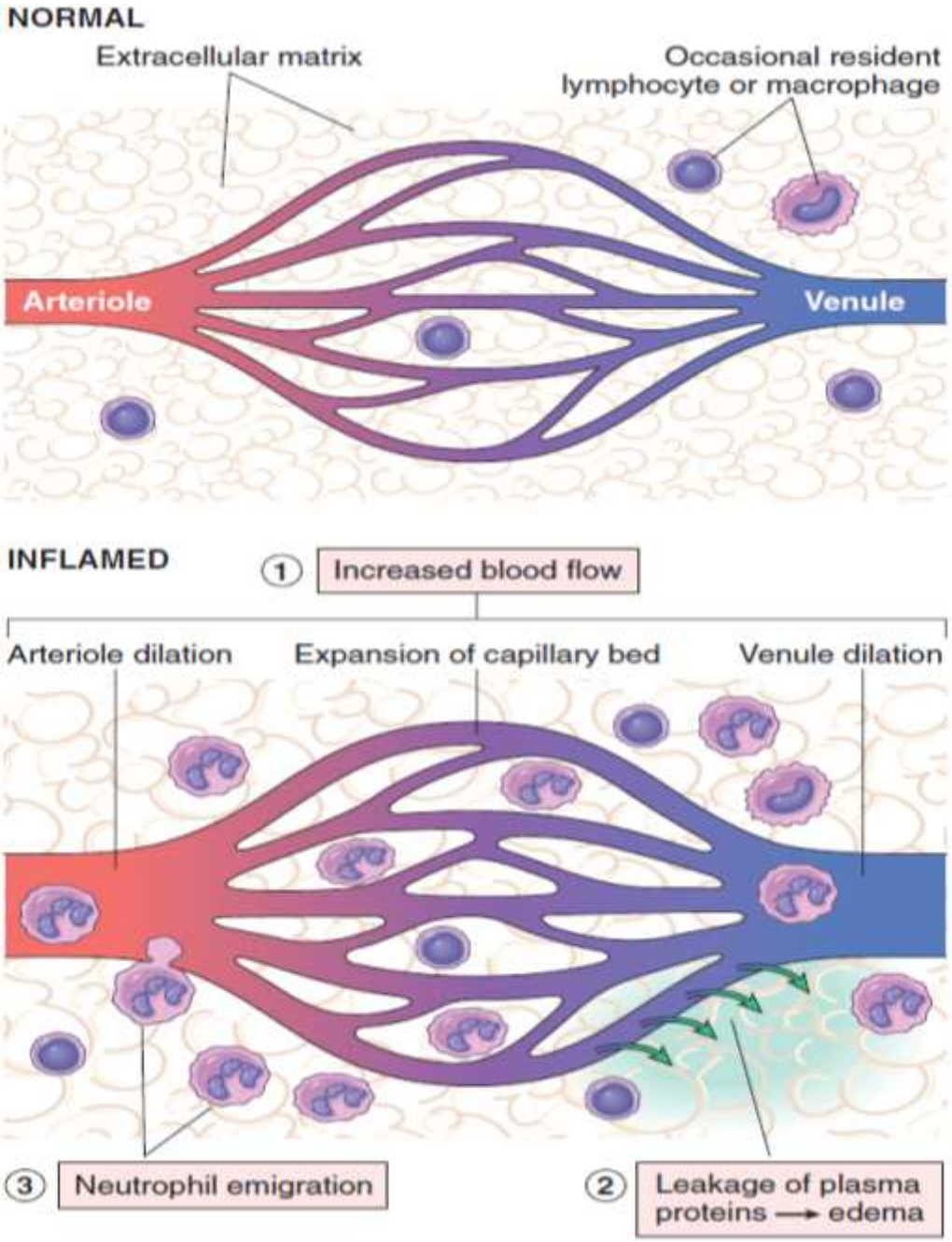

Figure 1-1: The major local manifestations of acute inflammation, compared to normal. 1) Vascular dilation and increased blood flow (causing erythema and warmth), 2) extravasation and deposition of plasma fluid and proteins (edema), and 3) leukocyte emigration and accumulation in the site of injury [2].

1.3.2 Chronic Inflammation

Chronic inflammation is a dysregulated form of inflammation [14]; it represents a pathological condition characterized by continued active inflammatory response and tissue destruction [15,16]. It may develop from unresolved symptomatic acute inflammation or may evolve insidiously over a period of months without apparent acute onset of clinical manifestations [17]. Chronic inflammation is characterized by a different set of reactions [2]:

1. Infiltration with mononuclear cells, including macrophages, lymphocytes, and plasma cells.
2. Tissue destruction, largely induced by the products of the inflammatory cells.
3. Repair, involving new vessel proliferation (angiogenesis) and fibrosis.

Based on histologic features chronic inflammation can be classified into the following two types [18].

1.3.2.1 Nonspecific Chronic Inflammation

Nonspecific chronic inflammation involves a diffuse accumulation of macrophages and lymphocytes at the site of injury that is usually productive with new fibrous tissue formations [18]. It is characterized by non-specific inflammatory cell infiltration. A variant of this type of chronic inflammatory response is chronic suppurative inflammation in which infiltration by polymorphs and abscess formation are additional features [19]. The outcome of non-specific chronic inflammation depends on whether local and systemic factors favor the injurious agent or the process of healing [20].

1.3.2.2 Granulomatous Inflammation

Granulomatous inflammation is a distinctive pattern of chronic inflammation in which cells of the mononuclear phagocyte system are predominant and take the form of macrophages, epithelioid cells and multinucleated giant cells [21,22]. Granuloma is a focal, compact (organized) collection of mature mononuclear phagocytes, which is not necessarily accompanied by accessory features such as necrosis [23-25]. Granulomas evolve in three stages:

1. The development of an infiltrate of young mononuclear phagocytes.
2. The maturation and aggregation of these cells into a mature granuloma.
3. Maturation of these cells into an epithelioid granuloma [23].

The granulomatous inflammatory response is a manifestation of many infective, toxic, allergic, autoimmune, neoplasm and conditions of unknown etiology [22]. Granuloma formation is usually regarded as a means of defending the host from persistent irritants of either exogenous or endogenous origin [21]. Granulomatous inflammations have provided much knowledge about the pathogenesis of granulomas, which had shown that both the nature of the irritant and host factors are important in governing the type of reaction that is produced [21].

All injected substances cause an initial influx of mononuclear cells by the phenomenon of chemotaxis. However, what happens next depends on the resistance of the irritant to degradation by macrophages. If it is a soluble substance that is easily digested, then the macrophages move away once degradation is complete [26]. However, if it is poorly soluble, persistent and undegradable granuloma is formed. The exception to this rule is that soluble materials can produce granulomas if they were

combined with endogenous macromolecules to form insoluble and non-degradable compounds [21,26].

1.4 Boron

1.4.1 Boron, the Element

Boron, ubiquitous in the earth's crust, can be found in most soil types as well as in fresh and salt water. While most of the earth's soils have <10 ppm Boron, the range is from 2-100 ppm with the average soil Boron concentration reported to be 10-20 ppm. While large areas of the world can be Boron deficient, high concentrations can also be found, for example, in parts of the western United States, throughout China, Brazil and Russia. The world's richest deposits of Boron are located in a geographic region that stretches from the Mediterranean countries inland to Kazakhstan. Seawater contains an average of 4.6 ppm Boron, but ranges from 0.5-9.6 ppm. Freshwaters normally range from <0.01-1.5 ppm, with higher concentrations in regions with high concentrations of Boron in soil [27]. Most essential elements that make their way into the human food and water supply are directly derived only from soil minerals. While most environmental sources of Boron are geogenic in nature, some trace elements such as Boron, iodine, and selenium are supplied in significant amounts to soils by atmospheric transport from the marine environment.

Deficiency problems associated with these elements are therefore generally less common in coastal areas than farther inland. It has been known for some time that Boron is an essential micronutrient for higher plants yet the mechanism through which Boron functioned in plants was, until recently, unknown [28,29]. While Boron accumulates in aquatic and

terrestrial plants it does not magnify through the food-chain. On the other hand, its deficiencies in plants are often observed. Boron is also a constituent in all phyla of living organisms and its role is in most obscure [30]. For some microorganisms, algae, and higher plants, Boron is essential. Although the quantities required are low they are also highly variable and species specific. In other species, including humans, knowing how much Boron is needed and what Boron does is still being determined.

Boron has long been recognized as an essential trace element for plants, but has only recently been considered to be possibly essential for humans. Boron appears to participate in hydroxylation reactions, which play a role in the synthesis of steroid hormones and vitamin D. In Australia, where much of the food is grown on soil deficient in this mineral, Boron supplements were popular as a treatment for osteoarthritis (OA), and were reportedly selling at a rate of 10,000 bottles per month before the Australian government removed the product from the market [31]. In a double-blind study, 20 Australians with OA were randomly assigned to receive Boron (6 mg per day as sodium tetraborate decahydrate) or a placebo for eight weeks [32]. Of those receiving Boron, 50 percent improved, compared with 10 percent of those given placebo. Because of the small sample size, this difference was not statistically significant. When the five subjects (25%) who dropped out of the study (mostly because of clinical deterioration) were excluded from the analysis, 71% of those in the Boron group improved, compared with 12.5 percent of those in the placebo group ($P < 0.05$). No side effects were seen and there were no significant changes in common laboratory parameters. These results suggest Boron supplementation may be helpful for individuals with OA whose diets are likely to be low in Boron.

Further research is needed to confirm this preliminary study and to determine whether individuals with a higher dietary intake of Boron can benefit from supplementation. The average American diet provides approximately 1-2 mg of Boron per day, primarily from fruits, vegetables, and nuts; however, according to German research, intake can vary from 0.3 to 41 mg per day. While the capacity of Boron to increase estrogen levels [33] might raise concerns about possible cancer risks with Boron supplementation, there is no evidence that populations with a high intake of Boron (such as the French) have an increased incidence of hormone-related cancers.

1.4.2 Pharmacokinetics of Boron

Boron is easily absorbed across the gastrointestinal epithelia in humans and animals [34], and across mucous membranes, such as the mouth, eyes, vagina, and anus. In 1998, Hunt reported that humans and animals absorb nearly 100% of supplemental inorganic Boron. Some organic forms of supplemental Boron may be inaccessible to animals because plants can only absorb organic forms of Boron in soils after mineralization [35]. Boron is primarily excreted in the urine, with about 2% lost in the feces, and lesser amounts lost in bile, sweat, and breath [36,37]. Tissue Boron concentrations are generally kept steady by a homeostatic mechanism, primarily through renal excretion, and higher Boron intakes do not significantly increase plasma levels [38]. A 167-day metabolic study of 11 postmenopausal women showed a rapid increase in urinary Boron when Boron intake increased from (0.36 mg/day) to (3.22 mg/day) [39]. Naghii and Samman [40] studied the effect of Boron supplementation on urinary excretion in healthy male subjects. When 18 healthy males remained on a habitual diet, urinary Boron excretion measured on two separate occasions ranged from 0.3 to 3.53 mg/day.

The difference in Boron values between the two 24-h urinary collections was not statistically significant, but slight variations within and between some subjects suggested differences in their daily Boron consumption. In a second study, when subjects were administered 10 mg/day of supplementary Boron for 4 weeks, urinary Boron increased from an average of 1.64±0.3 (at baseline) to 10.16±0.92 mg/day. This increase in urinary excretion, which occurred in every individual, was significant and represented 84% of the supplemented dose. These findings provide evidence that urinary Boron reflects Boron intake.

1.4.3 Role of Boron Intake on Health of the Population

The suggestion that Boron may be a factor in maintaining health is reasonable because there is evidence that many people consume less Boron than the necessary to promote bone and brain health. In human depletion-repletion experiments, subjects responded to a Boron supplement after consuming a diet supplying only 0.2-0.4 mg Boron/day for 63 days [41] suggesting that this intake of Boron is inadequate. Thus, a dietary Boron intake higher than 0.4 mg/day may be beneficial to bone and brain health. Extrapolation of data from animal experiments suggests that 1 mg Boron/day would provide optimal nutritional benefits for this element. The WHO suggested that an acceptable safe range of population mean intakes of Boron for adults could well be 1-13 mg/day [42] relying on both animal and human data. Based on published values for Boron in foods, it has been estimated that the median intake of Boron in the United States is 0.86 mg/day [43]. The 1994-1996 Continuing Survey of Food Intakes by Individuals indicated that Boron intakes ranged from a low of about (0.35 mg/day) to a high of about (3.25 mg/day) for adults [44]. The median intakes for various age groups of adults ranged from

(0.87 to 1.13 mg/day). The reported median intakes of 0.86 and 0.87 mg Boron/day suggest a significant number of people would benefit from increased Boron intakes. This suggestion is supported by a study of premenopausal women in eastern North Dakota [45]. Based on urinary excretion of Boron (a good indicator of Boron intake), two women apparently consumed an average of less than 0.5 mg Boron/day, and 14 women consumed between 0.5 and 1.0 mg Boron/day. There are only a few reports associating Boron intake or status with diseases other than some types of cancer described above. Low concentrations of Boron in hair [46] and low environmental Boron [47] have been associated with Kashin-Beck disease (Osteochondropathic) in China. Low Boron status has been associated with rheumatoid arthritis (RA) [48]. Based on the suggestion that a significant number of people may have a low Boron status, more epidemiological studies determining whether a low Boron intake is associated with some disorders of bone and brain seems prudent.

There are two reports describing no or limited responses by postmenopausal women to Boron deprivation, which may have resulted in negative impressions about the nutritional importance of Boron. Several aspects of the experimental designs of these studies, however, may have contributed to the lack of marked findings. In one experiment, the subjects were only equilibrated on the experimental diet for two days before starting the low dietary Boron regimen that lasted only 21 days [49]. The data (i.e. increasing urinary calcium) presented from only six subjects suggest that they were still adjusting from their self-selected diets to the experimental diet, and thus to changes in other nutrient intakes when they began receiving Boron supplementation of 21 days duration. Additionally, 21 days is an extremely short deprivation period

for an adult organism when the diet is not severely deficient and a small number of subjects limits statistical power.

In successful Boron deprivation experiments, 14 subjects were equilibrated to the experimental diet for 14 days, and the first 21 days of Boron deprivation were not included in the analysis because only minimal responses occurred during this time; the most marked effects were seen after 42 days of Boron deprivation [41,50]. Thus, short Boron deprivation periods of only 42 days in a Latin-square experimental design most likely contributed to finding a limited number of responses to Boron deprivation compared to other human studies [51]. In addition, varying dietary magnesium (deficient and adequate) may have obscured or blunted the effects of varying dietary Boron. These design concerns suggest that these two human studies are ill suited for assessing the nutritional relevance of Boron.

Many epidemiological and controlled animal and human experiments have provided evidence for the use of Boron as a safe and effective treatment for some forms of osteoarthritis (OA) [52]. By examining the relationship between Boron administration and OA prevalence around the world, researchers have discovered that in the areas where Boron intake is 1 mg or less per day, the estimated incidence of arthritis is between 20% and 70%. In contrast, in areas where Boron intake is usually 3–10 mg per day, the arthritis percentage is lower, ranging from zero to only 10%. This remarkable finding is a compelling evidence of the fact that abundant intake of dietary Boron can confer strong protection against the development of OA [53,54]. An analytical study showed that Boron concentration is lower in femur heads, bones, and synovial fluid of OA patients as compared with patients without OA. Moreover, surgeons have observed that the bones of patients that had used Boron supplementation

were harder to cut than those patients who had not used these supplements [55].

The most convincing evidence for Boron usage in the case of OA patients comes from a double-blind placebo Boron supplementation trial conducted in Australia [56,32] reporting that Boron supplementation may improve symptoms for people with OA and rheumatoid arthritis [32]. Experimental studies on arthritic rats have led to an emerging hypothesis suggesting that Boron reduces the risk of inflammatory disease by down-regulating enzymes of the inflammatory response and has a beneficial immunomodulatory effect in the arthritic rats [57-59].

C-reactive protein (CRP), one of the most useful markers of systemic inflammation, has recently been identified as a marker of OA with clinical significance. CRP levels are moderately high for patients with OA as compared with the normal controls [60,61]. Of great clinical significance are CRP levels, with reference values below 0.5 mg/dL in OA patients [62,63]. Increased levels have been associated with the disease evolution as well as with the clinical aggravation, as an unspecific response to inflammations and infections [64-66].

Calcium fructoborate (CF) is used as a recent non-pharmaceutical therapy for osteoarthritis treatment. CF is a complex of calcium, fructose, and Boron and is naturally found in fresh and dried fruits, vegetables, herbs, and wine. This form of Boron is not only safe but also bioavailable compared with other commercial forms of Boron. An open label pilot study, authored by Miljkovic and colleagues from the Orthopedic Clinic of the University of Novi Sad, Yugoslavia, was conducted. The purpose of the study was to investigate the effects of CF on OA symptoms. The study included 20 patients with mild, medium, or severe forms of OA.

Two criteria for assessment were used: the Western Ontario McMaster University Osteoarthritis Index and Newnham criteria. After the administration of CF, the results were quite impressive: the pain was strongly diminished, the joint rigidity disappeared, and mobility and flexibility were improved [67,68]. Previous investigations have been summarized in two other reviews [67,68] that have revealed an anti-inflammatory property of CF on cellular cultures. In addition, it has been hypothesized that CF might have dual roles as both an anti-inflammatory and anti-oxidant agent, with modifying effect on lipid metabolism [67,68].

The study investigates whether CF can relieve OA symptoms in selected subjects. Scientists have hypothesized that CF may have a role in diminishing inflammation-related pain, joint stiffness and other discomforts associated with OA [69-71]. Because OA discomfort is often invariably related to joint inflammation, this study approaches the CF effect on inflammatory blood markers levels such as CRP, fibrinogen, and on erythrocyte sedimentation rate (ESR) and on lipid metabolism markers because it has been suggested that Boron is involved in both mechanisms [67]. When analyzing inflammatory markers, the 2-week time interval for the CF dietary supplementation was long, enough to confirm previous results obtained in vitro. Because the general characteristic of the placebo effect has a slightly delayed onset, and a relatively short duration (from 2 to 6 weeks as cited in the literature) [72], a time interval of 2 weeks was chosen for this trial to more accurately observe the short-term efficacy of CF. This pilot study is only a bridge for a future, more complex research study regarding the effects of CF on OA symptoms.

1.4.4 Suggested Mechanisms for the Biological Effects of Boron

The diverse responses reported for animals and humans-deprived of Boron have made it difficult to identify a primary mechanism for its possibly beneficial activity. The wide range of responses is likely secondary to Boron influencing a cell signaling system and/or the formation and/or activity of entity that is involved in many biochemical processes. A plausible mechanism of action may be indicated by the biochemistry of Boron. Boric acid forms ester complexes with hydroxyl groups of organic compounds, which preferably occurs when the hydroxyl groups are adjacent and in a *cis*-orientation. This property results in the formation of complexes with several biologically important sugars. These sugars include ribose, which is a component of adenosine. Recent findings suggest that the diverse beneficial effects of Boron occur through affecting the presence or action of biomolecules containing adenosine or formed from adenosine precursors. These biomolecules include S-adenosylmethionine and diadenosine phosphates that have higher affinities for Boron than any other recognized Boron ligands in animal tissues [73].

Diadenosine phosphates are present in all animal cells and function as signal nucleotides involved with neuronal response. S-adenosylmethionine is one of the most frequently used enzyme substrates in the body [74]. About 95% of S-adenosylmethionine is used in methylation reactions, which influence the activity of DNA, RNA, proteins, phospholipids, hormones, and transmitters. The methylation reactions result in the formation of S-adenosylhomocysteine, which can be hydrolyzed into homocysteine. Support for the hypothesis that Boron

bioactivity is through an effect on S-adenosylmethionine formation and/or utilization are the findings that plasma homocysteine increased and liver S-adenosylmethionine decreased in rats fed (0.05-0.15 mg/kg) Boron compared to rats supplemented with (3 mg/kg) diet [75]. High circulating homocysteine and depleted S-adenosylmethionine have been implicated in many of the disorders that can be affected by nutritional intakes of Boron, including arthritis, osteoporosis, cancer, diabetes, and impaired brain function.

Further support for the hypothesis is that the bacterial quorum sensing signal molecule, autoinducer-2, is a furanosyl borate ester synthesized from S-adenosylmethionine [76]. Quorum sensing is the cell-to-cell communication between bacteria accomplished through the exchange of extracellular signaling molecules (auto-inducers). Moreover, Boron strongly binds the oxidized form of nicotinamide adenine dinucleotide (NAD^+) [73], and thus might influence reactions in which it is involved. One role of extracellular NAD^+ is to bind to the plasma membrane receptor CD38, an adenosine diphosphate ribosyl cyclase that converts NAD^+ to cyclic ADP ribose. Cyclic ADP ribose is released intracellularly and binds to the ryandodine receptor, which induces the release of calcium ions from the endoplasmic reticulum. Cell culture studies show that Boron binds to and is a reversible inhibitor of cyclic ADP ribose [77,78]. Concentrations of Boron found in blood are found to decrease Ca^{2+} release from ryandodine receptor-sensitive stores [78]. Thus, it has been hypothesized that Boron is bioactive through binding NAD^+ and/or cyclic ADP ribose and inhibiting the release of Ca^{2+}, which is a signal ion for many processes affected by Boron, including insulin release, bone formation, immune response, and brain function.

Studies with plants have resulted in another suggested plausible mechanism of action for Boron bioactivity. Boron might be bioactive through forming diester borate complexes with phosphoinositides, glycoproteins, and glycolipids in cellular membranes. Diester borate polyl complexes might act as calcium chelators and/or redox modifiers [79] that affect membrane integrity and function [80]. This modifying effect could alter the transduction of regulatory or signaling ions across membranes. Determination of such an effect in animals and humans has yet to be determined. However, the finding that the borate transporter NaBC1, which apparently is essential for Boron homeostasis in animal cells, conducts Na^+ and OH^- across cell membranes in the absence of Boron [81], supports the suggestion that Boron deprivation might affect the transduction of regulatory and signaling ions across cell membranes.

1.4.5 Boron and the Inflammatory or Immune Response

Several laboratories have found that Boron status affects the response to injury or infection. Among the findings is that of Boron status affecting the response to the injected antigens. When injected with an antigen (*M. butyricum* in mineral oil) to induce arthritis, Boron-supplemented (2.0 mg/kg diet) rats had less swelling of the paws and lower circulating concentrations of natural killer cells and $CD8a^+/CD4^-$ cells than did Boron-deficient (0.1 mg/kg diet) rats [57]. Another study found that Boron supplementation (20 mg/kg diet) of a Boron-low (0.2 mg/kg) diet significantly delayed the onset of adjuvant-induced (*M. tuberculosis*) arthritis in rats [57]. Pigs fed a Boron-low (1-2 mg/kg) diet for 95 days exhibited a significantly higher skinfold thickness response to an intradermal injection of phytohemagglutinin than pigs supplemented with Boron (5 mg/kg diet) [82]. Physiological amounts of Boron (3 mg/kg) supplemented to a Boron-low diet (0.2 mg/kg) more than doubled the

serum total antibody concentrations to injected antigen (human typhoid vaccine) in rats [83].

The suggestion that Boron may have a regulatory role in the inflammatory or immune response is supported by a study of mice infected with the nematode *H. bakeri* [84]. Boron deprivation down-regulated 30 of 31 cytokines or chemokines associated with the inflammatory response six days post-primary-infection. An opposite pattern was found, especially 21 days post-challenge; mice consuming low and marginal Boron-deficient diets had >100% increases in 23 of 31 cytokines determined. This finding is consistent with lower serum TNF-α and INF-γ after lipopolysaccharide injection in pigs fed a marginal Boron-deficient diet than in pigs supplemented with a 5 mg Boron/kg diet [85]. Boron also affects changes in immune cell populations induced by other dietary factors, which include dietary fatty acids. Supplementation of young healthy men with 6 g/day of the n-3 polyunsaturated fatty acid docosahexaenoic acid for 12 weeks decreased the number of white blood cells, mainly because of a decreased granulocyte number; the decreased granulocyte number resulted in an increased percentage of lymphocytes in the white blood cells [86]. In contrast, 1.5 g of the n-6 polyunsaturated fatty acid increased granulocyte numbers [87]. Compared with safflower oil (mostly n-6 polyunsaturated fatty acids), fish oil (high in n-3 polyunsaturated fatty acids) increased white blood cell numbers, with most of the increase in the lymphocyte fraction, in Boron-adequate (3 mg/kg diet) rats but not in Boron-deprived (0.1 mg/kg diet) rats [88]. Fish oil instead of safflower oil increased monocyte and basophil numbers in Boron-deprived but not in Boron-adequate rats. Similarly, canola oil (high in n-3 fatty acids) increased the percentages of white blood cells that were basophils and monocytes in Boron-deprived rats,

but not in Boron-adequate rats [89]. An effect on the inflammatory response might be the reason that Boron was found beneficial in a study of 20 patients with radiographically confirmed osteoarthritis consuming daily either a 6 mg Boron supplement or a placebo for 8 weeks in a double-blind trial [32]. The Boron-supplemented arthritic individuals self-reported substantial improvement in subjective measures of joint swelling, restricted movement, and fewer analgesics for pain relief. Affecting the immune response might be the reason that Boron intake has been associated with some cancers, for example breast cancer.

1.5 Aim of the Study

This study was designed to consider:

1. A key role player is Boron, and the aim of using it is to assess the anti-inflammatory effect either therapeutically or as a pre-treatment.
2. A strong dose and route relationship alone and/or in adjuvant with another anti-inflammatory agent in rat models which is fairly close to human in their physiologic, metabolic and anatomic body design, to give a clue on the effectiveness of Boron regarding their use as a future protective and therapeutic drug.

Chapter two

Materials and methods

Chapter Two

Materials and Methods

2.1 Materials

The specific chemicals, drugs and kits, which are used in the present study, are listed in table 2-1 with their manufacturers, while instruments used are listed in table 2-2.

Table 2-1: Chemicals, Reagents and their Producers

No.	Names	Producers
1	Disodium Tetrahydroborate	Riedel-deHaenag, Hannover, Germany
2	Dexamethasone Ampule	TAD, Germany
3	Rat Interleukin-1β (IL-1β) ELISA Kit	YH Bioresearch laboratory, Shanghai, China
4	Rat Tumor Necrosis Factor-α (TNF-α) ELISA kit	YH Bioresearch laboratory, Shanghai, China
5	Rat High Sensitivity C-Reactive Protein (hs-CRP) ELISA kit	YH Bioresearch laboratory, Shanghai, China
6	Formaldehyde	Merck. Germany
7	Diethyl Ether	SDFCL, Industrial Estate. Mumbai, India
8	Cotton-Wool	Sepa Co. Ltd, Turkey
9	D.W	Local production by Daihan Labtech distiller
10	Coulter Apparatus Reagents	ABXhoriba, UK

Table 2-2: Instruments and their Producers

No.	Equipment	Producer
1	MicroELISA Plate Reader	BioTek, USA
2	Centrifuge	Heraeus Labofuge 200, Germany
3	Incubator	INCD 2, memmert, Germany
4	Autoclave	LabTech, Korea
5	Water Stills/ Distiller	Daihan Labtech, India
6	Ultra Low-Temperature Freezer(-65 C)	SANYO, Japan
7	Refrigerator (-20)	Konka, China
8	Micropipettes (5-50 µl, 20-200 µl, 100-1000 µl)	Transferpette, brand, Germany
9	Sensitive Balance	MonoBloc inside/ METTLER TOLEDO, USA
10	Weight Measurement Balance	Turkey
11	Digital Vernier Caliper	China for CE Marketing
12	Petri Dish	Jordon
13	Forceps	Heyinovo, China
14	Glass Cylinder	Kartell, Italy
15	Beakers	Marienfeld , Germany
16	Automated Coulter Machine	Beckman, USA
17	Oral Gavage Needle	UK

2.2 Experimental Animals

Wistar rats weighing 150-300 g of both sexes aged 11-12 weeks were brought from the animal house of the College of medicine/Hawler Medical University in February 2014. They were housed in the animal house, School of Pharmacy, Faculty of Medical Sciences, University of Sulaimani in well ventilated plastic cages, maintained on normal conditions of temperature, humidity and light/dark cycle (at an ambient temperature 25±2°C and humidity of 55±5% under 12 hr dark-light cycle), between the period of February to July 2014. They were fed standard pellet diet and had free access to water. The experimental protocol was approved by the Ethical Committee of the Faculty of Medical Sciences, University of Sulaimani and the protocol comply with ethics and rules for laboratory animals [90].

2.3 Study Design

Sixty-six rats were used in the present study; the animals were randomly assigned to different groups as follows (Figure 2-1):

1. **Negative control group:** Six rats were assigned to this group, and treated with vehicle only without induction of inflammation.
2. **Positive control group:** Twelve rats were used, assigned into two different groups, each containing 6 rats, and treated with vehicle only and induced chronic and granulomatous inflammation.
3. **Boron supplemented group:** Twenty four rats were used, divided into four groups, each containing 6 rats; they were treated with two different doses of Boron (3 and 6 mg/kg BW) orally, for the study of the anti-inflammatory activity of Boron in rat model of formaldehyde-induced chronic inflammation and cotton pellet-induced granuloma.

4. **Dexamethasone treated group:** Twelve rats were used, divided into two groups, each containing 6 rats; they were treated with Dexamethasone (1 mg/kg BW) orally, for the study of the anti-inflammatory activity of Dexamethasone (as the standard anti-inflammatory agent) in rat model of formaldehyde-induced chronic inflammation and cotton pellet-induced granuloma.
5. **Dexamethasone-Boron group:** Twelve rats were used, assigned into two groups, each containing 6 rats; they were treated with combination of Boron (3 mg/kg BW) and Dexamethasone (1 mg/kg BW) orally, for the study of anti-inflammatory activity of both agents in rat model of formaldehyde-induced chronic inflammation and cotton pellet-induced granuloma.

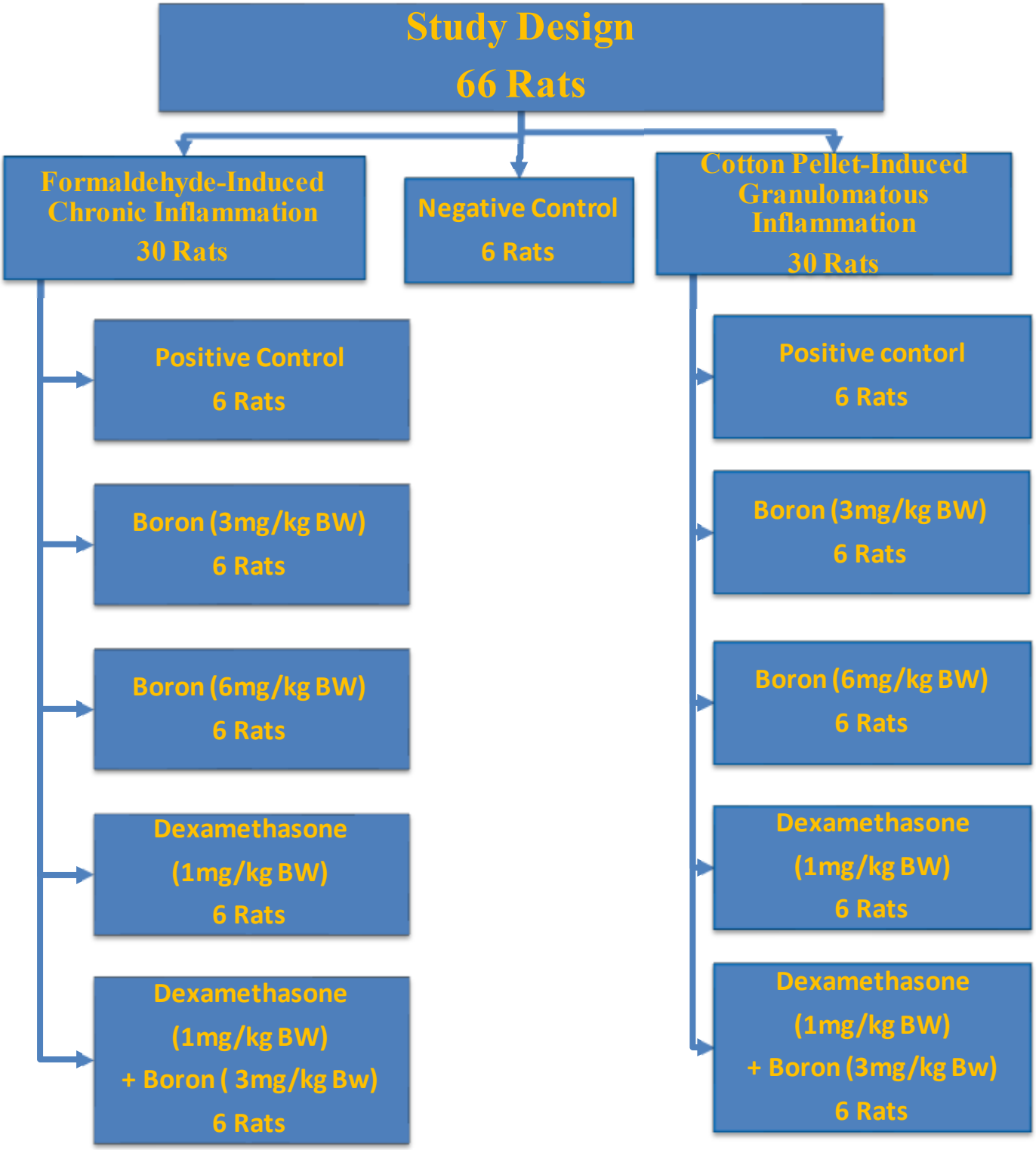

Figure 2-1: Study Design

2.4 Methods

2.4.1 Preparation of Boron Solution

Disodium tetrahydroborate powder that used in this study was dissolved in distilled water to produce a solution with a concentration of 25 mg/ml (for preparing the dose of 6 mg Boron/kg BW) and 12.5 mg/ml (for preparing the dose of 3 mg Boron/kg BW).

2.4.2 Body Weight Measurement of Rats

Measurement of body weight of each rat was done at the first day of the experiment for each group; at day seven before induction of inflammation, and at day fourteen. Weight was measured by weight measurement balance.

2.4.3 Study of the Effects of Boron in Rat Model of Formaldehyde-Induced Chronic Inflammation

The effects of Boron in chronic inflammation were evaluated utilizing formaldehyde-induced paw edema [91,92]. In this model, chronic inflammation was induced by injecting 0.1 ml of 2% formaldehyde subcutaneously in the planter region of the right hind paw of ether-anaesthetized rat at day seven. The test drug, both doses of Boron (3 and 6 mg/kg BW); the standard drug, Dexamethasone (1 mg/kg BW), and the vehicle distilled water (0.2 ml/100gm BW), were given 30 minutes prior to formaldehyde injection and continued for seven consecutive days.

Both doses of Boron and the vehicle were given as once daily oral doses. Boron was given for fourteen consecutive days; whereas Dexamethasone was given at the day of inducing inflammation, while before that they were given distilled water.

In this model, the increase in paw thickness (edema) was measured by the vernier caliper method. The paw thickness was measured before starting administration of drugs (first day), at day seven before induction

of inflammation, and at day fourteen, and presented as the mean increase in paw thickness (mm) [92,93]. The ability of the administered drugs to suppress paw inflammation was expressed as a percentage of inhibition of paw edema and this percentage can be calculated according to the following equation [94]:

$$\text{Percentage of inhibition } (\%) = (C - T)/C \times 100$$

Where C= mean increase in paw thickness of control group of rats and, T= mean increase in paw thickness of treated group of rats.

2.4.4 Study of the Effects of Boron in Rat Model of Cotton Pellet-Induced Granulomatous Chronic Inflammation

The cotton pellet-induced granuloma in rats was evaluated using the method of Winter and Porter [95]. The cotton pellets weighing 10±1 mg were sterilized in an autoclave for 30 minutes at 120°C under 15 Ib pressure. Four pellets were implanted subcutaneously (s.c.) into the ventral region, two in each side (left and right), in each rat under light ether anesthesia [96]. Boron (3 and 6 mg/kg BW), the standard drug Dexamethasone (1 mg/kg Bw), and the vehicle distilled water (0.2 ml/100 gm Bw), were given orally for seven consecutive days from the day of cotton pellet implantation. On the 8th day the animals were anaesthetized and the pellets together with the granuloma tissues were carefully removed and made free from extraneous tissues [34]. Figures 2-2 and 2-3 show pictures demonstrating the procedure of granuloma induction in this model.

Both drugs and the vehicle were given as once daily oral doses. Boron was given for fourteen consecutive days, whereas Dexamethasone was given at the day of implanting cotton pellet, while before that they were given distilled water. The wet pellets were weighed for the determination of wet weight, and then dried in an incubator at 60°C for eighteen hours until a constant weight was obtained (all the exudates dried), then the dried pellets were weighed again [96,97].

The exudate amount (weight of exudate in mg) was calculated by subtracting the constant dry weight of pellet from the immediate wet weight of pellet. The granulation tissue formation (dry weight of granuloma) was calculated after deducting the weight of cotton pellet (10 mg) from the constant dry weight of pellet and taken as a measure of granuloma tissue formation [96,98].

The percent inhibitions of exudate and granuloma tissue formation were determined as follows [98]:

$$\text{Exudate inhibition } (\%) = \left(1 - \frac{\text{Weight of Exudate in mg of treated group of rats}}{\text{Weight of Exudate in mg of control group of rats}}\right) \times 100$$

$$\text{Granuloma inhibition } (\%) = \left(1 - \frac{\text{Weight of granuloma in mg of treated group of rats}}{\text{Weight of granuloma in mg of control group of rats}}\right) \times 100$$

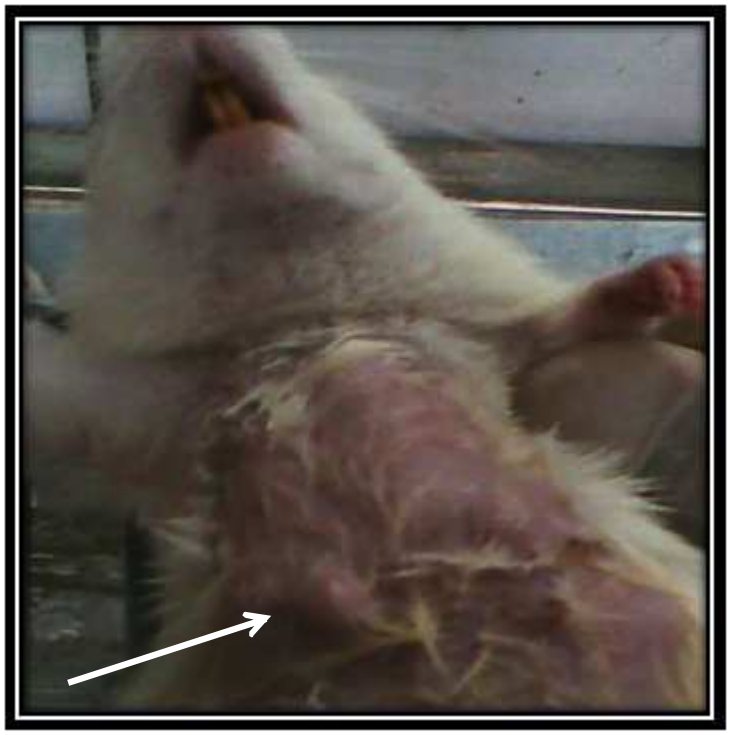

Figure 2-2: Granuloma formation in rats by implanting cotton pellet.

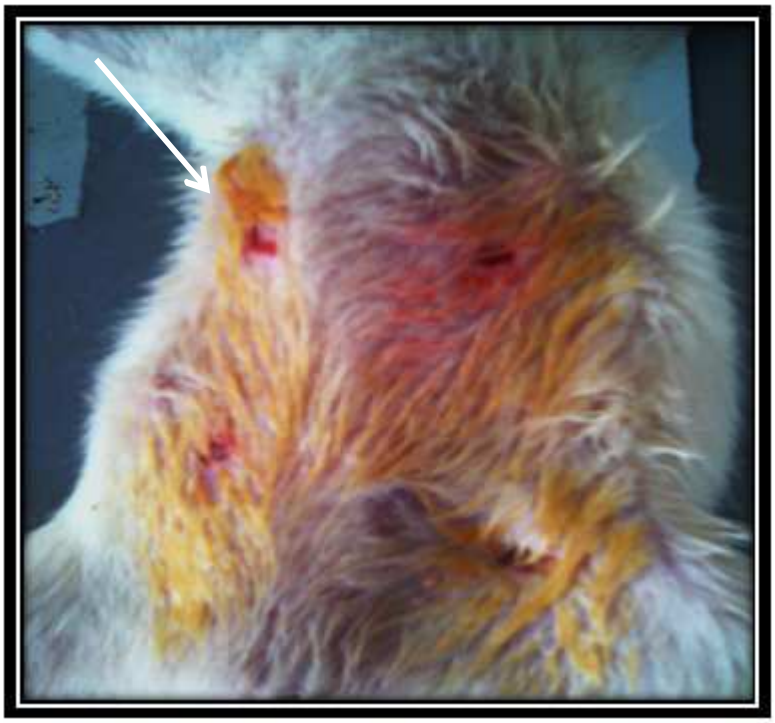

Figure 2-3: Taking off cotton pellet- induced granuloma in rats.

2.4.5 Blood Sample Collection

Approximately five milliliters of heart blood was drawn from each rat using disposable syringes. About 2 milliliter of the blood were collected in EDTA containing tubes and sent directly to the private laboratory for CBC analysis. The remaining milliliters of blood were put into plain tubes and allowed to clot for 20 minutes at room temperature. Serum was separated by centrifugation at 3000 rpm for approximately 20 minutes then stored at -20°C until assayed.

2.4.6 Measurement of Biochemical Markers

2.4.6.1 Tumor Necrosis Factor-α (TNF-α) Test

2.4.6.1.1 Principle of the Test

The rat Tumor necrosis factor-α (TNF-α) test uses enzyme-linked immune sorbent assay (ELISA) based on biotin double antibody sandwich technology to assay Rat Tumor necrosis factor-α. TNF-α is added to each well that are pre-coated with TNF-α monoclonal antibody and incubated. After incubation, anti TNF-α antibodies labeled with biotin are added to the unit with streptavidin-HRP, which forms the immune complex. Then unbound enzymes after incubation are removed by washing, after wards the plate is drained and substrate A and B are added to each well. The solution turns to blue and changes to yellow after the addition of the stop solution. The shades of the solution and the concentration of rat tumor necrosis factor-α (TNF-α) are positively correlated.

2.4.6.1.2 Reagents

1. Tumor necrosis factor- α monoclonal antibody coated ELISA plate.
2. Anti TNF-α antibodies labeled with biotin 1ml*1 tube.
3. Streptavidin-HRP (horseradish peroxidase) 6ml*1 tube.
4. Chromogenic reagent A 6ml*1 tube.
5. Chromogenic reagent B 6ml*1 tube.
6. Washing concentrate (20ml*30)*1 tube.
7. Standard solution (1280ng/ml). 0.5ml*1 tube.
8. Standard dilution 3ml*1 tube.
9. Stop solution 6ml*1 tube.

2.4.6.1.3 Assay Procedure

Table 2-3: Assay Procedure for Measuring TNF-α Test

Specimen	Blank	Standard	Assay
Serum			40microliter
Standard		50microliter	
Streptavidin-HRP	50microliter	50microliter	50microliter
Anti TNF-α Antibody	10microliter		10microliter
The plate was covered with seal membrane, mixed by shaking it gently and incubated for 60 minute at 37 °C, then washed with washing solution			
Chromogen Reagent A	50microliter	50microliter	50microliter
Chromogen Reagent B	50microliter	50microliter	50microliter
Both reagents were added, mixed and incubated for 10 minute at 37 °C for color development (blue)			
Stop Solution	50microliter	50microliter	50microliter
The absorbance measured at wavelength of 450 nm			

2.4.6.2 Interleukin - 1β (IL-1 β) Test

2.4.6.2.1 Principle of the Test

The rat Interleukin-1β (IL-1β) test uses enzyme-linked immune sorbent assay (ELISA) based on biotin double antibody sandwich technology to assay Rat Interleukin-1β. IL-1β is added to each well that are pre-coated with IL-1β monoclonal antibody and then incubated. After incubation, anti-IL-1β antibodies labeled with biotin are added to the unit with streptavidin-HRP, which forms the immune complex. Then unbound enzymes after incubation are removed by washing, after wards the plate is drained and substrate A and B are added to each well. The solution turns blue and changes to yellow after the addition of the stop solution. The shades of solution and the concentration of Rat Interleukin-1β (IL-1β) are positively correlated.

2.4.6.2.2 Reagents

1. Interleukin -1β monoclonal antibody coated ELISA plate.
2. Anti IL-1β antibodies labeled with biotin 1ml*1 tube.
3. Streptavidin-HRP (horseradish peroxidase) 6ml*1 tube.
4. Chromogenic reagent A 6ml*1 tube.
5. Chromogenic reagent B 6ml*1 tube.
6. Washing concentrate (20ml*30)*1 tube.
7. Standard solution (9600pg/ml). 0.5ml*1 tube.
8. Standard dilution 3ml*1 tube.
9. Stop solution 6ml*1 tube.

2.4.6.2.3 Assay Procedure

Table 2-4: Assay Procedure for Measuring IL-1β Test

Specimen	Blank	Standard	Assay
Serum			40 microliter
Standard		50 microliter	
Streptavidin-HRP	50 microliter	50 microliter	50 microliter
Anti IL-1β **Antibody**	10 microliter		10 microliter
The plate was covered with seal membrane, mixed by shaking it gently and incubated for 60 minute at 37 °C, then washed with washing solution			
Chromogen Reagent A	50microliter	50 microliter	50 microliter
Chromogen Reagent B	50 microliter	50 microliter	50 microliter
Both reagents were added, mixed and incubated for 10 minute at 37 °C for color development (blue)			
Stop Solution	50 microliter	50 microliter	50 microliter
The absorbance measured at wavelength of 450nm			

2.4.6.3 High Sensitivity C-Reactive Protein (hs-CRP) Test

2.4.6.3.1 Principle of the Test

The rat High sensitivity C-reactive protein (hs-CRP) test uses enzyme-linked immune sorbent assay (ELISA) based on biotin double antibody sandwich technology to assay Rat High sensitivity C-reactive protein. hs-CRP is added to each well that are pre-coated with hs-CRP monoclonal antibody and then incubated. After incubation, anti hs-CRP antibodies labeled with biotin are added to the unit with streptavidin-HRP, which forms the immune complex. Then unbound enzymes after incubation are removed by washing, after wards the plate is drained and substrate A and B are added to each well. The solution turns blue and changes to yellow after the addition of the stop solution. The shades of solution and the concentration of rat high sensitivity C-reactive protein (hs-CRP) are positively correlated.

2.4.6.3.2 Reagents

1. High sensitivity C - reactive protein monoclonal antibody coated ELISA plate.
2. Anti hs-CRP antibodies labeled with biotin 1ml*1 tube.
3. Streptavidin-HRP (horseradish peroxidase) 6ml*1 tube.
4. Chromogenic reagent A 6ml*1 tube.
5. Chromogenic reagent B 6ml*1 tube.
6. Washing concentrate (20ml*30)*1 tube.
7. Standard solution (2400ng/ml). 0.5ml*1 tube.
8. Standard dilution 3ml*1 tube.
9. Stop solution 6ml*1 tube.

2.4.6.3.3 Assay procedure

Table 2-5: Assay Procedure for Measuring hs-CRP Test

Specimen	Blank	Standard	Assay
Serum			40 microliter
Standard		50 microliter	
Streptavidin-HRP	50 microliter	50 microliter	50 microliter
Anti hs-CRP Antibody	10 microliter		10 microliter
The plate was covered with seal membrane, mixed by shaking it gently and incubated for 60 minute at 37 °C, then washed with washing solution			
Chromogen Reagent A	50 microliter	50 microliter	50 microliter
Chromogen Reagent B	50 microliter	50 microliter	50 microliter
Both reagents were added, mixed and incubated for 10 minute at 37 °C for color development (blue)			
Stop Solution	50 microliter	50 microliter	50microliter
The absorbance measured at wavelength of 450nm			

2.4.7 Measurement of White Blood Cells Using Coulter Method by Analyzing the Whole Blood Cells

2.4.7.1 Principle of CBC Analysis

As center for disease control stated, the Beckman Coulter method of sizing and counting particles uses measurable changes in electrical resistance produced by nonconductive particles suspended in an electrolyte. A suspension of blood cells passes through a small orifice simultaneously with an electric current. A small opening (aperture) between electrodes is the sensing zone through which suspended particles pass. In the sensing zone, each particle displaces its volume of electrolyte. Beckman Coulter measures the displaced volume as a voltage pulse, the height of each pulse being proportional to the volume of the particle. The quantity of suspension drawn through the aperture is for an exact reproducible volume. Beckman Coulter counts and sizes individual particles at a rate of several thousands per second. This method is independent of particle shape, color, and density.

2.4.8 Statistical Analysis

All the results are expressed as mean ± standard error of mean (SEM). The data is analyzed using GraphPad Prism 5.1 software (GraphPad Software Inc, San Diego, CA, USA). Paired *t*-test and one-way ANOVA followed by Bonferroni's *post hoc* test are utilized for the statistical evaluation of the differences between the means. *P* values<0.05 are considered statistically significant.

Chapter three

RESULTS

Chapter Three

Results

3.1 Effects of Different Doses of Boron Alone and in Adjuvant with Dexamethasone on Formaldehyde-Induced Chronic Inflammation in Rats

Injection of formaldehyde in rat's hind paw resulted in a significant increase of total WBC in the blood, and serum level of TNF-α, IL-1β, hsCRP and the diameter of paw thickness. This increase in the levels of the above parameters was attenuated by the 14 days pretreatment with orally supplemented Boron.

Table 3-1 shows that treatment with Boron significantly reduced the swelling of the paw ($P<0.05$) in dose-dependent patterns compared with controls, with the maximum effect produced by the 6 mg/kg BW of Boron (46.5%). Meanwhile, (1 mg/kg BW) Dexamethasone significantly inhibited the increase in paw thickness compared to controls (58.8%). Boron (3 mg/kg BW) in adjuvant with Dexamethasone (1 mg/kg BW) resulted in 66.3% inhibition in paw edema, which is significantly higher than the effects produced by Boron alone.

The two variances of the same groups as shown in figures 3-1 and 3-2 represent different presentations of rats paw thickness before and after injecting formaldehyde to induce chronic inflammation. It is obvious that pre- and post-treatment with Boron significantly depressed the increase in hind paw thickness of rats ($P<0.05$), in dose dependent pattern.

In figure 3-3, effects of different doses of Boron and in adjuvant with Dexamethasone on the percentage inhibition of edema in formaldehyde-induced inflammation was found to be significantly different. The maximum percentage of inhibition, using formaldehyde-induced paw edema, of Boron was achieved when Boron (3 mg/kg BW) was combined

with Dexamethasone (1 mg/kg BW); while when given alone, the percentage of inhibition was in dose-dependent pattern, as Boron (6 mg/kg BW) shows more percentage of inhibition than the dose (3 mg/kg BW).

Table 3-1: Effects of Different Doses of Boron Alone and in Adjuvant with Dexamethasone on Paw Thickness and Inhibition of Paw edema (%) in Formaldehyde-Induced Chronic Inflammation in Rats

Treatment Groups	Paw thickness (mm) zero time	Paw thickness (mm) after 7 days	Increase paw thickness (mm) after 7 days	Inhibition of edema (%)
Control (Distilled water)	4.46±0.13	7.67±0.21*	3.21 ± 0.16^{a}	---
Dexamethasone (1mg/kg BW)	4.37±0.10	5.69±0.08*	1.32 ± 0.16^{b}	58.8 ± 4.5^{a}
Boron (3mg/kg BW)	3.68±0.10	5.75±0.10*	2.07 ± 0.16^{c}	35.5 ± 5.6^{b}
Boron (6mg/kg Bw)	3.71±0.10	4.47±0.11*	$0.76\pm0.17^{b,c}$	$46.5\pm5.3^{a,b}$
Boron +Dexamethasone (3mg/kg +1mg/kg BW)	3.41±0.09	5.42±0.20*	$2.01\pm0.06^{b,d}$	$66.3\pm3.4^{a,c}$

Values are presented as mean±SEM; n=6 rats in each group; * significantly different compared with zero-time values ($P<0.05$) within the same group, using paired *t-test*; values with different superscripts (a,b,c,d) among different groups are significantly different ($P<0.05$), using ANOVA and *post hoc* test.

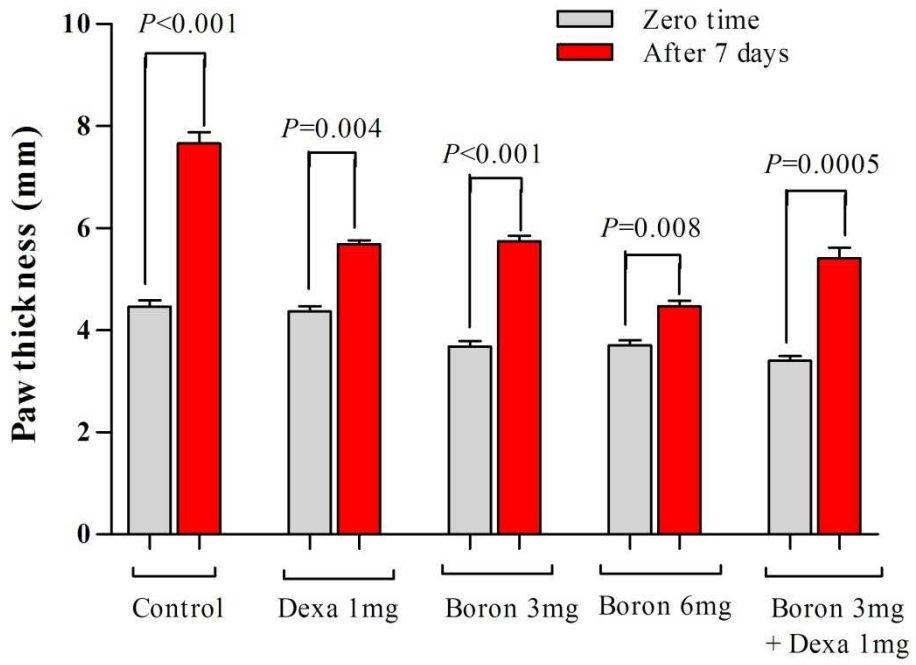

Figure 3-1: Effects of different doses of Boron, and in adjuvant with Dexamethasone on the edema formation in formaldehyde-induced chronic inflammation in rats; values are presented as mean±SEM; *n*= 6 rats in each group. *P*<0.05: significantly different compared with zero-time values within the same group using paired *t-test*.

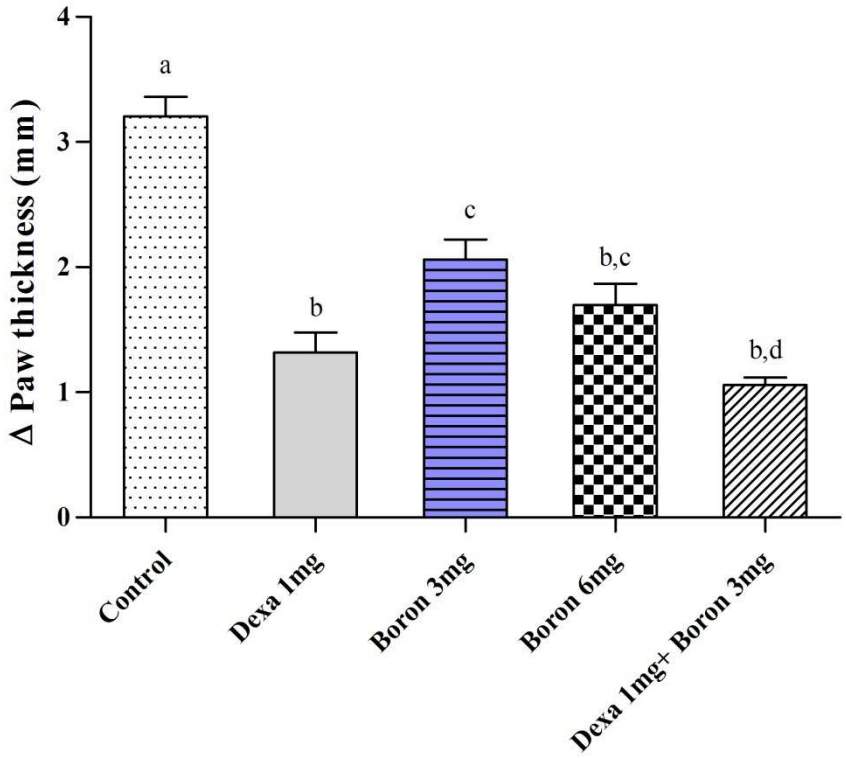

Figure 3-2: Effects of different doses of Boron, and in adjuvant with Dexamethasone on Δ paw thickness in formaldehyde-induced chronic inflammation in rats; values are presented as mean±SEM; *n*= 6 rats in each group. Values with different letters (a,b,c,d) among different groups are significantly different (*P*<0.05) using ANOVA and *post hoc* test.

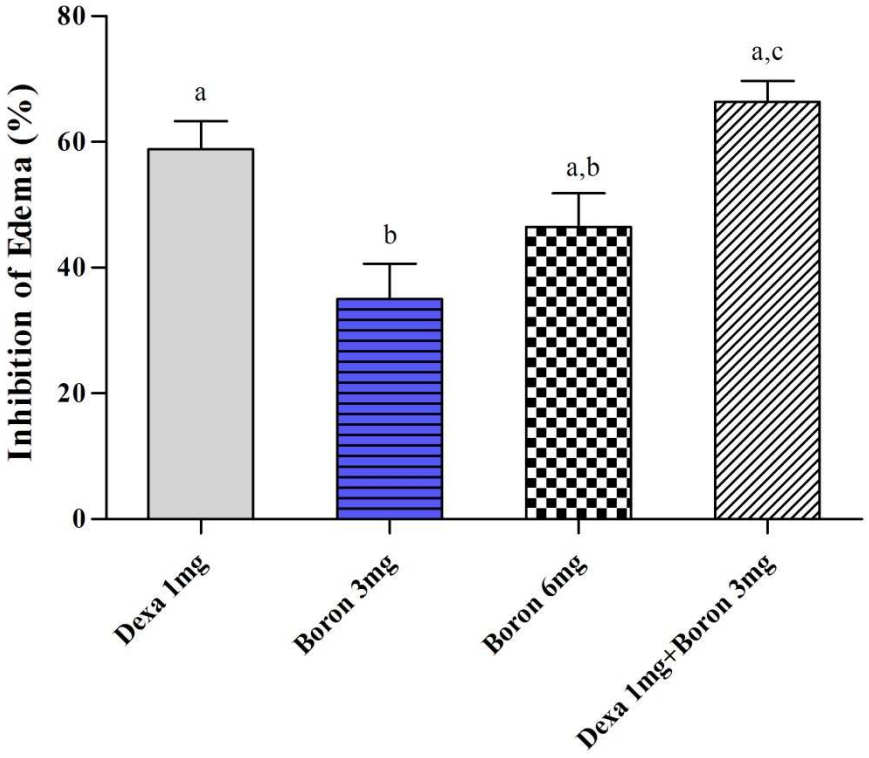

Figure 3-3: Effects of different doses of Boron, and in adjuvant with Dexamethasone on the percentage inhibition of edema in formaldehyde-induced chronic inflammation in rats; values are presented as mean±SEM; *n*= 6 rats in each group. Values with different letters (a,b,c) among different groups are significantly different (*P*<0.05) using ANOVA and *post hoc* test.

3.2 Effects of Different Doses of Boron, and in Adjuvant with Dexamethasone on Exudate Formation in Cotton Pellet-Induced Granuloma in Rats

The inhibitory activity of different doses of Boron and its adjuvant with the standard anti-inflammatory agent, Dexamethasone, on the exudate formation in cotton pellet-induced granuloma in rats is shown in table 3-2 and figures 3-4 and 3-5. The data presented in table 3-2 clearly shows that treatment with Boron alone significantly decreases the formation of inflammatory exudate, in a dose-dependent pattern, compared to controls; with the maximum percentage of inhibition produced by the dose (6 mg/kg BW) of Boron (23%). Meanwhile, administration of (1 mg/kg BW) of Dexamethasone significantly decreases the exudate formation compared to controls, reaching maximum effect of 31%.

Boron (3 mg/kg BW) in adjuvant with Dexamethasone (1 mg/kg BW) results in 34.3% decrease in exudate formation, which is significantly higher than the effects produced by different doses of Boron alone or Dexamethasone alone.

Table 3-2: Effects of Different Doses of Boron Alone, and in Adjuvant with Dexamethasone on Exudate Formation in Cotton Pellet-Induced Granuloma in Rats

Treatment Groups	Weight of Exudate (mg)	Inhibition of Exudate (%)
Control (Distilled water)	107.7± 5.6[a]	--
Dexamethasone (1mg/kg Bw)	74.8±4.4[b]	31.0±4.2[a]
Boron (3mg/kg Bw)	98.3±3.7[a]	9.3±5.4[b]
Boron (6mg/kg BW)	83.2±5.6[b]	23.0±3.9[a]
Boron+ Dexamethasone (3mg/kg+1mg/kg Bw)	69.0±3.2[b]	34.3±5.9[a]

Values are presented as mean±SEM; *n*=6 rats in each group; values with different superscripts (a,b) among different groups are significantly different ($P<0.05$), using ANOVA and *post hoc* test.

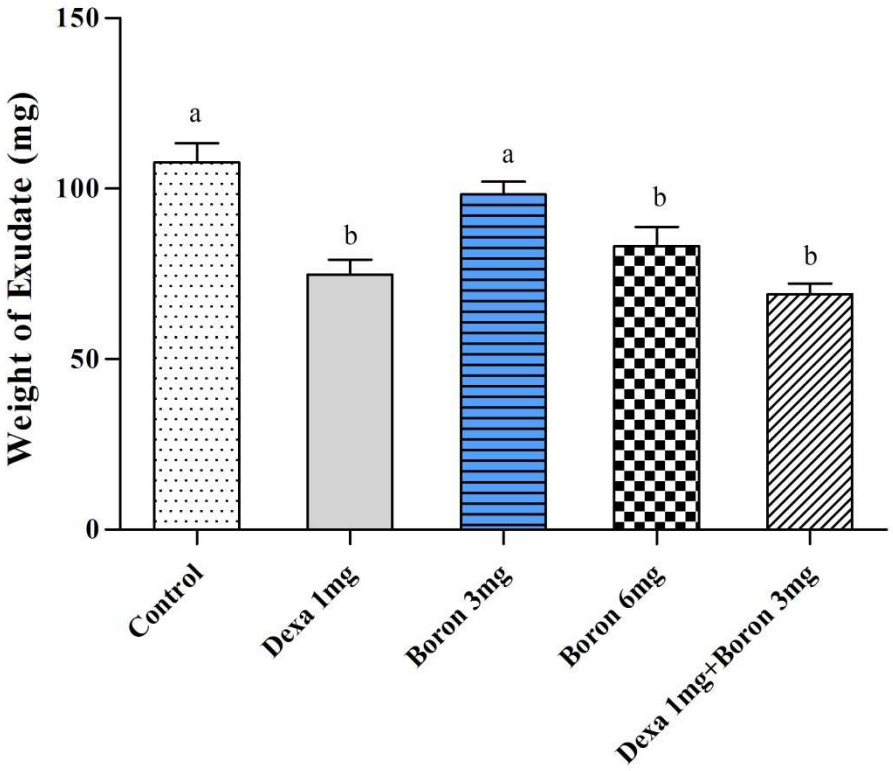

Figure 3-4: Effects of different doses of Boron, and in adjuvant with Dexamethasone on the exudate formation in cotton pellet-induced granuloma in rats; values are presented as mean±SEM; *n*= 6 rats in each group. Values with different letters (a,b) among different groups are significantly different (*P*<0.05) using ANOVA and *post hoc* test.

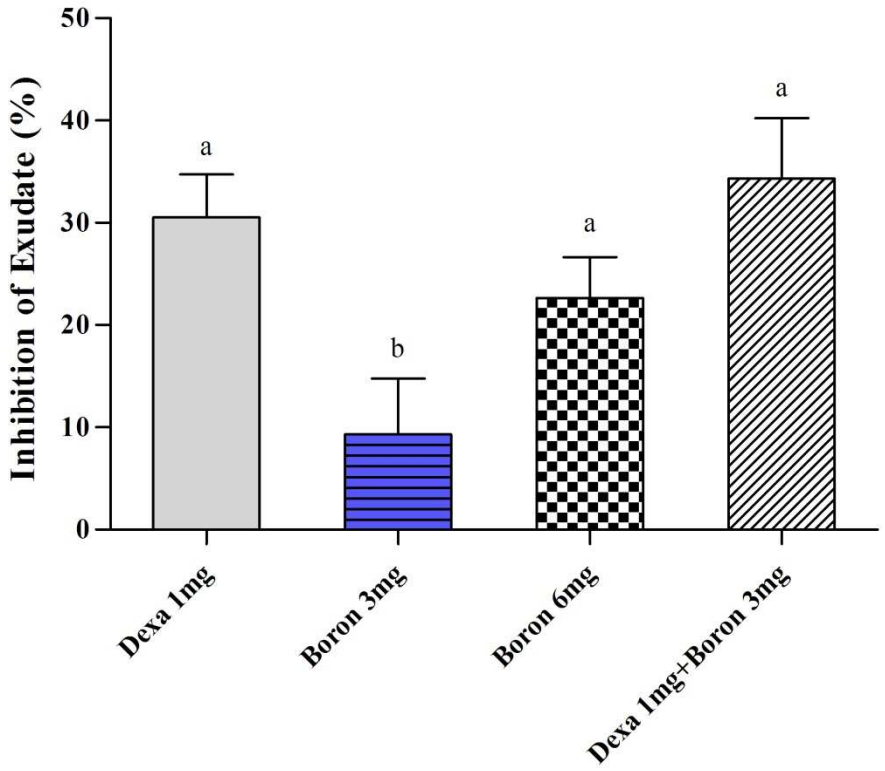

Figure 3-5: Effects of different doses of Boron, and in adjuvant with Dexamethasone on the percentage of exudate inhibition in cotton pellet-induced granuloma in rats; values are presented as mean±SEM; *n*= 6 rats in each group. Values with different letters (a,b) among different groups are significantly different (*P*<0.05) using ANOVA and *post hoc* test.

3.3 Effects of Different Doses of Boron, and in Adjuvant with Dexamethasone on Granuloma Formation in Cotton Pellet-Induced Granuloma in Rats

The inhibitory activity of different doses of Boron and its adjuvant with the standard anti-inflammatory agent, Dexamethasone, on granuloma formation in cotton pellet-induced granuloma in rats is shown in table 3-3 and figures 3-6 and 3-7.

The data presented in table 3-3 clearly shows that treatment with Boron significantly decreases the formation of inflammatory granuloma, in a dose-dependent pattern, compared with controls, with the maximum effect produced by Boron (6mg/kg BW) (36.5%). Meanwhile, (1 mg/kg BW) Dexamethasone attenuates significantly the formation of granuloma compared to controls (58%), as presented in figures 3-6 and 3-7. Boron (3 mg/kg BW) in adjuvant with Dexamethasone (1 mg/kg BW) results in 62% decrease in the formation of granuloma, which was significantly higher than the effects produced by the two doses of Boron when administered alone.

Table 3-3: Effects of Different Doses of Boron, and in Adjuvant with Dexamethasone on the Formation of Granuloma in Cotton Pellet-Induced Granuloma in Rats

Treatment Groups	Weight of Granuloma (mg)	Inhibition of Granuloma (%)
Control (Distilled water)	46.0± 4.1[a]	--
Dexamethasone (1mg/kg BW)	19.0± 1.1[b]	58.0±2.8[a]
Boron (3mg/kg Bw)	33.7± 2.6[c]	24.3±8.4 [b]
Boron (6mg/kg Bw)	28.3± 1.8[d]	36.5±5.7[c]
Boron+ Dexamethasone (3mg/kg+1mg/kg Bw)	16.8± 0.8[b]	62.1±3.4[a]

Values are presented as mean±SEM; n=6 rats in each group; values with different superscripts (a,b) among different groups are significantly different (P<0.05), using ANOVA and *post hoc* test.

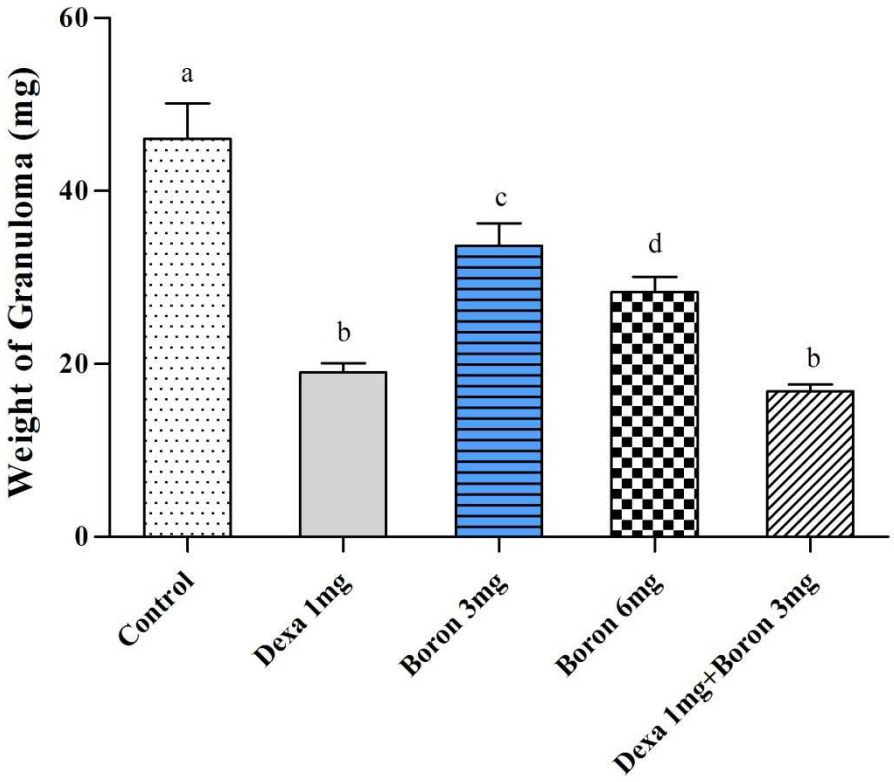

Figure 3-6: Effects of different doses of Boron, and in adjuvant with Dexamethasone on the formation of granuloma in cotton pellet-induced granuloma in rats. Values are presented as mean±SEM; *n*= 6 rats in each group; values with different letters (a,b,c,d) among different groups are significantly different (*P*<0.05) using ANOVA and *post hoc* test.

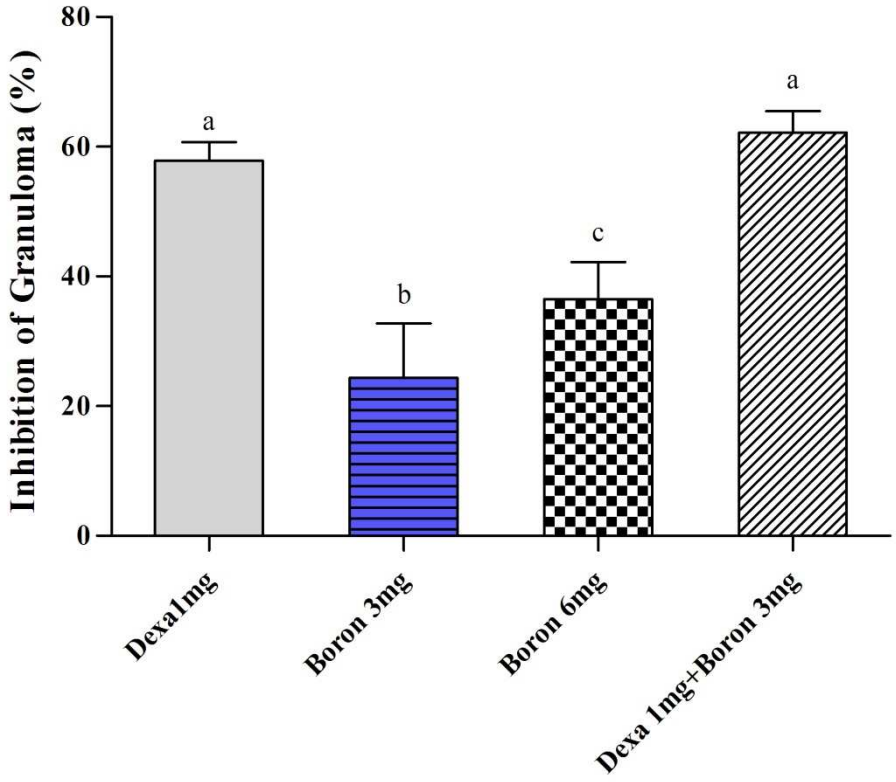

Figure 3-7: Effects of different doses of Boron, and in adjuvant with Dexamethasone on the percentage of granuloma inhibition in cotton pellet-induced granuloma in rats; values are presented as mean±SEM; *n*= 6 rats in each group. Values with different letters (a,b) among different groups are significantly different (*P*<0.05) using ANOVA and *post hoc* test.

3.4 Effects of Different Doses of Boron, and in Adjuvant with Dexamethasone on the Total WBC Count in Formaldehyde-Induced Chronic Inflammation in Rats

Figure 3-8 clearly shows that treatment with Boron alone has no significant effects on the total white blood cell count in formaldehyde-induced chronic inflammation. Meanwhile, the maximum reduction in total white blood cell count was reported due to the administration of Dexamethasone (1 mg/kg BW).

3.5 Effects of Different Doses of Boron, and in Adjuvant with Dexamethasone on the Total WBC Count in Cotton Pellet-Induced Granuloma in Rats

Figure 3-9 shows that administration of Boron (3 mg/kg BW) in adjuvant with the standard anti-inflammatory agent Dexamethasone (1 mg/kg BW) significantly reduced total white blood cell count in cotton pellet-induced granuloma. Meanwhile, the maximum reduction of total white blood cell count was produced by Dexamethasone (1 mg/kg BW).

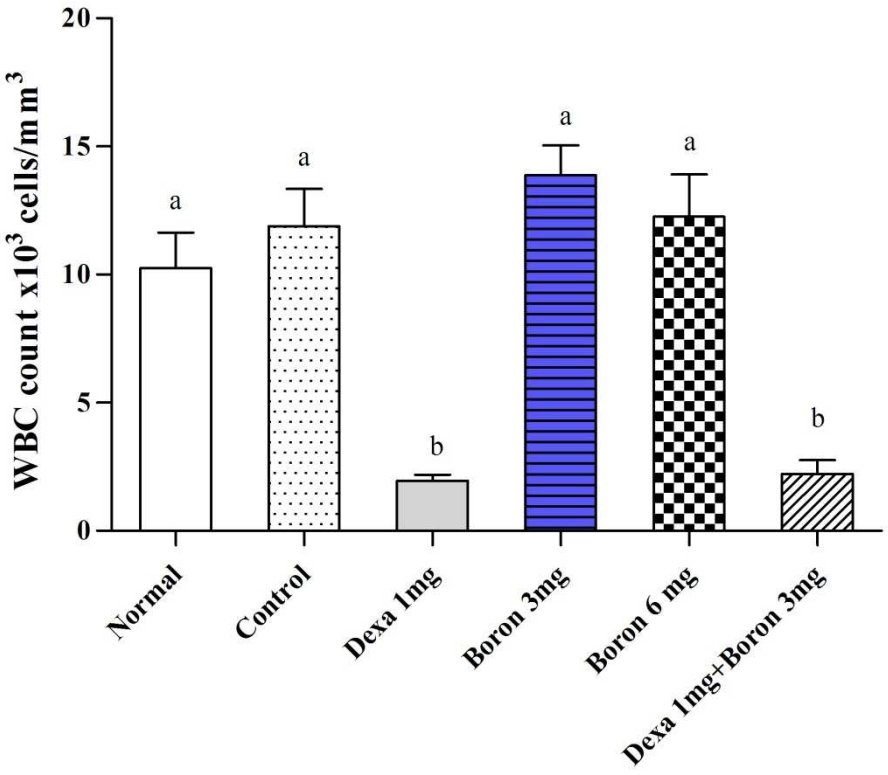

Figure 3-8: Effects of different doses of Boron, and in adjuvant with Dexamethasone on the total WBC count in formaldehyde-induced chronic inflammation in rats; values are presented as mean±SEM; *n*= 6 rats in each group. Values with different letters (a,b) among different groups are significantly different (*P*<0.05) using ANOVA and *post hoc* test.

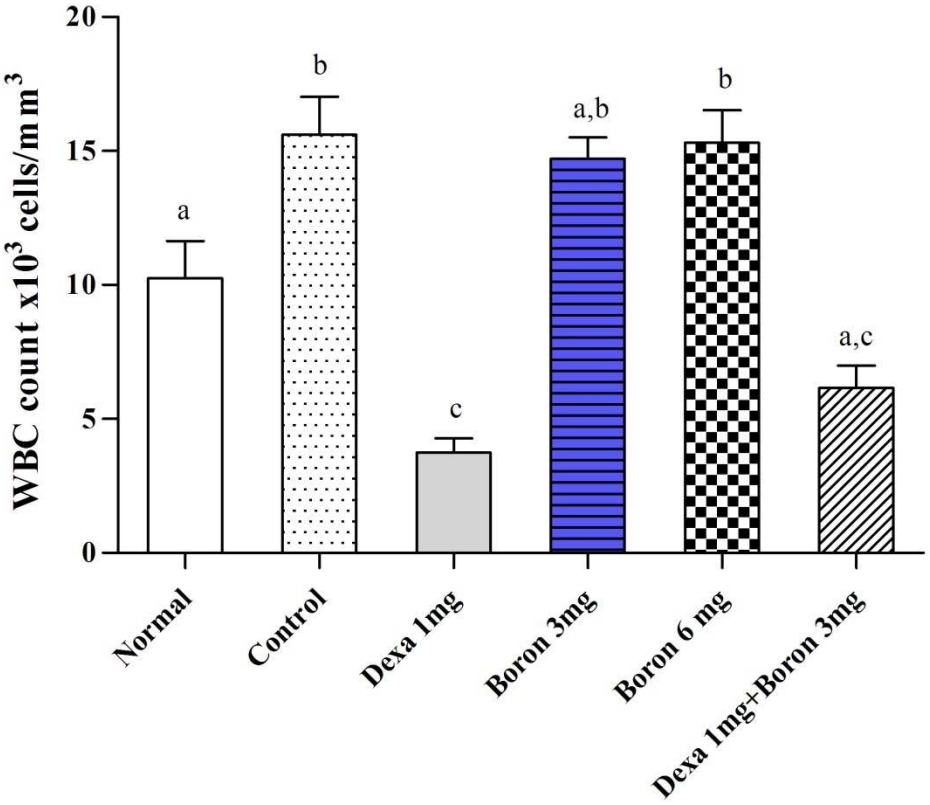

Figure 3-9: Effects of different doses of Boron, and in adjuvant with Dexamethasone on the total WBC count in cotton pellet-induced granuloma in rats; values are presented as mean±SEM; *n*= 6 rats in each group. Values with different letters (a,b,c) among different groups are significantly different (*P*<0.05) using ANOVA and *post hoc* test.

3.6 Effects of Boron (3 and 6 mg/kg BW), Dexamethasone (1 mg/kg BW) and their Adjuvant on the Serum Levels of TNF-α in Rat's Model of Formaldehyde-Induced Chronic Inflammation

The effects of treatment with different doses of Boron (3 and 6 mg/kg BW), Dexamethasone (1 mg/kg BW) and their adjuvant on the serum levels of TNF-α were analyzed after induction of chronic inflammation in rat's paw with formaldehyde. The results presented in figure 3-10 shows that the treatment with Boron (3 mg/kg BW) significantly reduced serum TNF-α level, compared with controls. Meanwhile, treatment with Boron (6 mg/kg BW) produces greater reduction in TNF-α level in challenged rats, which was nearly comparable to the effect of Dexamethasone (1mg/kg BW) alone. Moreover, the highest degree of serum TNF-α level suppression was achieved by co-administration of Boron (3 mg/kg BW) with Dexamethasone (1 mg/kg BW).

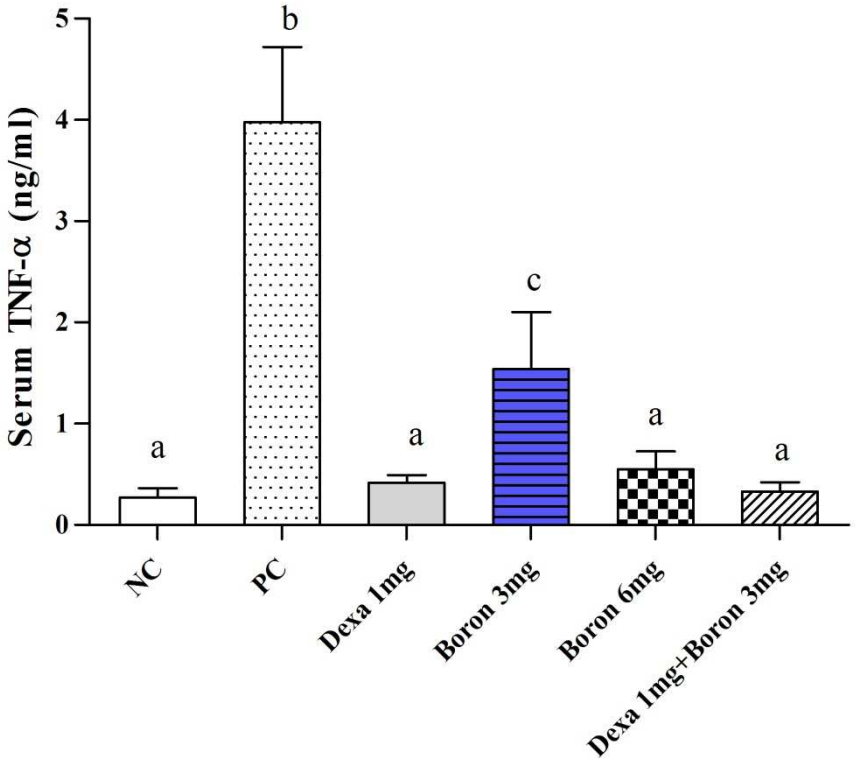

Figure 3-10: Effects of Boron (3 and 6 mg/kg BW), Dexamethasone (1 mg/kg BW) and their adjuvant, on the serum levels of TNF-α in rat's model of formaldehyde-induced chronic inflammation; number of rats= 6 in each group. Values with non-identical letters (a,b,c) are significantly different using ANOVA and *post hoc* test ($P<0.05$). NC: negative control. PC: positive control.

3.7 Effects of Boron (3 and 6 mg/kg BW), Dexamethasone (1 mg/kg BW) and their Adjuvant on the Serum Levels of IL-1β in Rat'S Model of Formaldehyde-Induced Chronic Inflammation

In figure 3-11, administration of different doses of Boron (3 and 6 mg/kg BW) significantly decreases serum IL-1β level after formaldehyde-induced inflammation in rats, compared with the positive control (PC) group ($P<0.05$). More specifically, the dose of Boron at (6 mg/kg BW) reduced the levels of serum IL-1β, which is approximately equivalent to that produced by Dexamethasone, when administered at a dose level of (1 mg/kg BW). Whereas, the highest degree of IL-1β level suppression was obtained by the co-administration of Boron (3 mg/kg BW) with Dexamethasone (1 mg/kg BW).

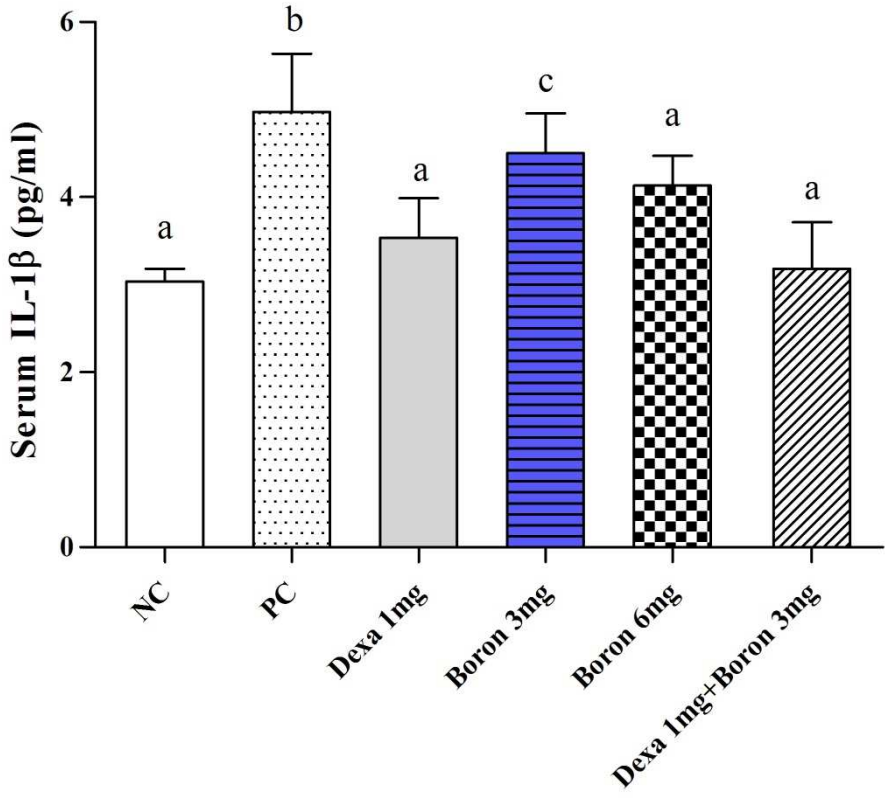

Figure 3-11: Effects of Boron (3 and 6 mg/kg BW), Dexamethasone (1 mg/kg BW) and their adjuvant on the serum levels of IL-1β in rat's model of formaldehyde-induced chronic inflammation; number of rats= 6 in each group. Values with non-identical letters (a,b,c) are significantly different using ANOVA and *post hoc* test (P<0.05). NC: negative control; PC: positive control.

3.8 Effects of Boron (3 and 6 mg/kg BW), Dexamethasone (1 mg/kg BW) and their Adjuvant on the Serum Levels of hsCRP in Rat's Model of formaldehyde-Induced Chronic Inflammation

The effects of treatment with different doses of Boron (3 and 6 mg/kg BW), Dexamethasone (1 mg/kg BW) and their adjuvant on the serum levels of hsCRP were analyzed after induction of chronic inflammation in rat's paw with formaldehyde. The results presented in figure 3-12 shows that the serum level of hsCRP was significantly elevated in the positive control group, compared with negative control. Meanwhile, treatment with Boron (6 mg/kg BW), Dexamethasone (1 mg/kg BW) and their adjuvant produces significant decrease in serum hsCRP level in challenged rats, with maximum effect produced by the Dexamethasone and its adjuvant with (3 mg/kg BW) of Boron, where both approaches showed comparable effects. In this regard, Boron alone at the dose level of (3 mg/kg BW) did not change serum hsCRP level significantly compared with PC group ($P>0.05$).

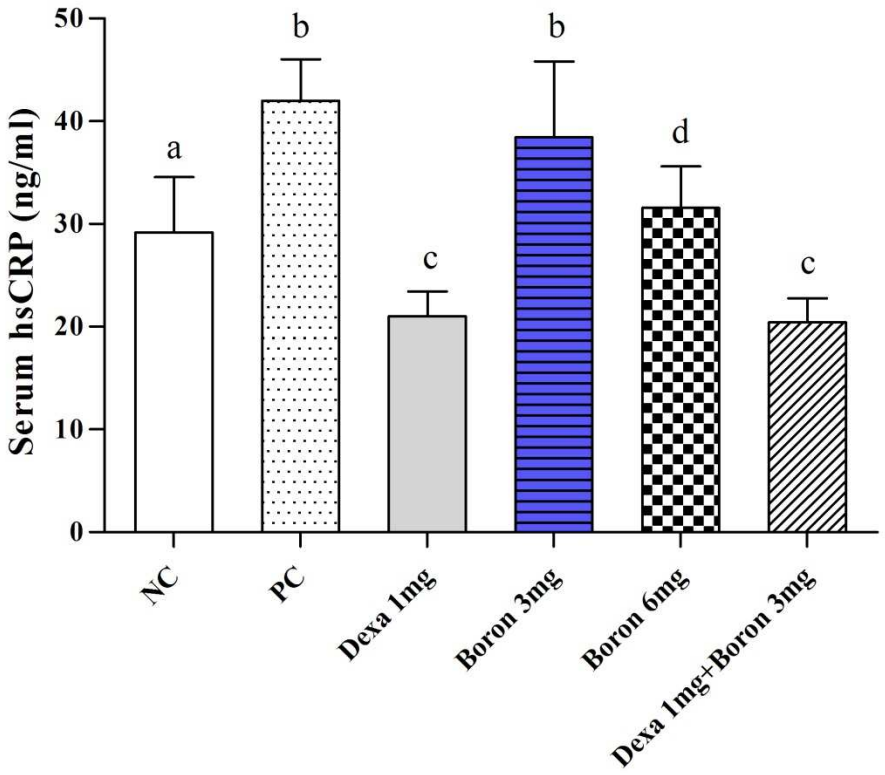

Figure 3.12: Effects of Boron (3 and 6 mg/kg BW), Dexamethasone (1 mg/kg BW) and their adjuvant on the serum levels of hsCRP in rat's model of formaldehyde-induced chronic inflammation; number of rats= 6 in each group. Values with non-identical letters (a,b,c,d) are significantly different using ANOVA and *post hoc* test (P<0.05). NC: negative control; PC: positive control.

3.9 Effects of Boron (3 and 6 mg/kg BW), Dexamethasone (1 mg/kg BW) and their Adjuvant on the Serum Levels of TNF-α in Rat's Model of Cotton Pellet-Induced Granulomatous Inflammation

As shown in figure 3-13, treatment with different doses of Boron (3 and 6 mg/kg BW) attenuates the production of TNF-α in the rat's model of cotton pellet-induced granuloma, which is significantly different compared with positive control group ($P<0.05$). The high dose of Boron (6 mg/kg BW) showed more obvious suppressing effect on TNF-α level, which indicates that the anti-inflammatory effect had positive correlation with the dose of Boron. The highest level of reduction in serum TNF-α was achieved by the co-administration of Dexamethasone (1 mg/kg BW) with Boron (3 mg/kg BW). Comparing the effects of Boron with the standard drug, the anti-inflammatory effect of Boron (6 mg/kg BW) was nearly equivalent to the effect of (1 mg/kg BW) Dexamethasone.

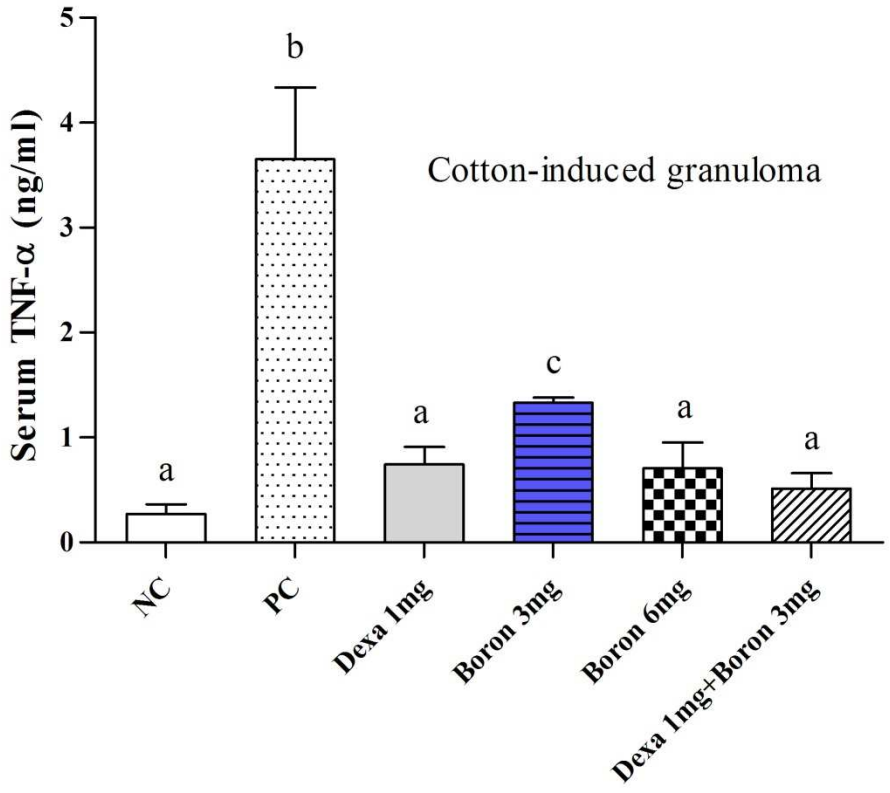

Figure 3-13: Effects of Boron (3 and 6 mg/kg BW), Dexamethasone (1 mg/kg BW) and their adjuvant on the serum levels of TNF-α in rat's model of cotton pellet-induced granulomatous inflammation; number of rats= 6 in each group. Values with non-identical letters (a,b,c) are significantly different using ANOVA and *post hoc* test ($P<0.05$). NC: negative control; PC: positive control.

3.10 Effects of Boron (3 and 6 mg/kg BW), Dexamethasone (1 mg/kg BW) and their Adjuvant on the Serum Levels of IL-1β in Rat's Model of Cotton Pellet-Induced Granulomatous Inflammation

In figure 3-14, implanting of cotton pellet into s.c pocket in the ventral region of the rats produced significant increase in the serum level of IL-1β, compared with negative control ($P<0.05$). However, the orally administered Boron (6 mg/kg BW) significantly reduced the level of IL-1β ($P<0.05$), compared with the positive control group, while the lower dose (3 mg/kg BW) did not show such effect. This effect of Boron (6 mg/kg BW) was comparable to that produced by Dexamethasone (1 mg/kg BW), though the dose of Boron (3mg/kg BW) has half potency of combination of Dexamethasone with Boron in reducing serum level of IL-1β. Meanwhile the co-administration of (3 mg/kg BW) Boron with the standard drug produced the greatest inhibition on the serum level of IL-1β.

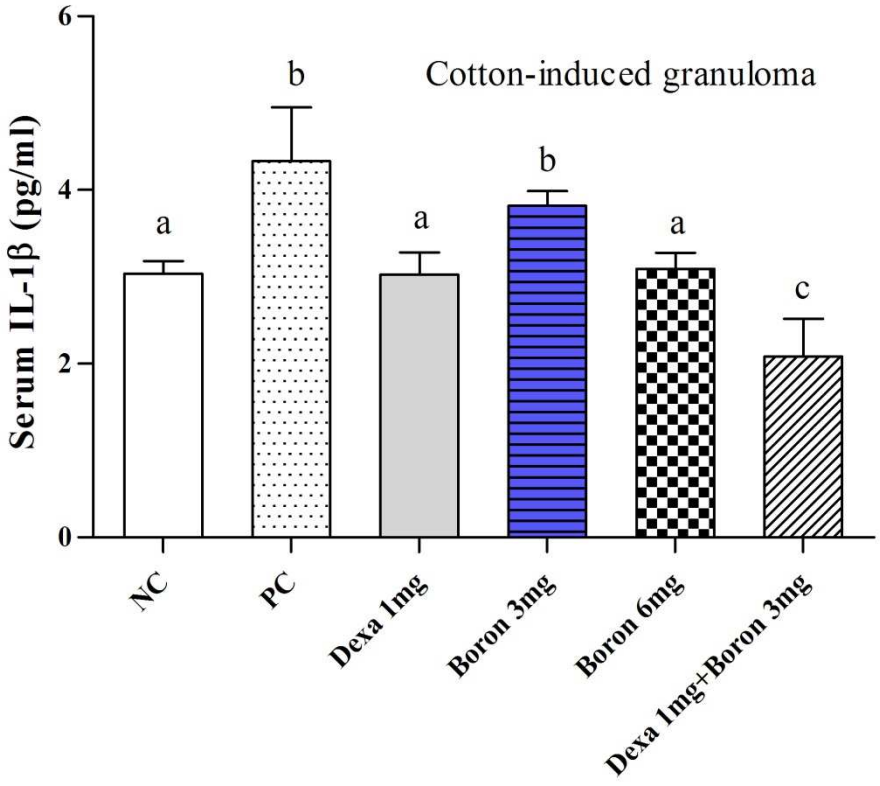

Figure 3-14: Effects of Boron (3 and 6 mg/kg BW), Dexamethasone (1 mg/kg BW) and their adjuvant on the serum levels of IL-1β in rat's model of cotton pellet-induced granulomatous inflammation; number of rats= 6 in each group. Values with non-identical letters (a,b,c) are significantly different using ANOVA and *post hoc* test (P<0.05). NC: negative control; PC: positive control.

3.11 Effects of Boron (3 and 6 mg/kg BW), Dexamethasone (1 mg/kg BW) and their Adjuvant on the Serum Levels of hsCRP in Rat's Model of Cotton Pellet-Induced Granulomatous Inflammation

Highly sensitive C-reactive protein (hsCRP), as a sensitive biochemical marker of inflammation than traditional CRP, showed significant increase in PC group compared with NC group in the rat's model of cotton pellet-induced granuloma as seen in figure 3-15. The administered doses of Boron (3 and 6 mg/kg BW) significantly attenuated the elevation in serum hsCRP level, compared with PC group. Moreover, this effect was not dose-dependent, where both doses produced comparable effects in this regard ($P>0.05$). Although the adjuvant of Boron (3 mg/kg BW) with Dexamethasone (1 mg/kg BW) produced the highest reduction in serum hsCRP level, it was not significantly different compared with other treatment approaches followed in the present study.

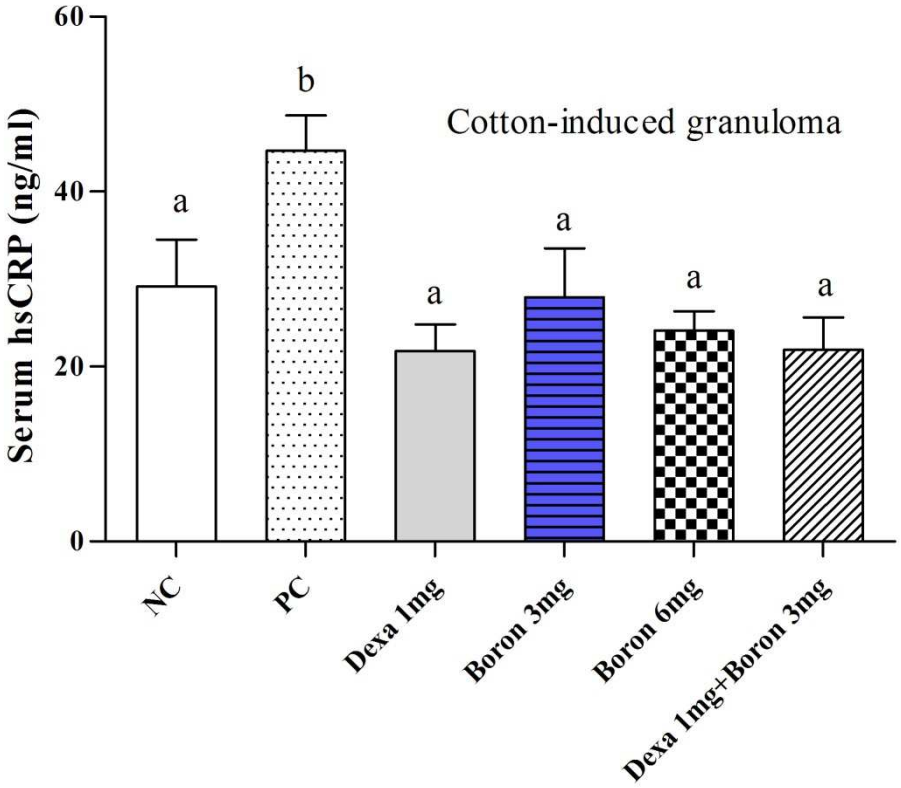

Figure 3-15: Effects of Boron (3 and 6 mg/kg BW), Dexamethasone (1 mg/kg BW) and their adjuvant on the serum levels of hsCRP in rat's model of cotton pellet-induced granulomatous inflammation; number of rats= 6 in each group. Values with non-identical letters (a,b) are significantly different using ANOVA and *post hoc* test (P<0.05). NC: negative control; PC: positive control.

Chapter four

DISCUSSION AND CONCLUSION

Chapter Four

Discussion and Conclusion

4.1 Discussion

Trace elements are essential nutrients for human being. Though often overlooked by medical providers, they play a significant role in human metabolism and can lead to serious complications when deficient or in excess. The possibility of preventing or decreasing inflammatory processes through the daily administration of trace element enriched food with therapeutic properties and low side effects is an attractive alternative to medical therapy [99]. The interest in Boron and Boron-containing organic complexes as possible therapeutic drugs for inflammatory disorders is not a new issue [100]. A class of Boron-containing antibacterial agents (borinic acid picolinate esters) was previously reported [101], and a related antibacterial agent was prepared, which has additional activity against pro-inflammatory cytokines [102]. Such adjuvant of activities is ideal for the treatment of topical infections with inflammatory consequences.

Moreover, topical therapeutic application of Boron-containing compounds has been one of the options [103], and two Boron-containing PDE4 inhibitors (AN2728 and AN2898) have been identified as anti-inflammatory agents undergoing clinical development for potential topical treatment of psoriasis and atopic dermatitis [104]. According to the available evidence about the inhibitory effect of Boron on chronic inflammation, this research evaluates its dose-dependent effect using experimental animal models of chronic inflammation. The study design is based on hypothesis that on administering Boron to the experimentally inflamed rats, the inflammatory biochemical markers are stymied.

As expected, Boron blunted chronic inflammation spike in inflamed rats. Subsequently, chronic inflammation attenuation by Boron was elevated by increasing the dose of Boron or when co-administered with Dexamethasone. However, in the light of our study, the two rat models used to induce chronic inflammation (formaldehyde-induced chronic inflammation and cotton pellet-induced granuloma) are widely accepted as sensitive and reliable phlogistic tools for investigating potential anti-inflammatory agents [4,105].

It is well known that inhibition of formaldehyde- induced paw edema in rats is one of the most suitable test procedures to screen anti-arthritic and anti-inflammatory agents, as it closely resembles human arthritis. Thus, formaldehyde-induced arthritis is a model used for the evaluation of an agent with anti-inflammatory activity; therefore, accordingly, we utilized this model to evaluate the dose-response relationship of Boron with expected anti-inflammatory activity [91,106].

Localized inflammation, induced by injection of formaldehyde subcutaneously into right hind paw of rats, is biphasic. It produces a painful response; the first phase represents response to direct stimulation of the nerve endings and increased release of mediators, such as substance P, bradykinin, and excitatory amino acids. The second phase represents a tonic response to subsequent inflammation, where histamine, prostaglandins (PGs), 5-HT, and bradykinin are known to be involved. This leads to the development of a local inflammatory reaction and progressive functional changes in the body system, with subsequent alterations at higher levels of the tissues and cells. Therefore, significant changes occurs by increasing the TNF-α mRNA level in the inflamed tissue of the rat hind paw [107-109].

In the present study, using formaldehyde-induced chronic inflammation in rats, different doses of Boron (3mg/kg and 6mg/kg Bw)

produced significant ($P<0.05$) anti-inflammatory activity compared to the control group; this anti-inflammatory activity increased by increasing the dose. The trace element Boron (3mg/kg BW) when adjunctly used with Dexamethasone (1mg/kg BW) seems to be the best approach to achieve the highest anti-inflammatory activity compared to the other approaches. This may be attributed to the effects of both agents on the same or alternative pathways through which they thought to produce their anti-inflammatory activity. Although the results of the present study are consistent with many other previously reported, the dose response relationship could be considered as a new insight in this regard.

Nielsen (2008) provides a comprehensive review of Boron in human health referencing the positive effects of Boron in human bone, brain, inflammation and hormone function, and clearly adding to the body of knowledge needed to confirm Boron as essential in human nutrition. However, essentiality hinges on knowing a defined biochemical role for Boron in addition to demonstrable signs of impaired functions in humans with Boron deficiencies [110]. Moreover, Newnham discussed the observed improvements in arthritic dogs treated with boric acid [111], and shed a light on data from human studies that suggest Boron a safe and effective treatment for some forms of arthritis. Formaldehyde-induces paw edema is a well-established rat model which has been extensively used in the evaluation of anti-inflammatory effects of various agents in preclinical research.

Moreover, inflammatory reactions induced by formaldehyde injection are similar to those reported during arthritis, and it is a standard model for the evaluation of therapeutic agents with suspected anti-proliferative and anti-arthritic activities [112,113]. In the present study, Boron significantly attenuated the increase in inflammatory reactions in this model of inflammation, and can be proposed that it may possess anti-proliferative

and anti-arthritic activities. The present study is in agreement with others, where pigs that consumed Boron-supplemented diets showed a decreased inflammatory response to an intradermal injection of phytohemagglutinin [82]. The mechanism behind the ability of Boron to reduce inflammation is unclear, though many ideas are suggested to explain such activity based on both experimental and clinical data. In this regard, Hunt and Idso (1999) reported that paw swelling is reduced in adjuvant-induced arthritic rats that received supplemental Boron [57], and hypothesized that Boron may decrease the inflammatory response, due to attenuating the production of pro-inflammatory cytokines by the monocyte/macrophage lineage.

Moreover, Boron may also lower the level of oxidative damage, which is accomplished by decreasing the production of NADPH and the activity of λ-glutamyl transpeptidase; this action could possibly increase the amount of glutathione (GSH) in the body [114], which plays a role in protecting cells from toxic oxygen radicals [115]. Boron's anti-inflammatory actions have been attributed to various mechanisms. These include suppression of serine proteases released by inflammation-activated white blood cells, inhibition of leukotriene synthesis, reduction of reactive oxygen species generated during neutrophil's respiratory burst, and suppression of T-cell activity and antibody concentrations [114].

Tissue injury induces a cascade of cellular reactions in the lesion area, accompanied with the release of pro-inflammatory cytokines, such as TNF-α, IL-1β, IL-6, IL-8 and other substances [116]; hydrogen peroxide can be then degraded by glutathione peroxidase. The activities of superoxide dismutase and glutathione peroxidase have been increased by Boron supplementation, and the mechanism whereby Boron affects activity of these enzymes is unknown [117]. Another possible explanation for the decrease in the inflammatory response in Boron-pretreated rats

might be related to the interference with the production of cytokines, specifically IL-1β and TNF-α, and the supplemented Boron may reduce the production of IL-1β and TNF-α from monocytes and macrophages [82].

Cotton pellet-induced granuloma is an animal model based on the foreign body granuloma, which is induced by subcutaneous implantation of sterilized compressed cotton pellets in rats, and has been accepted as a useful tool for investigating drugs suspected to have anti-inflammatory activity [118,119]. Preventing generation of collagen fibers and suppression of mucopolysaccharids are considered as indicators for the anti-proliferative effects of the anti-inflammatory agents, where monocytes infiltration and fibroblast proliferation are the major events in chronic inflammation instead of neutrophils infiltration and fluid exudation [120].

In the cotton pellet-induced granuloma model, after a short period of acute inflammation, proliferative cells develop and the inflammation becomes chronic. This model is an indication for the proliferative phase of inflammation; it involves monocyte-macrophages infiltration and proliferation of neutrophils and fibroblasts, which are the basic sources of granulation tissue. During this process, monocyte migration, liquid accumulation, apoptosis, damage and adjoining of multinucleated giant cells will occur in the surrounding tissue of the pellets, with consequent formation of granulation tissue that covers the pellets. Hence, the decrease in the weight of granuloma indicates that the proliferative phase is effectively attenuated by the tested compound (Boron) [120].

By using such model of chronic inflammation, the results showed that both doses of Boron (3 and 6 mg/kg BW) possesses marked and significant ($P<0.05$) anti-inflammatory activity against cotton pellet-induced granuloma in rats compared to controls. Boron, in a dose-

dependent pattern, showed significant ($P<0.05$) anti-inflammatory activity probably through reducing the formation of exudate and granuloma during the second phase of the inflammatory reaction. Although these results are clearly within the limitations of the utilized method, the previously reported data raise many doubts about the effect of Boron in this regard; they reported that dietary Boron supplementation increases production of cytokines following stress, which indicates a role for Boron in the immune system. However, these data do not explain the reduction in localized inflammation following an antigen challenge in pigs [85]. Such differences in the behavior of Boron may be attributed to the variation in the doses and methods of administration followed during the experiments.

Utilization of sensitive biochemical markers (including the serum level of the inflammatory mediators like (TNF-α, IL-1β, and hs-CRP) may give more evidence in this respect. Orally administered Boron (3 mg/kg BW) as adjuvant with the standard drug, Dexamethasone (1 mg/kg BW), decreases formation of granuloma, which was significantly higher than the effects produced by using each one of them alone. Again, these results support the thesis hypothesis that using a adjuvant of Boron with corticosteroids or non-steroidal anti-inflammatory drugs (NSAIDs) as adjuvant therapy for resistant cases of chronic inflammatory disorders like RA, may enable reducing the doses of corticosteroids or NSAIDs, and decrease the chance of side effects. Moreover, the correlation studies of the dose-dependent anti-inflammatory activity of Boron, as indicated by the evaluated markers (edema, exudate, and granuloma) reveals highly positive and significant relationships; these results clearly indicate the anti-inflammatory properties of the non-complexed Boron.

The cotton pellet -induced granuloma is widely used to evaluate the transudative and proliferative components of chronic inflammation. The

weight of the wet cotton pellets correlates with transudate, while the weight of dry pellet correlates with the amount of granulomatous tissue formation [119,121]. The non-steroidal anti-inflammatory drugs decrease the size of granulation tissue, which results from cellular reaction by inhibiting granulocyte infiltration, preventing generation of collagen fibers and suppressing mucopolysaccharids [119]. Accordingly, the efficacy of an anti-inflammatory agent in chronic inflammatory states is indicated by its ability to inhibit the increase in the number of fibroblasts, and synthesis of collagen and mucopolysaccharids during granuloma tissue formation. Therefore, orally supplemented Boron decreases granuloma formation by inhibiting the weight of the dry and wet cotton pellet in a dose dependent manner, which is very close to the inhibitory effect of Dexamethasone; this may indicate the suppression of the proliferative phase (synthesis of collagen by the fibroblasts) of the inflammatory events [121,122]. These results are in tune with the previous reports, which indicate that pigs consumed Boron-supplemented diets had a decreased inflammatory response to an intradermal injection of phytohemagglutinin [82]; however, the mechanism behind the ability of Boron to reduce inflammation is unclear.

The previous and currently presented data, which indicate decreased localized inflammatory response following Boron supplementation [123] cannot be explained by decreased cytokine production due to Boron supplementation in the current and previous studies [85,124]. Therefore, a mechanism other than decreased cytokine production by Boron might explain the decreased local tissue swelling following an intradermal injection of irritant substances. Hunt and Idso (1999) suggested that the reduced inflammation in rats that received Boron-supplemented diets might be explained by Boron-induced down-regulation of certain

enzymes involved in the respiratory burst cascade [57]. This would result in a decrease in the production of reactive oxygen species.

Dexamethasone, a synthetic glucocorticoid, inhibits expression of inflammatory mediators via macrophages and other cells, and is used in the treatment of immune-related inflammatory condition [125]. Dexamethasone modulate transcription of the gene expression via GC receptors, a member of nuclear superfamily hormone receptor [126], and interferes with the capability of NF-κB and AP1 to induce transcription of inflammatory mediators [127,128].

It has been reported that Boron is required for bone, mineral, lipid, and energy metabolisms, immune and endocrine functions, and the defense mechanisms against lipid peroxidation and DNA damage. Boron may act as a metabolic regulator in many enzymatic systems. However, biochemical functions of Boron are not fully understood. In the present study, serum TNF-α was significantly lowered by Boron supplementation alone (3mg/kg and 6mg/kg BW), and when used as adjuvant with Dexamethasone (3mg/kg BW Boron and 1mg/kg BW Dexamethasone). It also resulted in a highly significant decrease in the serum levels of TNF-α in both models of chronic inflammation. However, serum levels of IL-1β and hsCRP did not show any significant decrease compared to that reported with TNF-α. Meanwhile, adjuvant of Boron with Dexamethasone shows greater response in this regard, and may enable the conclusion that Boron may augment the effects of glucocorticoids. This assumption is in agreement with the finding that Boron has an impact on steroid hormone metabolism, and the finding that it is necessary for the hydroxylation step in the formation of specific steroid hormones [129]. Naghii and Samman, who reported that steroid hormones level was increased in rats that consumed an equivalent dose of 2 mg Boron/day, have claimed this assumption. The increased levels of

steroid hormones support the hypothesis that Boron enhances the hydroxylation of the steroid rings [130]. While the capacity of Boron to increase estrogen levels might raise concerns about possible cancer risks with Boron supplementation [131], there is no evidence that populations with a high intake of Boron (such as the French) have an increased incidence of hormone-related cancers.

4.2 Conclusion

According to the presented data, we can conclude the following:

1. Boron has a significant effect in decreasing chronic inflammatory conditions in animal models (rats) of formaldehyde-induced edema and cotton pellet-induced granuloma.
2. The anti-inflammatory activity of the orally supplemented Boron is dose-dependent.
3. Adjuvant use of Boron with Dexamethasone enhances its anti-inflammatory activity in the rat's model of formaldehyde-induced edema and cotton pellet-induced granuloma.

4.3 Recommendations for Further Study

1. Evaluate different doses and/or routes of Boron administration, and utilize appropriate measurements of more sensitive markers of inflammatory reactions.

2. Evaluate the inclusion of Boron in adjuvants of other potent anti-inflammatory medication to explore any expected potentiation or synergistic activity.

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الخلاصة

الأساس:

الآثار الجانبية للأدوية المضادة للالتهاب المتوفرة في الوقت الحاضر تمثل معضلة عظمى أثناء الاستخدام السريري. لذلك، فان من الضروري أن تأخذ بالحسبان عند تطوير عدد أكثر و احدث من هذه الأدوية. مؤخراً حصل تقدم هام لتطوير أدوية فعالة مضادة للالتهاب عن طريق استخدام بعض المركبات التي تحتوي على البورون، خالية من الآثار الجانبية نسبياً و يمكن استخدامها بصورة فعالة كمكملات غذائية. صممت هذه الدراسة لتقييم العلاقة بين الجرعة و الاستجابة للفعالية المضادة للالتهاب لمادة البورون في نموذج الجرذ للالتهاب المزمن المستحدث مع مقارنتها بالفعالية المضادة للالتهاب لمادة الديكساميثازون و التي استخدمت كدواء قياسي، و تقييم الفعالية المضادة للالتهاب للأدوية المساعدة عند إعطائها مؤتلفة مع مادة الديكساميثازون.

طرق العمل :

استعمل في هذه الدراسة ستة وستين جرذ مختبري؛ تم تقسيم الحيوانات الى ٥ مجموعات، أول مجموعة: ٦ جرذان تعامل معها بالسائل المذيب (vehicle) فقط دون تحريض التهاب كمجموعة مراقبة سلبية. المجموعة الثانية: ١٢ جرذا قسمت إلى مجموعتين فرعية، كل منها تحتوي على ٦ جرذان، وتم تعاملها مع المذيب فقط مع تحريض الالتهاب المزمن والورم الحبيبي، كمجموعة مراقبة ايجابية. المجموعة الثالثة: ٢٤ جرذا تم تقسيمها إلى أربع مجموعات، كل منها تحتوي على ٦ جرذان، لدراسة الفعالية المضادة للالتهابات من جرعات مختلفة من البورون (٣ و ٦ ملغم / كلغم من وزن الجسم) في كلا النموذجين للالتهاب. المجموعة الرابعة: ١٢ جرذا استخدمت لدراسة فعالية الديكساميثازون المضادة للالتهابات (١ ملغم/كلغم من وزن الجسم) في نفس النماذج. المجموعة الخامسة: تم استخدام ١٢ جرذان مختبريه لدراسة فعالية البورون المضادة للالتهاب (٣ ملغم/كلغم من وزن الجسم) عند إعطائها مؤتلفة مع مادة الديكساميثازون (١ملغم / كلغم من وزن الجسم) في نفس النماذج.

النتائج :

أشارت نتائج الدراسة الحالية الى أن البورون في نمط يعتمد على الجرعة (٣ و ٦ ملغم / كلغم من وزن الجسم) ثبط بشكل كبير الالتهاب المزمن المستحدث بمادة الفورمالديهايد والورم الحبيبي المستحدث بواسطة قطع القطن في الجرذان. كما بينت الدراسة انه عند إعطاء مادة البورون (٣ ملغم/كلغم من وزن الجسم) مؤتلفة مع مادة الديكساميثازون (١ ملغم/كلغم من وزن الجسم) فإنها تعطي فعالية ذات فرق معنوي في تثبيط الالتهاب في نموذج الجرذ للالتهاب المزمن المستحدث

بمادة الفورمالديهايد و نموذج الجرذ للورم الحبيبي المستحدث بواسطة قطع القطن بصورة أعلى من التأثيرات المنتجة بواسطة الطرق الاخرى للعلاج في حال استعمال مادة البورون وحده.

الاستنتاجات :

ان البورون، في نمط يعتمد على الجرعة، كان فعالا في الحد من تأثير الالتهاب المزمن الناجم عن الفورمالديهايد والورم الحبيبي الذي تسببه القطع القطنية في الجرذان. وبالتالي، فإنه قد يعتبر كعلاج محتمل لحالات الالتهابات المزمنة في الإنسان. البورون، كمكمل علاجي مع العامل القياسي و المضادة للالتهاب (الديكساميثازون) يحسن من النشاط المضاد للالتهابات لهذه الأدوية، مع وجود فرصة لتقليل الجرعة.

حكومة إقليم كردستان/العراق
وزارة التعليم العالي و البحث العلمي
جامعة السليمانية
كلية العلوم الطبية
كلية الطب

التأثيرات المضادة للالتهابات لمادة البورون لوحده أو كعلاج مساعد مع الديكساميثازون، في النماذج الحيوانية للإلتهاب المزمن والحبيبي

رسالة

مقدمة الى فرع الادوية و لجنة الدراسات العليا في كلية العلوم الطبية / كلية الطب في جامعة السليمانية كجزء من متطلبات الحصول على شهادة الماجستير في علم الأدوية (الفارماكولوجي)

من قبل

هه ناو نصرالدين محمد أمين
بكالوريوس صيدلة (٢٠٠٩)

بإشراف
الأستاذ الدكتور سعد عبدالرحمن حسين
دكتوراه في علم الأدوية والسموم

پوختە

بنچینە:

تێبینی کراوە کاریگەریە لاوەکییەکانی ئەوماددە دژە هەوکەرانەی کە ئێستا لەبەردەستدان کێشەیەکی گەورەیە لەکاتی بەکارهێنانی کلینکی دا. لەبەرئەوە، دۆزینەوەی نوێترین ماددەی دژەهەوکەری بێ زیان وکاریگەر گرنگە کە ڕەچاوبکرێت و بایەخی پێ بدرێت. لەم دواییەدا، لەڕێگەی بەکارهێنانی ئەو تێکەڵانەی بۆرۆن لە پێکهاتەکەیدایە، کە بەکاردێن وەک ماددەی دژەهەوکەر، پەرەسەندنێکی بەرچاوی بەخۆیەوە بینی، کە کاریگەرە وە بەبەراورد لەگەڵ ماددەکانی تردا بێ زیانە و دەتوانرێت بە ئاسانی بەکاربهێنرێت وەک هاوپێچێکی دەرمان. ئەم لێکۆڵینەوەیە تەرخانکراوە بۆ هەڵسانگاندنی کاریگەری ژمەدەرمان لەسەر لەش، بۆ چالاکی ماددەی دژەهەوکەری (بۆرۆن) لە هەوکردنی درێژخایەنی جرجی تاقیگەیی دا، و بەراوردکردنی بەو دەرئەنجامەی دروست دەبێت لەکاتی بەکارهێنانی دەرمانی پێوانەیی (دێکسامیسازۆن)، و هەڵسەنگاندنی توانای دژەهەوکردنی بۆرۆن کاتێک بەکاردەهێنرێت لەگەڵ دێکسامیسازۆن.

ڕێگاکانی کارکردن :

لەم لێکۆڵینەوەیەدا شەست و شەش جرجی تاقیگەیی بەکارهێنران، دابەشکران بەسەر ٥ کۆمەڵ دا؛ کۆمەڵەی یەکەم: پێکهاتبوو لە ٦ جرج، هیچ جۆرە هەوکردنێکیان تیادروست نەکرا و ڕۆژانە (vehicle) یان پێدەدرا، ئەژمارکران بە(negative control). کۆمەڵەی دووەم: پێکهاتبوو لە ١٢ جرج دابەشکران بەسەر ٢ کۆمەڵەدا، ڕۆژانە (vehicle) یان پێدەدرا و هەوکردنی درێژخایەن و هەوکردنی لووی دەنکۆڵەیی (granulomatous)یان تیا دروست کرا و ئەژمارکران بە (positive control). کۆمەڵەی سێهەم: ٢٤ جرج دابەش کران بەسەر ٤ کۆمەڵەدا،هەریەک لەکۆمەڵەکان ٦ جرجی تێدابوو، بۆ لێکۆڵینەوەی چالاکی دژەهەوکردنی جەند ژمەە دەرمانێکی جیاوازی بۆرۆن (٣ و ٦ ملگم/ کگم کێشی لەش) لە ڕێگای دەمەوە لە هەردوو جۆری هەوکردنەکەدا.

کۆمەڵەی چوارەم: ١٢ جرج بەکارهێنرا بۆ لێکۆڵینەوەی چالاکی دژە هەوکردنی دێکسامیسازۆن(١ملگم /کگم کێشی لەش) بۆهەمان جۆری هەوکردنی کۆمەڵەی پێشوو. کۆمەڵەی پێنجەم: ١٢ جرج بەکارهێنرا بۆ لێکۆڵینەوەی چالاکی دژەهەوکردنی بۆرۆن (٣ ملگم / کگم کێشی لەش) کاتێک وەک دەرمان بەهێزکەر بەکارهات لەگەڵ دێکسامیسازۆن (١ملگم / کگم کێشی لەش) بۆهەمان جۆری هەوکردن.

ئەنجامەکان :

ئەنجامەکانی ئەم لێکۆڵینەوەیە دەریدەخەن کە (بۆرۆن) بەپێی شێوازی پشت بەستن بە ژەم (٣ و ٦ ملگم / کگم کێشی لەش) دروستبوونی هەوکردن لە جرجی تاقیگەیی دا بە بەکارهێنانی ماددەی (فۆرماڵین) بۆ دروستبوونی هەوکردنی درێژخایەن و کڵۆی لۆکە بۆ دروستبوونی لووی دەنکۆڵەیی (granuloma) بەشێوەیەکی بەرچاو کەم دەکاتەوە. هەروەها پێدانی بۆرۆن (٣ ملگم/کگم کێشی لەش) لەگەڵ دێکسامیسازۆن (١ ملگم/کگم کێشی لەش) دروستبوونی هەوکردن لە جرجی تاقیگەیی دا بە هۆی بەکارهێنەنی ماددەی (فۆرماڵین) بۆ دروستبوونی هەوکردنی درێژخایەن و کڵۆی لۆکە بۆ دروستبوونی لووی دەنکۆڵەیی (granuloma) بەشێوەیەکی بەرچاو کەمکردەوە، کە ئەمەش زۆرترین کەمبونەوەیە بە بەراورد ئەو کاریگەریانەی دروستبوون بەرێگا جیاوازەکانی چارەسەرکردن کاتێک بۆرۆن بەتەنها بەکارهات.

دەرئەنجامەکان :

بۆرۆن، بەپێی شێوازی پشت بەستن بە ژەم، بەشێوەیەکی بەرچاو کاریگەرە لە کەمکردنەوەی دروستبوونی هەوکردنی درێژخایەن بەماددەی فۆرماڵین و دروستبوونی لووی دەنکۆڵەیی(granuloma) بە کڵۆی لۆکە لە جرجی تاقیگەیی دا؛ لەبەر ئەوە، دەتوانرێت وەک چارەسەرکردنێک دابنرێت بۆ دۆخی هەوکردنی درێژخایەن لە مرۆڤ دا. بۆرۆن، وەک دەرمان بەهێزکەر لەگەڵ ماددەی دژەهەوکردنی پێوانەیی، دێکسامیسازۆن، دەبێتە هۆی بەهێزکردنی توانای دژەهەوکردنی دێکسامیسازۆن، لەگەڵ هەڵی کەمکردنەوی ژەمەدەرمانەکەی.

حکومەتی هەرێمی کوردستان/عێراق
وەزارەتی خوێندنی باڵاوتوێژینەوەی زانستی
زانکۆی سلێمانی
فاکەڵتی زانستە پزیشکیەکان
سکوڵی پزیشکی

" کاریگەری دژەهەوکردنی بۆرۆن بەتەنها یان وەک دەرمان بەهێزکەرێک لەگەڵ دێکسامیسازۆن، لە هەوکردنی درێژخایەن ولووی دەنکۆڵەیی (Granulomatous) بە بەکارهێنانی ئاژەڵی تاقیگەیی زانستی"

لێکۆڵینەوەکە پێشکەش بەلقی فارماکۆلۆجی و لقی خوێندنی باڵای سکوڵی پزیشکی/ فاکەڵتی زانستە پزیشکی یەکانی زانکۆی سلێمانی کراوە وەک بەشێک لەپێداویستیەکانی بەدەستهێنانی پروانامەی ماستەر لە زانستی فارماکۆلۆجی

لەلایەن
هەناو نەسرەدین محمد ئەمین
بەکالۆریۆس لەدەرمانسازی ٢٠٠٩

سەرپەرشتیار
پرۆفیسۆر
دکتۆر سعد عبدالرحمان حسین
دکتۆرا لەفارماکۆلۆجی و ژەهرناسی

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