ORIGINAL RESEARCH PAPER

Biology

EFFECT OF OCIMUM BASILICUM EXTRACT ON SODIUM HYPOCHLORITE-INDUCED BIOCHEMICAL ALTERATIONS IN RABBITS

KEY WORDS: Sod. Hypochlorite, Ocimum Basilicum, Hematology, Transaminases, Rabbit.

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ABSTRACT

Sodium hypochlorite (SODH) is used as a disinfectant and bleach for both domestic and industrial purposes. On the other hand, it showed different toxicity in mammalian animals. Basil or sweet basil (Ocimum basilicum) is a plant which showed many pharmacological effects. The present work studied the potential effect of O. basilicum extract on SODH -induced Biochemical alterations in rabbits. Animals were divided into 4 groups. Group 1 served as controls, group 2 given O. basilicum aqueous extract (0.2mg/ml, for a week), group 3 were given SODH (20 ppm for 3 minutes daily for 4 weeks), group 4 given SODH for 3 weeks and given O. basilicum for another week. The results showed that exposing animals to SODH induced significant decrease in RBCs count, haemoglobin, hematocrit percentage and blood platelets. On the other hand, the WBCs count, lymphocytes, monocytes and neutrophils were increased. Triglycerides, cholesterol and transaminases (ALT and AST) were increased in the sera of treated rabbits. Rabbits treated with SODH for 3 weeks and given O. basilicum extract for another week showed an improvement of the haematological and biochemical parameters. It is concluded from the present results that O. basilicum extract modulated toxicity of SODH by the antioxidant activity of flavonoids constituent.

INTRODUCTION

Chlorine as SODH is used as a disinfectant and bleach for both domestic and industrial purposes, and is also widely used to disinfect drinking water and swimming pool water and to control bacteria and odors in the food industry. On the other hand, SODH, bleach (5.25%) may result in various degrees of toxicity including mucosal irritation, nausea, vomiting, diarrhea, corrosive injury to the oesophagus and gastrointestinal tract, acidosis and even death.[1] The presence of residual chlorine interferes with a number of biochemical events in the body. [2] reported that A single gavage dose of 143 mg/kg chlorine (as SODH) given to rats resulted in morphological and biochemical changes in the liver within a 2-day period. Decreased thyroxin, increased plasma cholesterol, and increased heart weight have been reported in pigeons consuming 2 to 15 mg/l chlorine (as sodium hypochlorite) in drinking water for three months.[3] SODH produced chromosomal aberrations in Chinese hamster fibroblast cells without metabolic activation.[4] Excess addition of SODH into drinking water may prove toxic to the birds [5]. It was reported that in human population in different regions of world, increase in certain congenital malformations, a short gestation duration and smaller, head circumference has been observed in newborns in regions where SODH was used for water anitation.[6].Chlorinated water has been implicated in bladder and colon cancer in humans.[7]

Recently, many traditional medicines are in use and derived from medicinal plants constituents. Over 50% of all modern clinical drugs are of natural product origin and natural products play an important role in drug development programs. *Ocimum basilicum* L., Basil, belongs to the family Lamiaceae, is widely distributed in the tropical and warm regions of the world.[8] It is mainly used as a spice to flavour foods and meats. The components of *O. gratissimum* has biological activity such as antidiabetic, antiseptic, antitussive, antihelmintic, antispasmodic and antimicrobial.[9, 10] Supplementation of O. sanctum leaf reduced the severity of hydropericardium, hepatitis, myocarditis, oedema in lungs, lymphocytic depletion in lymphoid organs and focal interstitial nephritis.[11] In a study of patients with chronic bronchitis, exposure to essential oils of basil caused lowering of plasma levels of dienic conjugates and ketones and activation of catalase in red cells characteristic of antioxidant effects.[12] Aqueous suspension of *Ocimum* sanctum contains a variety of effective compounds that exhibit anticancer effect.[13] The present work was carried out to study the effect of *O. basilicum* extract on SODH induced biochemical changes in albino rabbits.

2. MATERIAL AND METHODS

2.1 TEST ANIMALS

Adult male New Zealand rabbits weighing 500-700 g were used in the present experiments. The rabbits were kept in universal galvanized wire cages at room temperature (22 ± 2C°) and in a photoperiod of 12:12 light/dark cycle, 50% ± 5% humidity. They were acclimatized for 2 weeks prior to the start of the experiment. Animals were fed freely on pellets of standard rabbit diet and free access to water *ad libitum*. They were divided into four groups: The rabbits fed freely

Group 1: animals of this group (4 rabbits) served as controls.
Group 2: At the 4th week, animals of this group (4 rabbits) were orally given basil aqueous extract at a dose level of 0.2mg/ml, for a week. Freshly prepared basil extract was prepared by diluting 20g of blended basil with 1000 ml of distilled water.

Group 3: animals of this group (8 rabbits) were given 20 ppm of SODH in the drinking water for 3 weeks.

Group 4: animals of this group (8 rabbits) were given 20 ppm of SODH for 3 weeks and then given basil aqueous extract for another week.

2.2 BIOCHEMICAL ASSAYS

The treated animals and their controls were sacrificed by decapitation after 4 weeks of treatment. For haematological study, blood was collected from control and treated animals. The haematological parameters: red blood cells count (RBCS), haemoglobin value (Hb), hematocrit value (HCT %), white blood cells count (WBCS) and blood platelets number were measured by a fully automated Coulter counter (Coulter Electronics Limited, England). For biochemical assays, blood samples were collected from animals and sera were obtained by centrifugation of the blood sample and stored at -20°C. For biochemical assays, blood samples were collected from animals and sera were obtained by centrifugation of the blood sample and stored at -20°C. Aspartate aminotransferase (AST) and alanine aminotransferase (ALT) were determined colourimetrically according to [14]. Alkaline phosphatase was determined by the method of [15]. Cholesterol and triglycerides level was estimated according to methods of [16, 17], respectively.

2.3 STATISTICAL ANALYSIS

Data were expressed as mean values ± SD and the criterion for statistical significance was set at $P < 0.05$. All statistical analyses were performed using SPSS statistical version 16 software package (SPSS ® 4 Inc., USA).

3. RESULTS

Effects of oral administration of SODH and supplementation of basil extract on rabbits haematological parameter is illustrated in Table 1. Administration of SODH showed significant decrease in RBCs, haemoglobin, hematocrit value and platelets ($P<0.05$)

when compared to control animals. While, the administration of basil extract with SODH groups maintained the normal levels of these values similar to control. On the other hand, treating animals with SODH caused an increase in WBSCs and the differential count, monocytes, neutrophils (Table 2).Figures 1-3 represent the activities of serum AST, ALT and ALP in animals treated with SODH. Administration of SODH significantly increased the activities of ALT (*P<0.05*), AST (*P<0.05*) and ALP (*P<0.05*) when compared to control. There was a decrease in activity of all these enzymes in rabbits given SODH and basil. Cholesterol and triglycerides elevated in sera of animals administered SODH and decreased after treatment with basil extract (Figs. 4&5).

4. DISCUSSION

Excess addition of SODH into drinking water may prove toxic to different animals and human. The present study reveals that the administration of SODH was significantly caused decrease in RBCs, haemoglobin, hematocrit and platelets. Similarly, decreased hematocrit, erythrocyte, haemoglobin, leukocytes, plasma proteins and globulin were recorded in Japanese quail (*Coturnix japonica*) given SODH in drinking water.[18] [19]reported that residual chlorine administration decreases haemoglobin concentrations in rats. In addition, [20] reported that residual chlorine in water may damage the hematopoietic system and cause anemia. Total WBCs, neutrophils, lymphocytes and monocytes increased in rabbits given SODH. [21] observed that males receiving 60 mg/kg-day of sodium chlorite exhibited significantly decreased hematocrit and haemoglobin levels and increased methemoglobin and neutrophil levels, whereas in the females, methemoglobin levels were significantly decreased.

Transaminases are intracellular enzymes and the most sensitive biomarkers, released into the circulation after damage and necrosis of hepatocytes.[22] Measurement of the activities of marker enzymes, like AST and ALT can be used in the assessment of liver function. The current study revealed a significant increase in the activity of ALT and AST in the serum of SODH treated rabbits which may be a sign of impaired liver function. [23] examined the effects of subchronic exposure to sodium chlorite in drinking water on hematologic and serum clinical chemistry parameters in African green monkeys. They observed decreases in erythrocyte levels and cell indices, and increases in aspartate aminotransferase. AlP activity increased in sera of animals treated with SODH. It is well known the ALP enzyme activity dephosphorylated, phosphorylated organic substrates in the animal tissues. ALP is a hydrolase and a transphosphorylase in function associated with cell membranes.[24] Increase in the specific activity of this enzyme, therefore suggested the existence of a greater depohsphorylation potential within the animal cell.

In the present study, oral administration of SODH caused the increase in serum cholesterol and triglycerides which indicates a loss of membrane integrity.[25] In agreement with this result, [3] reported that SODH in drinking water led to increase cholesterol in pigeons. This raised cholesterol level may be also due to decreased utilization of cholesterol under stress. [26] have also suggested that the elevation of cholesterol levels may be attributed to enhanced cholesterol and triglyceride synthesis and/or reduced cholesterol catabolism.

Our results showed that administration of *O. basilicum* with SODH improved the haematological and biochemical alterations induced by SODH. The importance of *Ocimum* as a medicinal plant has been previously documented in several studies. Concerning, the haematological results, [27]reported that that oral administration of *O. gratissimum* increases RBC, PCV, Hb, platelet count, and neutrophils in rats. In addition,[28] reported that packed cell volume, red blood cell count and haemoglobin concentration were significantly increased in the diabetic rats treated with *Ocimum* when compared with the diabetic group.

Ocimum treatment induced a decrease in activity of ALT,AST and ALP compared with Sod. perchlorite group. In this concern, [29] found that injection subcutaneously with CCl_4 to Sprague Dawley rats caused increase in ALT and AST, oral doses of basil extract significantly decreased the higher levels of serum of ALT and AST. [30] found that sodium arsenite-induced higher level of serum ALT and AST in rats and Treatment with leaf extracts of *O. basilicum* before or after sodium arsenite led to reduction in the level ALT and AST activities. [31] revealed that diazinon treatment was accompanied by a significant increase in activity of ALT and AST as compared with control group. On the other hand, treatment with diazinon and *O. basilicum* extract showed a reduction in the activity of these two enzymes and improves the histological changes of the liver.

Treating animals with SODH and *O. basilicum* extract improved cholesterol and triglycerides levels. [32] recorded significant reduction in the levels of total cholesterol, low-density lipoprotein-cholesterol, very low-density lipoprotein cholesterol and triglycerides in diabetic rats administered with *Ocemium*. [33] reported a reduction of cholesterol levels during treatment with whole basil leaves (*Ocimum sanctum* and *O. album*) in patients with non-insulin- dependent diabetes mellitus. Consumption of basil or basil oil has been associated with a reduction in total cholesterol, low-density lipoprotein and triglyceride levels.[34]

It was reported that the therapeutic effect of *Ocimum* extract is attributed to its antioxidant activity. [35] found that *O. basilicum* increased the activity of xenobiotic metabolizing phase 1 and phase 11 enzymes, elevating antioxidant-enzyme response by increasing significantly the hepatic glutathione reductase, superoxide dismutase, and catalase activities increasing glutathione content and decreasing lipid peroxidation and lactate dehydrogenase activity in the liver of mice. [36] reported that administration of *O. sanctum* extract before and after cadmium intoxication resulted in a significant decrease in LPO levels and significant increase in SOD, CAT, GPx, GSH and ascorbate levels. Moreover, [37] indicated that *Ocimum basilicum* increased the antioxidant activity (SOD, CAT, GSH, GPx, GST) and decreased cholesterol, TAG level, Blood urea and ALT following exposure to gibberellic acid in broilers chicken.

Leaves of *O. basilicum* are rich source of flavonoids which have been shown to possess various biological properties related to antioxidant mechanisms. [38] reported that the main components of *O. basilicum* were: linalool (29.68%) , (Z)- cinnamic acid methyl ester (21.49%) , cyclohexene (4.41%) , alpha- cadinol (3.99%), 2,4-diisopropenyl-1-methyl-1- vinylcyclohexane (2.27%), 3,5-pyridine-dicarboxylic acid, 2,6- dimethyl-diethyl ester (2.01%), beta-cubebene (1.97%), guaia- 1(10),11-diene (1.58%), cadinene (1.41%), (E)-cinnamic acid methyl ester (1.36%) and beta-guaiene (1.30%). It is concluded from the present study that SODH caused hematobiochemical changes in rabbits and the flavonoids of *O. basilicum* extract have protective effects.

Table (1) Effect of SODH and /or basil on haematological parameters of male rabbits

Treatment Parameters	Control	Basil	Sod. hypochlorite	Sod.hypo+ Basil
RBCs 10^6/mm^3	6.56±0.3	6.13±0.33	4.77±0.18*	6.62±0.16
RBCs 10^6/mm^3	6.56±0.3	6.13±0.33	4.77±0.18*	6.62±0.16
Hb gm/dl	12.4±0.3	12.5±0.35	8.2±0.45*	13.5±0.48
HCT %	41.5±0.65	38.1±0.4	22±0.4*	40.3±0.58
Platelets 10^3/L	312±2.58	314±2.8	200±2.33*	246±3.1
MPV (fl)	4.6± 0.38	5.1 ±0.28	3.3±0.23*	4.1±0.28

-Values are expressed as mean ± SEM, (*) Significant at p<0.05

Table (2) Effect of SODH and /or basil on WBCs and differential count of male rabbits

Treatment Parameters	Control	Basil	Sod. hypochlorite	Sod.hypo+ Basil
WBCs 103/mm3	4.85±0.23	4.95±0.25	6.31±0.35*	5.07±0.2
Neutrophil%	1.39±0.005	1.26±0.003	3.20±0.03*	1.40±0.01
Lymphocytes%	3.3±0.01	3.40±0.05	4.19±0.08*	3.10±0.08
Monocytes %	0.33±0.008	0.41±0.05	0.96±0.01*	0.30±0.08

-Values are expressed as mean ± SEM, (*) Significant at p<0.05

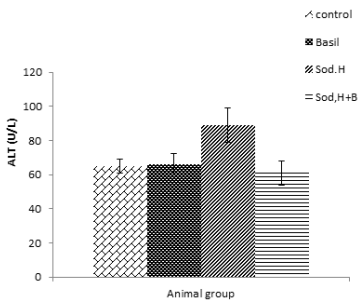

Fig.1. Effect of different treatments on serum ALT.

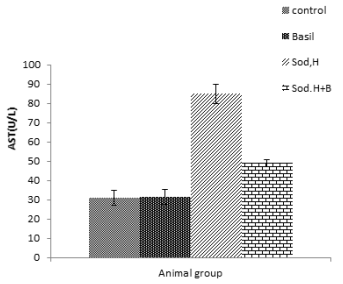

Fig.2. Effect of different treatments on serum AST.

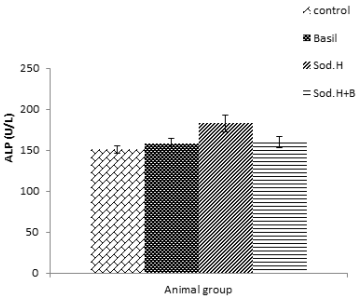

Fig.3. Effect of different treatments on serum ALP

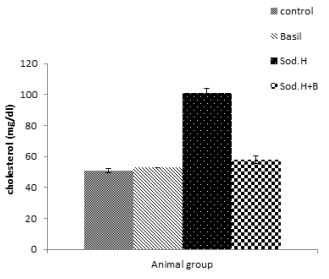

Fig.4. Effect of different treatments on serum Cholesterol

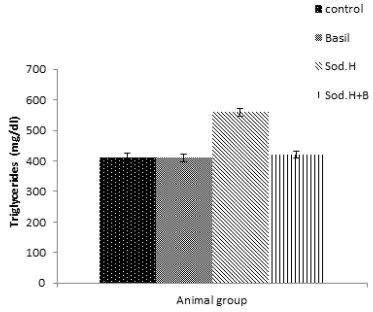

Fig.5. Effect of different treatments on serum triglycerides

5. CONCLUSION

In conclusion, the exposure of rabbits to sod. Hypochlorite at low dose (20 ppm) causes a marked increase in WBCs count, triglycerides, cholesterol and liver enzymes (ALT and AST). Meanwhile, RBCs count, percentage of haemoglobin, haematocrit, and platelets values showed marked decrease. These parameters were improved when the rabbits treated with aqueous solution of Ocimum basilicum at (20mg/ml).

COMPETING INTEREST

The author have declared that no competing interest exist.

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