

## BIOCHEMICAL ANALYSIS OF BLOOD AND TISSUES OF RATS ADMINISTERED SOME FOOD PRESERVATIVES

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### ABSTRACT

Biochemical aspects of blood and histopathological examination of tissues from rats given some food preservatives were investigated. This study was carried out on 120 white male albino rats to study the effect of sorbitol 0.1%, sodium nitrite 0.2% and citric acid 0.02% as food additives to rat diets for 3 months on some liver and kidney function tests also on histopathological changes in liver and kidney. The results revealed a highly significant increase in the activity of serum AST, ALT and ALP in rats received food preservatives for 3 months and significant increase in serum urea after one, two and three months and creatinine levels after the third month in rats received nitrite preservatives diet. A significant increase was also recorded in serum sodium level in rats received preservatives diets for 3 months, except sodium nitrite group, also uric acid in rats treated for one and two months in all experimental groups (II, III and IV) except after first month in sodium nitrite group. In contrast, result revealed non significant decrease in serum sodium level after one and two months also uric acid of 3rd group after one and three months. Significant decrease in serum potassium level in all groups of rats for one, two and three months were observed except after first month in the 2nd group and after third month in the 4th group respectively. The histopathological examination revealed pathological changes in the liver and kidney which reaches in some cases to necrosis. It could be concluded from this study that liver and kidney functions were affected by the addition of sodium nitrite, sorbitol and citric acid as preservatives for long periods. Therefore, it is advisable to reduce the frequent use of these food preservatives which constitute, at times, public health hazards.

### INTRODUCTION

Food preservatives can be defined as agents that extend the storage life of foods, by retarding or inhibiting the undesirable changes in flavour, nutritive value, odour, colour, texture and other organoleptic properties. Some of these undesirable changes are due to either microbial spoilage or enzymatic reaction<sup>(1)</sup>.

Chemical preservatives are commonly employed in food processing and manufacture to inhibit or control a variety of deteriorative reactions in foods<sup>(2)</sup>.

Acid and salt preservatives are used for inhibiting or retarding the growth of various microorganisms in foods. They exert acidifying, alkalinizing, antidrying, antifoaming, antihardening, antisticking, bleaching, buffering, chill proofing, clarifying, colour retaining, colouring, enhancing flavour and firming<sup>(3,4)</sup>.

Several investigators have reported that addition of chemical preservatives to food may interfere with or change the blood parameters of human and animals which reflect the impairment of some organ functions. The values of serum sodium and potassium in blood were decreased when sodium citrate was added to rat's diet for 21, 28 and 56 days<sup>(5)</sup>.

Whereas, it has been reported that serum

transaminases activities were significantly increased in rats received citrate mixed diet<sup>(6)</sup>. A significant increase of serum creatinine level in patients received citrate in foods has been reported<sup>(7)</sup>.

Recently, an increase of renal urea and creatinine clearance in rats received citrate in foods has been recorded<sup>(8)</sup>.

Moreover, higher values of serum transaminases activities in rats feeding high amounts of sorbitol preservatives mixed diets were reported<sup>(9)</sup>.

Nitrite is included in meat curing mixtures primarily to develop and fix colour and flavour of cured meat. It has been found that nitrite can react with certain amine compounds in the intestine and other conditions, to form nitrosamines which are strong carcinogenic compounds<sup>(10-12)</sup>.

The effects of nitrite on erythrocytic count, liver and kidney functions in poultry were studied<sup>(13)</sup> observed that all parameters of the hepatorenal function were affected after feeding of sodium nitrite mixed diets.

Accordingly, it is necessary to adapt this work to investigate the effect of some food preservatives (sorbitol, sodium nitrite and citric acid) on some blood parameters, renal and hepatic functions of rats as well as histopathological changes in tissue of some organs (liver and kidney). However,

determination of such parameters as well as histopathological examination of liver and kidney help to clarify the metabolic changes which occur as a result of the addition of food preservatives which may affect animal and human health. Therefore this work was carried out to determine some serum parameters for renal and hepatic functions such as, aspartate aminotransferase, alanine aminotransferase, alkaline phosphatase, urea, uric acid, creatinine as well as sodium and potassium. In addition to histopathological examination of liver and kidney.

#### MATERIAL AND METHODS

One hundred and twenty male albino rats of two months old and weighing 120-150 gm were employed for this study. They were kept at a constant environmental and nutritional condition throughout the period of the experiment.

Rats were randomly divided into four equal groups according to type of preservative added to their diets each group consists of 30 rats and was placed in individual cages as follows :

**Group I :** Used as control group given a basal diet free from any preservative.

**Group II :** Given sorbitol preservative diet 0.1 g%.

**Group III :** Receiving diet containing sodium nitrite 0.2%.

**Group IV :** Given citric acid preservative diet 0.02 g%.

The doses of these preservatives were calculated (14) while control rats fed a basal diet ad-libitum (15). The experiment continue for 3 months.

Blood samples were collected monthly for a period of 3 months from 10 rats of each group after sacrificing of rats, sera were separated and kept in a deep freeze at -20°C until used for the following biochemical analysis :

- Aspartate amino transferase (AST) and Alanine amino transferase (ALT) (16).
- Alkaline phosphatase (ALP) (17)
- Sodium (18) - Potassium (19)
- Urea (20) - Uric acid (21) - Creatinine (22)

#### Histopathological examination :

Immediately after decapitation liver, kidney, specimens were isolated and kept in 10% neutral formalin for histopathological examination.

The data obtained were statistically analysed using Students "t" test (23).

#### RESULTS AND DISCUSSION

The obtained results in Table (1) indicates that ~~there~~ was a significant increase in serum aspartate ~~aminotransferase~~ activity (AST) ( $P < 0.05$  -  $P < 0.001$ )

in rats received (sorbitol, Na nitrate and citric acid preservatives) in their ration for one two and three months, except group of citric acid after 3 months which showed non significant effect. It was clear that there was a highly significant increase in the activity of serum (ALT) of all treated groups collected at the end of the first and second months of preservatives administration whereas, after 3 months the increase was seen only in the group of sorbitol. Such results were in accordance with those previously reported (6). They found that there was a marked increase of serum transaminases activities (AST and ALT) of mice injected intraperitoneally by ferric citrate. Similar results were also observed (23) in calves administered sodium nitrite (13.5 mg/kg body weight) intra ruminal and nitrite poisoning was induced. Moreover, our results were supported by those obtained (13), they stated that serum AST activity was markedly increased in the nitrite fed birds (1.7 gm/kg ration) for a duration of four weeks due to impaired liver function. It seems conceivable that the increased AST and ALT activities might be attributed to the pathological changes of the liver evidenced in this work by nitrites (Fig. 2). Moreover, nitrate reacts with amines in the blood forming cytotoxic and carcinogenic nitrosamines which mediated many of the toxic effects attributed to nitrite (24).

The obtained results in Table (1) revealed also that the serum alkaline phosphatase activity was significantly increased in rats administered preservatives mixed diet for a duration of three months in all the experimental groups except group of citric acid after the third month which showed no significant effect.

These results were in agreement with those previously reported (25). They reported that biochemical parameters in the liver were altered in rats received sodium nitrite as preservatives, (26) recorded the effect of sodium nitrite 10 mg/liter in drinking water of rabbits on biochemical parameters of the liver. The probable reason for the increase of alkaline phosphatase activity was the pathological changes in liver and impaired liver function as confirmed by the work of (27) recorded by the histopathological findings evidenced in our work. The adverse effects of nitrite on the liver function of birds which are involved in aetiology of the liver disease, and was attributed to oxidation of important iron-containing enzymes such as cytochrome responsible for cellular respiration and other oxidation reduction processes. At the meantime (28) studied the adverse effects of nitrite in birds received sodium nitrite mixed diet and they recorded a

**Table (I) :** Mean values  $\pm$  S. E. of serum Aspartate aminotransferase , Alanine aminotransferase and alkaline phosphatase activities in rats received preservative mixed diets .

Parameters	Group		Control group I	Sorbitol group II	Sodium nitrite group III	Citric acid group V
	Duration					
Aspartate aminotransferase (AST) (u/ml)	One month		30.75 $\pm$ 1.48	50.674 $\pm$ 3.23***	42.33 $\pm$ 2.67**	54.55 $\pm$ 3.27***
	Two months		31.33 $\pm$ 2.06	56.67 $\pm$ 6.97*	40.33 $\pm$ 1.69*	38.5 $\pm$ 3.86
	Three months		38.15 $\pm$ 3.17	52.2 $\pm$ 2.8*	77.9 $\pm$ 2.17***	54.53 $\pm$ 2.38**
Alanine aminotransferase (ALT) ( u/ml)	One month		26.0 $\pm$ 0.96	34.4 $\pm$ 1.84***	37.8 $\pm$ 1.94 ***	36.5 $\pm$ 1.01***
	Two months		31.1 $\pm$ 1.37	47.18 $\pm$ 4.34***	40.4 $\pm$ 2.45***	45.6 $\pm$ 4.29***
	Three months		40.9 $\pm$ 1.5	46.8 $\pm$ 1.24***	43.9 $\pm$ 1.12	43.1 $\pm$ 1.93
Alkaline phosphatase (ALP) ( u/l)	One month		19.82 $\pm$ 0.47	28.47 $\pm$ 1.93***	24.5 $\pm$ 1.14***	33.84 $\pm$ 1.54***
	Two months		21.8 $\pm$ 0.72	29.97 $\pm$ 1.61***	28.99 $\pm$ 2.2**	43.94 $\pm$ 3.13***
	Three months		31.92 $\pm$ 1.48	76.9 $\pm$ 6.02***	67.92 $\pm$ 4.2 ***	37.92 $\pm$ 5.72

\* ( P<0.05)      \*\* ( P<0.01)      \*\*\*( P<0.001)

pathological changes in liver and kidney and impaired liver function.

The data of the present investigation (Table 2) revealed that the serum sodium level in rats received sorbitol mixed diet showed a significant increase after three months. These results agreed with the results (29) for human, they observed a marginal clinically insignificant metabolic effects of orally administered physiological amount of sorbitol in rats and human (100-400 gm/kg body weight). The recorded significant increase of serum sodium in rats received sorbitol mixed diet could be attributed to the diuretic effect of polyols which was considered responsible for the changes in the monovalent ion metabolism.

Concerning the results of rats received citric acid in their ration for one and two months it was clear that there was a highly significant decrease in serum sodium level. Such results agreed well with those of (5), they reported that the values of serum sodium were decreased in the experimental diabetic rats and other group received saline citrate, and a significant increase in serum sodium output. The authors attributed this to renal hyperatrophy evidenced to our work (as shown in Fig. 1). Moreover, the tubular atrophy was increased in long term treated patients with multiple dosage daily of lithium citrate (30). The obtained data in Table (2) indicated that there was a highly significant decrease in serum potassium level in all groups of rats received sorbitol, sodium nitrite and citric acid preservatives mixed diet, except for the group of sorbitol after one month which showed a slight decrease. The decrease in serum potassium level of rats received citric acid preservatives mixed diet may be attributed to renal tubular atrophy (29) as shown in Fig. (1).

Table (3) shows that there was a marked increase of serum urea level in rats received sodium nitrite preservative diet after one, two and three months of administration. These results agreed well with those reported before (31) who stated that the blood urea nitrogen was markedly increased in male calves experimentally fed on sodium nitrite, and in birds. The authors also showed that nitrate and nitrite have adverse effects on growth, immunological states, erythrocytes, liver and kidney of birds. The increased level of serum urea of rats received sodium nitrite may possibly be due to impaired kidney function as confirmed (28), they showed that nitrate and nitrite caused pathological changes in liver and kidney of the pheasant. Moreover, it has been reported that the nitrate react with amines in the food to form potentially cytotoxic and carcinogenic

nitrosamines which mediate many of the toxic effects (24). The author also added that the toxicological effect of nitrite and nitrate in different mammalian species includes carcinogenesis, hepatotoxicity impaired reproductive function, endocrine disturbances, growth retardation, reduction of vitamin A, methaemoglobinemia and impairment of certain defence mechanism linked to the inflammatory response and tissue injury.

It was evident from Table (3) that the serum uric acid level revealed a significant increase in rats received preservative mixed diet for one and two months in all experimental group except sodium nitrite group after one month which showed non-significant increase. Our result were not in accordance with (32), they reported that the daily ingestion of 10 gm of mannitol and sorbitol in man for one month produced no significant change of serum non protein nitrogenous constituents (uric acid). Moreover, (33) concluded that the serum uric acid level does not differ from the normal physiological range in dog received hexitol (sorbitol) feeding diet, these differences might be attributed to species differences, changes in age and dose given.

Our results agreed with those (13) who stated that the serum uric acid level was markedly increased in birds fed on diet containing sodium nitrite (1.7 g/kg) compared with the control birds received basal diet. The increased serum uric acid level could be attributed to the adverse effects of nitrite on kidney function (24) who reported that nitrate react with amines in the food to form potentially cytotoxic and carcinogen nitrosamine which mediate many of the toxic effects.

The increased serum uric acid level in rats received citric acid preservative mixed diet agreed with (8) in human who observed that the renal clearance of uric acid was significantly reduced in patients with different degrees of renal insufficiency as a result of citrate treatment. The increase of serum uric acid level may be due to tubular atrophy shown in Fig. (1) confirmed by (30) who reported that the significant increase in tubular atrophy was observed in patients treated with multiple dosage of lithium citrate daily.

Table (3) shows that there was a significant increase ( $P < 0.05$ ) of serum creatinine level of rats received sodium nitrite preservative mixed diet for three months. Similar results were recorded (13). They reported that serum creatinine values were markedly increased in birds fed on diet containing sodium nitrite when compared with the control birds given a basal diet. The increase of serum creatinine

**Table (2) :** Mean values  $\pm$  S. E. of serum sodium and potassium levels in rats received preservatives mixed diet in ( m. Eq/l).

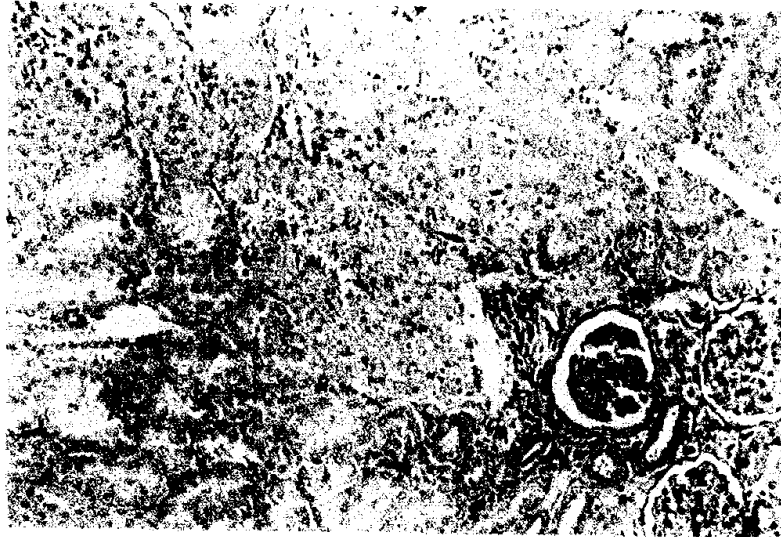
Parameters	Group		Control group I	Sorbitol group II	Sodium nitrite group III	Citric acid group V
	Duration					
Sodium (m. Eq/l)	One month		130.5 $\pm$ 6.5	122.4 $\pm$ 1.5	119.8 $\pm$ 1.2	104.5 $\pm$ 1.1***
	Two months		122.5 $\pm$ 1.4	121.4 $\pm$ 1.8	122.8 $\pm$ 1.3	109.3 $\pm$ 3.8**
	Three months		133.2 $\pm$ 1.8	142.2 $\pm$ 3.8*	134.4 $\pm$ 2.8	156.7 $\pm$ 4.03***
Potassium (m. Eq/l)	One month		7.9 $\pm$ 0.3	6.1 $\pm$ 1.52	5.6 $\pm$ 0.29***	6.3 $\pm$ 0.38***
	Two months		7.8 $\pm$ 0.28	6.1 $\pm$ 0.54***	4.6 $\pm$ 0.24**	5.3 $\pm$ 0.5***
	Three months		6.2 $\pm$ 0.5	4.3 $\pm$ 0.35**	3.7 $\pm$ 0.20**	5.2 $\pm$ 0.56

\* ( P<0.05)    \*\* ( P<0.01)    \*\*\* ( P<0.001)

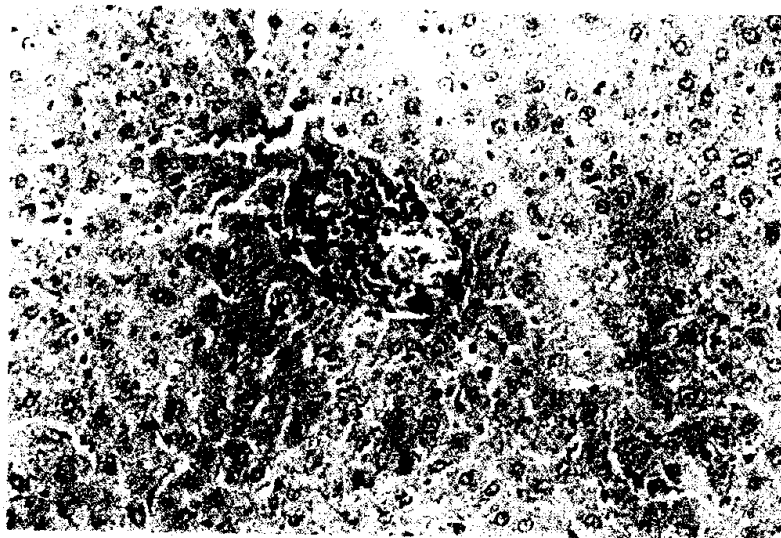
**Table (3) :** Mean values  $\pm$  S. E. of serum urea , uric acid and creatinine levels in rats received preservatives mixed diet

Parameters	Group		Control group I	Sorbitol group II	Sodium nitrite group III	Citric acid group V
		Duration				
urea (mg/dl)	One month		26.51 $\pm$ 0.97	28.67 $\pm$ 1.20	38.75 $\pm$ 2.08**	28.67 $\pm$ 1.20
	Two months		29.92 $\pm$ 1.11	30.58 $\pm$ 1.37	39.75 $\pm$ 0.30***	30.58 $\pm$ 1.37
	Three months		30.51 $\pm$ 0.14	31.40 $\pm$ 0.13	44.31 $\pm$ 0.26***	31.40 $\pm$ 0.13
uric acid (mg/dl)	One month		4.04 $\pm$ 0.90	6.26 $\pm$ 0.734*	5.43 $\pm$ 0.26	6.26 $\pm$ 0.734*
	Two months		4.05 $\pm$ 0.58	6.46 $\pm$ 0.81*	5.63 $\pm$ 0.62***	6.46 $\pm$ 0.81*
	Three months		4.27 $\pm$ 2.48	4.55 $\pm$ 1.46	4.16 $\pm$ 0.74	4.55 $\pm$ 1.46
creatinine (mg/dl)	One month		0.60 $\pm$ 0.09	0.59 $\pm$ 0.18	0.63 $\pm$ 0.11	0.59 $\pm$ 0.18
	Two months		0.84 $\pm$ 0.09	0.93 $\pm$ 0.04	0.90 $\pm$ 0.08	0.93 $\pm$ 0.04
	Three months		0.86 $\pm$ 0.06	0.92 $\pm$ 0.03	1.54 $\pm$ 0.19**	0.92 $\pm$ 0.03

\* ( P<0.05)      \*\* ( P<0.01)      \*\*\* ( P<0.001)



**Fig. (1) :** Section in kidney showing intravascular thrombosis (H & E stain x 150)



**Fig. (2) :** Section in Liver showing perivascular mononuclear cellular aggregation (H & E stain x 150)

levels in rats received sodium nitrite might be attributed to the direct effect of nitrate and nitrite on kidney function.

The histopathological findings showed that in case of sodium nitrite preservative mixed diet, periglomerular, perivascular, interstitial lymphocytic cellular infiltration with renal blood vessel thrombus were observed. These observations were in partial agreement with the result of (34) who found tubular nephrosis in kidney of rats received sodium nitrite as a preservative.

Moreover, our results showed hepatic degeneration and necrosis in cases of sodium nitrite preservative mixed diet. These findings were in a complete accordance with those reported before (34-36).

Our results showed renal B.V. congestion, hyaline cast in renal tubules with mononuclear leucocytic cellular aggregation among hepatic parenchyma in rats received sorbitol mixed diet. These results were conflicted with those of (32) who found no pathological changes in both kidneys and liver of monkey fed 3 g/day of sorbitol over a period of 3 months. These differences may be due to species and or dose variations.

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### التحليل الكيمائي الحيوي لدم وأنسجة الفئران المعطاة بعض المواد الحافظة

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يهدف هذا البحث إلى دراسة تأثير إضافة بعض المواد الحافظة إلى الأغذية على بعض التغيرات البيوكيميائية والهستويثولوجية لمكونات الدم والأنسجة . من أمثلة المواد الحافظة التي تم الدراسة عليها السوربيتول ، نيتريت الصوديوم وحمض الستريك . أجرى هذا البحث على عدد ١٢٠ فأر أبيض قسمت إلى أربعة مجموعات كلأ منها ٣٠ فأراً طبقاً لنوع المواد الحافظة المستخدمة كالآتى :-

- ١- المجموعة الأولى (الضابطة) : تم تغذيتها على غذاء أساسى خالى تماماً من المواد الحافظة طوال فترة التجربة .
  - ٢ - المجموعة الثانية : تم تغذيتها على غذاء مضاف إليه السوربيتول ٠.١ جم / جم .
  - ٣ - المجموعة الثالثة : تم تغذيتها على غذاء مضاف إليه نيتريت الصوديوم ٠.٢ جم / جم .
  - ٤ - المجموعة الرابعة : كانت تتغذى على غذاء مضاف إليه حمض الستريك ٠.٢ جم / جم .
- وقد تم تعاطى هذه المواد في غذاء فئران المجموعات الثانية والثالثة والرابعة لمدة ثلاثة شهور من بداية التجربة . وقد تضمن هذا البحث تحليل كيميائى وحيوى لدم وأنسجة الفئران في المجموع الأربعة .

وأظهرت النتائج مايلى :

زيادة معنوية عالية في نشاط خميرتى الاسبرتات أمينوترانس فيراز والالنين أمينوترانس فيراز في المجموع الثلاثة المعالجة . كما سجل أيضاً نشاط خميرة (الفوسفاتيز القاعدى) زيادة معنوية كبيره في كل مجموعات الفئران المعطاه المواد الحافظة لمدة شهر وشهرين وثلاثة شهور ماعدا بعد الشهر الثالث في مجموعة حمض الستريك . ولقد سجلت النتائج زيادة معنوية في الصوديوم في فئران المجموعة الرابعة بعد الشهر الثالث ونقص معنوى بعد الشهر الأول والثانى . كما أظهرت النتائج وجود نقص معنوي في مستوى البوتاسيوم في جميع مجموعات الفئران المعطاه مواد حافظة لمدة شهر وشهرين وثلاثة شهور ماعدا بعد الشهر الأول في المجموعة الثانية والشهر الثالث في المجموعة الرابعة . كما كان هناك زيادة معنوية في مستوى اليوريا في فئران المجموعة الثالثة ولكن المستوى أظهر نقصاً غير معنوى في فئران المجموعة الرابعة . كما أظهرت النتائج أن كل مجموعات الفئران المعطاه مواد حافظة لمدة شهر وشهرين سجلت زيادة معنوية في مستوى حمض البوليك في المجموعة الثانية والثالثة والرابعة ماعدا بعد الشهر الأول في مجموعة نيتريت الصوديوم . كما سجل الكرياتينين زيادة معنوية في فئران المجموعة الثالثة بعد الشهر الثالث . أظهر الفحص المجهرى وجود تغيرات مرضية في كل من الكبد والكلى وصلت في بعض الحالات إلى مرحلة التنكز . يتضح من النتائج أن إضافة نيتريت الصوديوم أو السوربيتول أو حامض الستريك يؤثر على وظائف كلاً من الكبد والكلى . لذلك يجب أن نقتل من الاستخدام لهذه المواد الحافظة والتي تؤثر على الصحة العامة .