

The Protective Role Of Royal Jelly Against Sodium Nitrate And Sun-Set Yellow Toxicity In Albino Rats

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Abstract

In last few years, all over the world, food preservatives and favorable food colour are used in wide scale. However, their use in food still controversial.

It causes and will cause severe tension to the consumers as the sensitivity of people to general health increases.

This work was carried out to study the possible toxic effect of the interaction of one of food preservatives (sod. nitrite) and one of the most favorable food colour (sun-set yellow) on rats. To study the effect of this interaction, a mixture of 1/10 of the limited dose of NaNO_3 and sun-set yellow was daily administered to rats. Other group was supplemented with royal jelly in combination with the mixture to evaluate its possible protective role during the course of experiment. Treatments were continued to 30 days, then half of the animals were sacrificed, the other half was left for 15 days after the last dose without any additional treatment (as a recovery period). The result can be summarized as follows:

1. Administration of sod. nitrite and sun-set yellow produced a significant decrease in percentage of body weigh, W.B.Cs, R.B.Cs, Hb, Hct, inorganic phosphorus, serum protein and serum albumin of rats.
2. A marked increase in respiration rate, serum glucose, T_3 , T_4 , calcium, γ -GT, LDH, CPK, alk. ph., serum cholesterol and (brain, liver and heart cholesterol) was recorded during treatment with the mixture.
3. Insignificant change in organ / b.wt., heart beat, rectal temperature, serum and tissue AST and ALT, serum acid phosphates, tissue proteins, serum and tissue total lipids muscle and kidney cholesterol and serum triglycerides was determined.
4. Administration of royal jelly and to some extent a recovery period ameliorated many hazards produced by using food additives. So, this study threw light on the bad behavior and its hazards of using food additives and food colour in the same time by our kids. It is also clear that royal jelly as a natural product nearly ameliorate these damage. So, it's advisable to administered royal jelly to children and prevent if possible the using of additive and synthetic colour in their food.

Introduction

Nitrites are used as human food additives, mainly for production of specific flavour, formation of characteristic colour and for preservation of meat products and canned meat under prescribed limitation against the changes of botulism (Wolff and Wasserman, 1972, and Laver, 1991). For the same purposes, nitrites are added to some extent to fish and dairy products.

Nitrites could be formed in food stuffs, as well as in the environment and in the human body itself by nitrifying and denitrifying micro-organisms (Asali-na *et al.*, 1971 and Friedman *et al.*, 1972).

The formation of nitrosamines has been demonstrated under natural conditions from secondary amines and nitrite in the presence of certain bacteria present in the humans digestive tract and urinary tract infection (Alam *et al.*, 1971).

Colorants play a significant role in enhancing the aesthetic appeal of food. Foods that are aesthetically pleasing are more likely to be consumed and to contribute to varied diet and hence better nutrition (Newsome, 1968). Although the importance of food colorants, a wave of awareness and concern about many adverse effects of synthetic food colorants on human health is growing. In Egypt, there has been a sharp increase in the use of synthetic food colorants in the past few years (Saleh, 1994).

Dangers of food preservatives and dyes, urged for searching for natural substances that may have an antagonistic action against the hazardous effects of food colorants. So in this demonstration, royal jelly was tested as a protective agent. Royal jelly is a thick milky food secreted by young nursing

worker of bee. It is composed of moisture (66.05%), protein (12.34 %), total reducing substances (12.49 %), and vitamins (Young and Cho, 1977). Ether extract from royal jelly was free fatty acid, (68.5%) of which was 10-OH-2-decenoic acid which is considered as the purity index of the royal jelly. It also contained abundant vital minerals such as Na, K, P, Mg, Ca, Mn, Fe and Zn (Wen and Hwany, 1994). Organic acid of royal jelly has bacteriostatic activity (Bonvehi and Jouda, 1991). Sayer *et al.* (1996) reported the immune modulator potential of royal jelly in rats and mice.

It has been noticed that children used to have food and drinks which contain food preservatives and colorants at the same time. This behavior attracted my attention to study the interaction between food preservatives and colorants. Since, sodium nitrite is one of the most famous food preservative and sunset yellow is one of the most attractive colorants. I mixed limited dose of each and tested it on rats. I found that the interaction of the limited dose of each gave a new compound with lethal dose. Hence, this study was planned to investigate the effect of this new compound on physical, hematological and biochemical parameters of male albino rats, as well as the effect of royal jelly as antidote to this new compound. And to see if it could ameliorate the expected hazards.

Material and methods

Thirty growing male albino rats aged about one month were used in this work. They were randomly divided into three groups of 10 rats each. All groups were kept in wire cages under the same condition. Fresh tap water and standard

rodent diet were provided *ad libitum* throughout the experimental periods.

The first group served as control group. The second group were received 10 mg NaNO₃ and 0.5 mg sun-set yellow (S.S.Y) /kg daily for a month. The third group received the same dose of NaNO₃ and S.S.Y in addition to 100 mg/kg royal jelly (R.J). All treated doses were given by gastric intubations to each rat.

Body weights, respiration rate, heart beats and rectal temperature were recorded once a week throughout the experimental period. After 30 days of treatment, Five animals of each group were decapitated. While the other half of each group was kept for two weeks, without any additional treatment for recovery.

At the end of the experiment, the animals were weighed and killed by decapitation. Liver, brain, kidney, heart and testis were removed, cleaned from adherent tissues and weighed at once. Pieces of liver, skeletal muscle, kidney, heart and brain were weighed and put in an appropriate amount of 30 % potassium hydroxide for total protein determination. Another pieces were put in concentrated sulfuric acid for total lipid determination.

Blood samples were collected for hematological and biochemical analysis. EDTA, an anticoagulant, was added to the collected blood for hematological parameters, while the blood samples for biochemical parameters were centrifuged for 10 min. at 5000 rpm and supernatant sera were separated for analysis without storage or delay. Hemoglobin concentration was determined according to Van-Kampen and Zulstra (1961). Red and white blood cells were counted and Haematocrit values (Hct) were estimated using the technique of Rodak (1995). The

biochemical analysis were carried out on the blood sera.

Glucose determination was based on the enzymatic method described by Siest and Schielf (1991). AST and ALT activities were accomplished using the method of Reitman and Frankel (1975). Gamma-glutamyl transpeptidase was estimated by the method of Meister *et al.* (1981). While, Lactic dehydrogenase (LDH) activity was determined according to Raabo (1963). Creatin phosphokinase assay was performed using sigma chemical company reagent kits (St. Louis Mo). Alkaline phosphatase activity was measured according to the method of Belfield and Goldberg (1971). The activity of plasma acid phosphatase was determined according to the method of Tietz (1986). Thyroid hormones assay were determined by using the enzyme-linked immunosorbent assay (ELISA) (Whitly *et al.*, 1996). Inorganic phosphorus was determined according to the method of Fisk and Subbarow (1952) and serum calcium (Ca⁺⁺) concentration was estimated according to the method adopted by Ray Sarker and Chauhan (1967). Total proteins were estimated using the Biuret method as described by Doumas (1975). Albumin was determined according to the method of Webster (1977). Total lipids were determined according to the method of Knight *et al.* (1972), while serum cholesterol was determined as mentioned by Fossati and Medici (1987). And serum triglycerides were determined by the method of Rojkin *et al.* (1974).

Student t-test was used to compare between means of the different experimental animal groups. Significant differences between means of the control and treated groups were considered only at P<0.05 (Sokal and Rahif, 1981).

Results

The present findings indicated that the group supplemented with sod. nitrite and sun-set yellow (S.S.Y) showed significant body weight loss ($P<.01$) after feeding for 30 days, and showed a significant reduction in body weight gain after 15 days of treatment stoppage (post effect) when compared with the weights of the control group. While, royal jelly treated group showed a significant decrease ($P<.01$) of the mean body weight gain for the two periods (Table, 1).

There was insignificant changes in the percentage of the mean relative kidney, brain, heart, liver and testis weight of all treated groups (Table, 1).

Furthermore, no significant differences were recorded either in rectal temperature, in respiration rate or heart rate of all treated groups as compared with the control one at different experimental periods (Table,2).

The number of WBCs as well RBCs, HB% and Hct % were significantly decreased ($(P<.01)$) after 30 days of treatment with both NaNO_3 and S.S.Y. This decrease continued even after the 15 days period where the animals were left for recovery. Daily treatment with royal jelly for 30 days produces insignificant change in all hematological parameters tested when compared with the control group even after the recovery period (Table, 3).

The group treatment with both sodium nitrite and sun-set yellow induced a significant increase of serum glucose level throughout the experimental period. When animals were administered royal jelly in addition to the mixture of additives, no significant change in serum glucose was recorded (Table, 4).

In relation to thyroid function, the data obtained showed that NaNO_3

and S.S.Y ingestion for 30 days led to a significant increase ($P<.01$) as indicated by higher serum T3 and T4 concentrations than that in control group (Table, 4). On the other hand, royal jelly caused insignificant change in serum T3 and T4 level as compared with the control group (Table, 4).

Table (4) showed calcium and inorganic phosphorous contents in control, a mixture ($\text{NaNO}_3 + \text{S.S.Y}$), and in mixture with royal jelly treated groups after 30 days of treatment and 15 days post-treatment. Serum calcium level was significantly increase ($P<.01$) after both treated and recovery periods. Rats treated with royal jelly in combination with food additives showed insignificant change in serum calcium level throughout the experimental period. The data showed that the inorganic phosphorus level increased significantly ($P<.01$) in the groups treated with ($\text{NaNO}_3 + \text{S.S.Y}$) and ($\text{NaNO}_3 + \text{S.S.Y} + \text{R.J}$) as compared with the control group. After the recovery period, serum inorganic phosphorus level in treated group ($\text{NaNO}_3 + \text{S.S.Y}$) showed a significant increase ($P<.01$), while the group treated with royal jelly, in addition to the mixture, showed insignificant change in comparison with the control group.

Concerning AST and ALT activities of serum and tissues, no appreciable changes have been recorded (data was in normal value) in all treated groups throughout the experimental period (Tables, 5 & 6).

Table (7) depict enzyme activities in serum of different rat groups. CPK, γ -GT and alk. phosphates activities were found to be significantly increased ($P<.01$) in rats treated with ($\text{NaNO}_3 + \text{S.S.Y}$). The effect of the mixture ($\text{NaNO}_3 + \text{S.S.Y}$) on serum

LDH activity was significant increase ($P<.01$) after treated period only. Furthermore, serum acid phosphates activity was insignificant in all treated groups as compared with the control group. On the other hand, no detectable changes were observed in all the detected enzyme activities of our study in the group treated with royal jelly in addition to ($\text{NaNO}_3 + \text{S.S.Y}$) after the two periods of the experiment.

The treated group with sod. Nitrite and S.S.Y for one month caused significant decrease ($P<.01$) in total serum protein and serum albumin (Table, 8). The animals fed with royal jelly for one month of treatment with ($\text{NaNO}_3 + \text{S.S.Y}$), no significant changes were observed in the concentration of serum total protein and serum albumin. Moreover, no significant change was recorded after the recovery period (15 days). The serum globulin level of all treated groups was not changed after 30 days of treatment. Yet, it is significantly decreased ($P<.05$) in all treated groups after the recovery period. The A/G ratio level in rats treated with mixture of ($\text{NaNO}_3 + \text{S.S.Y}$) induced insignificant change throughout the experimental period. When rats treated with the mixture and royal jelly for one month, the level of A/G ratio was significantly increased ($P<.05$). This decrease was more pronounced ($P<.01$) after the recovery period. On the other hand, total protein level in all tested organs was insignificantly change in all groups till the end of the experiment (Table, 8).

The treatment of rats with the mixture ($\text{NaNO}_3 + \text{S.S.Y}$) or the mixture with royal jelly induced insignificant changes of serum and tissues total lipids level of all treated animal group till the end of the experiment (Table, 9). Total cholesterol in serum was significantly elevated ($P<.05$) in rats treated with

($\text{NaNO}_3 + \text{S.S.Y}$) after 30 days of treatment. Total cholesterol in brain, liver and heart, however, recorded highly significant elevation ($P<.01$) after 30 days of treatment with the mixture. On the other hand, muscle and kidney cholesterol did not induce any significant change in all treated groups. After the recovery period (15 days post-treatment) cholesterol level of serum, liver, brain and heart was turn back to the normal value.

In conclusion, using of the royal jelly ameliorated the hazard effects of the mixture on serum and tissues cholesterol level, where no significant change were recorded (Table, 10).

Discussion

The present results revealed a significant decrease in body weight after one month of treatment and 15 days of recovery for all treated groups. These results were in agreement with those reported by Lachikawa *et al.* (1971) and Hirose *et al.* (1993). They recorded a decease in body weight gain of rats receiving different doses of sodium nitrite. Yet, Greenblatt and Lijinsky (1972) working on male mice and Van logten *et al.* (1972) working on rat observed a decrease in body weight gain in the rats receiving a canned meat treated with sodium nitrite. Yoshida *et al.* (1994) recorded that rats treated with ascorbic acid or sodium ascorbate individually or in presence of sodium nitrite showed a reduction of final body weight. In addition, Shaker and coworkers (1989) found that the supplementation of chocolate brown to the casein diet of rats significantly reduce their body weight after 15 days of feeding. The present results did not show statistically significant changes of the relative weight of the tested organs of the male rats treated with sod. nitrite

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and sun-set yellow or with sod. nitrite, sun-set yellow and royal jelly. Hirose *et al.* (1993); Fujitani (1993) and Yoshida *et al.* (1994) reported an increase of absolute and relative liver and kidney weights of rats treated with sod. nitrite. These results are not in harmony with those of the present study.

Concerning heart beat, rectal temperature and respiratory rate in all treated groups, no significant changes were recorded allover the experimental period.

Alterations of the various hematological parameters in blood of the studied rats were also investigated. These changes induced by NaNO_3 and food colorant may be due to the prevention of red blood cell synthesis *via* inhibition of erythropoiesis in the bone marrow. In agreement with the present work, Mason *et al.* (1974), demonstrated a reduction of hemoglobin when carmoisine was administered to the diet of mice. The present observation of low hemoglobin level may be due to decrease of food intake which leads to insufficiency of protein and / or iron content and their lesser utilization, that hemoglobin synthesis depends on. These observation may be due to that nitrite causes changes in the size of red blood cells. These changes may be related to its effect on hemoglobin (Shuval and Gruener, 1972).

The present results for hemoglobin and hematocrite values are in agreement with those reported by Til *et al.* (1988). Also, Grant and Butler (1989) observed that hemoglobin content and hematocrite value showed a tendency toward a lower values in rats received different doses of nitrite.

Treating rats with both sod. nitrite and sun-set yellow induced a significant increase in the blood sugar level. However, one can expect that sod.

nitrite produced hyperglycemia due to deficiency of insulin release. It is known that nitric oxide is formed from nitrites at least by the vascular epithelial cells (Harrison and Bates, 1993 and Katzung 1995). Both nitric oxide and nitrites open potassium channel (Katzung, 1995), which through closing voltage gated calcium channels decrease intracellular calcium. Calcium is known to trigger insulin secretion, calcium channel blockers are known to produce hyperglycemia (Katzung, 1995).

The present results go in parallel with those reported on the hyperglycemic effect of sodium nitrite in rats (Abdel-Rahim *et al.*, 1988 and Shelpov *et al.*, 1991). They reported that in presence of nitric ion, the activity of amylase increases beside an inhibition of adrenaline-induced activation of phosphorylase. This results in the liberation of glucose from glycogen, so blood glucose increases while liver glycogen decreases.

The elevation of level of serum glucose was also interpreted by the effect of sun-set yellow on enzyme system of the glycolytic pathway. It is not surprising to find an enhanced hyperglycemia due to the new compound resulted from the reaction of sodium nitrite and sun-set yellow treatment of the rats. The observed improvement shown in glucose in royal jelly treated group may be due to its action as antioxidant.

The degradation process of body weight in rats fed on NaNO_3 and S.S.Y paralleled the significant increase in activity of thyroxin T_3 and T_4 hormones of the thyroid gland. The present investigation is supported by the findings of El-Saadany (1991) who found that synthetic colorants (Chocolate; Indigo-carmine and Carmoisine) significantly increased T_4 hormone. He suggested that hyperthyroidism may play a signi-

ficant role in the genesis of decreased serum lipids content of rats fed on these synthetic chocolate colorants. There exists a positive correlation between relative body weight and plasma concentrations of lipids (Harper *et al.*, 1993). Therefore, the reduction of body weight may be attributed to the stimulation of thyroid gland through alteration in the pituitary-thyroid axis as a consequence of the stressing effects induced by the feed additives which may be stern an increase in rate of release or formation of thyroid stimulation hormone by the pituitary gland, elevating T₄ value, ther -eby increasing energy expenditure whi -ch would account for decreasing the fat pattern metabolism (Abdel Rahim *et al.*, 1993)

In the current study, the mechanism by which sodium nitrite altered thyroid function is still uncer -tain. It could be proposed that nitrite may enhance intrathyroidal synthesis of thyroid hormones and increases the extrathyroidal conversion of T₃ and T₄ meanwhile, nitrites may attenuate the binding capacity of thyroid binding proteins for thyroid hormones (Heiba -shy and Abd El- Moneim, 1999).

The present study revealed that administration of sod. nitrite and sun-set yellow to rats caused variable degree of stimulation of thyroid gland function after treated and recovery periods. This was proved by the significant increase in serum thyroid hormones T₃ and T₄. The interaction between sod. nitrite and sun-set yellow may give a new chemical component, which has a stimulatory effect on thyroid gland. It is also clear that royal jelly ameliorate this effect due to its antioxidant property. This could be by blocking the generation and propagation of free radicals.

The result of this study demo -nstrated that the administration of

(NaNO₃ + S.S.Y) caused a significant increase in serum calcium level. This observation was similarly recorded by Sharma (1989), who recorded higher values of minerals in rats treated with metanil yellow. On the other hand, the present results showed a significant decrease in serum phosphate after treat -ment with the mixture of (NaNO₃ + S.S.Y) during the treated period, while after the recovery period it turned back to the normal value in royal jelly treated group. Helal *et al.* (2000) stated that S.S.Y did not affect serum level of both Ca and Ph. So, the present results may be due to the new compound resulted from the reaction between both NaNO₃ and S.S.Y.

Measurements of certain enzy -me activities in the blood are of great value in liver diseases but their investigation is complicated by many factors including the existence of isoenz -ymes, their rate of disappearance by excretion or destruction, their rate of release from cells due to increased perm -eability or obstruction of the ducts of secretory organs, as well as increased enzyme synthesis, perhaps associated with regeneration.

AST and ALT activities of serum and tissue were still in normal range in all treated groups. In harmony with these findings, (Ford *et al.*, 1987), stated that tartrazine and carmoisine caused insignificant changes in rat serum AST and ALT. The liver cells also play an important role in both synthesis and secretion of γ -GT and alk. phosphates. Therefore, the alteration in their activities are attributed to early cholestatic liver damage which prim -arily affects the liver parenchyma, thus making alk. phosphatase and γ -GT sensitive indices in the early diagnosis of infiltrate diseases (El-Elaimy and El-Nabi, 1990).

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In the present work, both γ -GT and alk. phosphatase were increased in the group of rats given (NaNO₃⁺ S.S.Y). However, the significant reduction in γ -GT and alk. phosphatase activities in royal jelly treated group may be due to its protective role against the marked effect of the mixture on liver.

The elevation of serum CPK and LDH in a mixture (NaNO₃⁺ S.S.Y) treated rats, could be attributed to a generalized increase in membrane permeability and is particularly useful in the diagnosis of muscular disorder, especially progressive muscular dystrophy (Doran and Wilkinson, 1975; Ebashi *et al.*, 1959). The insignificant increase of acid phosphatase in the present findings is in accordance with Singh and Khanna (1979), who found no marked alteration in the hepatic and serum activities of GPT, GOT and acid phosphatase in rats fed orange II.

A significant decrease of the total serum protein was recorded after treatment with the mixture of NaNO₃⁺ S.S.Y for one month. The decrease of total serum protein due to the mixture treatment was reflected on serum albumin level where a remarkable decrease was recorded. This decrease may be resulted from liver function impairment induced by the nitrite. The globulin fraction, on the other hand, was not affected generally at the same time, but it was affected after the recovery period. Supplementation rats with both royal jelly and the mixture lead to a decrease in globulin all over the experimental time and an increase A/G ratio after the recovery period.

Eremin and Yocharina (1981) reported the harmful effect of nitrite which reflected on the biosynthesis of protein. They recorded a decrease in serum protein of rats due to the stimulatory effect of the nitrite on the thyroid and adrenal gland, which leads to block

of protein synthesis while fast break-down, occurs. In agreement with our results, Sharma (1989) and Mackenzie *et al.*, (1992) recorded a significant reduction in serum protein after administration of metanil yellow or caramel to rats, respectively

Effects of nitrite and sun-set yellow on most of the tested parameters were significantly counteracted by oral administration of royal jelly as compared with the non treated control group.

The lipid profile of the studied rats revealed a significant increase in serum, brain, liver and heart cholesterol, while no significant change was recorded in serum and organs total lipids. The present hypercholesterolemia in combination with normal total lipids level may be resulted in elevation of the risk ratio in the mixture treated rats, make the new component (within the mixture) a potential incriminates for risk of ischemic heart disease. In accordance with these observations it was reported that hypercholesterolemia (Schaefer *et al.*, 1981) has been considered as risk factors and important diagnostic criteria in differentiating individuals with coronary heart disease. Royal jelly supplementation prevented the elevation of cholesterol profile. Also, after the recovery period cholesterol levels of serum, brain, liver and heart were return back to their baseline values. So, it is advisable to supplement royal jelly simultaneously to kids who are at high risk of food additives interaction to alleviate their toxic effects.

It is known that in Egypt there is sometimes uncontrolled use of food additives particularly in food consumed mainly by children, hence the safe use of those compounds should be investigated. Cooperation between public health authorities and other ministries concerned with food safety is necessary legislation and specification of

food additions should be always carried out.

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Table (1): The percentage change in body weight and the organ /somatic ratio in control, treated with (NaNO₃ + S.S.Y) and treated with (NaNO₃+ S.S.Y + royal jelly) after treated and recovery periods in young albino rats.

Parameter		Treated period (30 days)			Recovery period (15 days)		
		Control	NaNO ₃ + S. S. Y	NaNO ₃ + S.S.Y and royal jelly	Control	NaNO ₃ + S. S. Y	NaNO ₃ + S. S. Y + Royal jelly
% of body weight change	X S.E P	19.6 0.26	-11.9 0.69 <.01	16.4 0.60 <.01	18.94 0.40	10.6 0.40 <.01	15 0.70 <.01
Kidney/ b.wt	X S.E P	0.70 0.12	0.57 0.12 ---	0.57 0.13 ---	0.60 0.13	0.57 0.12 ---	0.58 0.14 ---
Brain / b.wt	X S.E P	0.86 0.13	0.86 0.13 ---	0.89 0.13 ---	0.90 0.12	0.85 0.14 ---	0.87 0.14 ---
Cardio- somatic index	X S.E P	0.60 0.12	0.54 0.12	0.55 0.13 ---	0.62 0.14	0.55 0.12 ---	0.55 0.12 ---
Hepato- somatic Index	X S.E P	3.2 0.19	3.1 0.10 ---	3.1 0.19 ---	3 0.10	2.9 0.10 ---	2.98 0.19 ---
Gonado- somatic index	X S.E P	1.07 0.12	.9 0.15	0.95 0.14	1.1 0.12	.95 0.15	.94 0.14

Table (2): Changes in respiration rate, heart beats and rectal temperature in control, treated with food additives and treated with (Food additives and royal jelly) in young rats after both treated and recovery periods.

Parameter		Treated period (1 month)			Recovery period (15 days)		
		Control	NaNO ₃ + S.S.Y	NaNO ₃ + S.S.Y. and royal jelly	Control	NaNO ₃ + S.S.Y.	NaNO ₃ + S.S.Y. + Royal jelly
Respiration rate (breath/min)	X S.E P	49 1.8	65 0.50 <.01	46.6 1 -	48.2 1.2	48.6 0.90 -	45.8 1.2 -
Heart beats (beat /min)	X S.E P	136 1.8	139.6 0.70 -	139.6 1.1 -	134 1.8	133.6 1.1 -	132.4 1.1 -
Rectal temperature, °C	X S.E P	34.72 0.09	34.54 0.70 -	34.76 0.30 -	34.64 0.15	33.94 0.40 -	34.4 0.30 -

Table (3): Changes in some haematological parameters (W.B.Cs, R.B.Cs, Hb% and Hct% in rats treated with food additives, food additives and royal jelly and their control after treated (30 days) and recovery (15 days) periods.

Parameter		Treated period (30 days)			Recovery period (15 days)		
		Control	NaNO ₃ + S.S.Y	NaNO ₃ + S.S.Y. and royal jelly	Control	NaNO ₃ + S.S.Y.	NaNO ₃ + S.S.Y. + Royal jelly
W.B.Cs X10 ⁶	X S.E P	8.5 0.20	6.9 0.10 <.01	8.08 0.10 -	8.82 0.05	7.16 0.10 <.01	8.76 0.09 -
R.B.Cs X10 ³	X S.E P	5.88 0.05	4.9 0.05 <.01	5.9 0.07 -	5.9 0.07	5.08 0.06 <.01	5.88 0.09 -
Hb%	X S.E P	16 0.10	13.78 0.10 <.01	15.98 0.15	15.82 0.10	14.2 0.20 <.01	15.66 0.09 -
Hct %	X S.E P	41.4 0.90	36.8 0.50 <.01	39.4 0.70 -	42 0.90	37.2 0.50 <.01	41 0.80 --

Table (4): Biochemical and hormonal changes in serum of rats treated with food additives, food additives and royal jelly and their control after treated and recovery periods.

Parameter		Treated period			Recovery period		
		Control	NaNO ₃ + S.S.Y	NaNO ₃ + S.S.Y. and royal jelly	Control	NaNO ₃ + S.S.Y.	NaNO ₃ + S.S.Y. + Royal jelly
Glucose Mg/d L	X S.E P	93.6 2	134.4 1.9 <.01	90.2 2.1 -	92.4 1.7	105.4 1.7 <.01	8.9 1.8 -
T3 mg/d L	X S.E P	166 1.8	189 1.8 <.01	162 1.9 -	157.8 1.1	168.8 0.90 <.01	160 1.5 -
T4 mg/d L	X S.E P	7.64 0.30	9.5 0.10 <.01	7.44 0.20 -	6.84 0.09	7.4 0.10 <.01	6.7 0.12 -
Calcium mg/d L	X S.E P	7.9 0.20	9.875 0.20 <.01	7.82 0.20 -	7.5 0.18	8.7 0.20 <.01	7.48 0.20 -
Inorganic phosphorus mg/d L	X S.E P	12.04 0.18	9.9 0.20 <.01	10.94 0.20 <.01	11.94 0.18	10.8 0.20 <.01	11.6 0.18 -

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Table (5): AST activity in serum, liver, muscle, kidney and heart in food additives, food additives and royal-jelly treated rats in comparison with their control after treated and recovery periods

Parameter		Treated period (30 days)			Recovery period (15days)		
		Control	NaNo ₃ + S.S.Y	NaNo ₃ + S.S.Y and royal jelly	Control	NaNo ₃ + S.S.Y	NaNo ₃ +S.S.Y + Royal jelly
Serum AST u/L	X S.E P	23.8 0.70	37 1.2 normal value	21.6 0.90 normal value	21.2 1	27.4 1 normal value	18.4 0.90 normal value
Brain AST u/g	X S.E P	15 0.40	24.4 0.90 normal value	16.5 0.60 normal value	14 0.70	21.6 0.50 normal value	16 0.80 normal value
Liver AST u/g	X S.E P	18 1.1	26.2 0.50 normal value.	18 0.50 normal value	17.2 0.80	17.2 1.1 normal value	17 0.60 normal value
Muscle AST u/g	X S.E P	16.6 1.4	21 1.1 normal value	17 0.90 normal value	16.8 1.7	14.8 0.90 normal value.	15 0.40 normal value
Kidney AST u/g	X S.E P	16.8 1.2	23 1.5 normal value.	14 0.20 normal value	17 0.70	16.6 1.6 normal value	15.2 0.80 normal value
Heart AST u/g	X S.E P	13.4 0.50	20.6 1.4 normal value	18 0.08 normal value	13.8 0.90	18.4 0.70 normal value	15 0.30 normal value

Table (6): ALT activity in serum of rats, in brain, liver, muscle, kidney and heart treated with food additives or food additives and royal jelly and their control after treated and recovery periods.

Parameter		Treated period (30 days)			Recovery period (15days)		
		Control	NaNo ₃ + S.S.Y	NaNo ₃ + S.S.Y. and royal jelly	Control	NaNo ₃ + S.S.Y.	NaNo ₃ + S.S.Y. + Royal jelly
Serum ALT u/L	X S.E P	22.2 1	28 1 normal value	21.6 0.90 normal value	21.2 1	27.4 1.0 normal value	18.4 0.90 normal value
Brain AST u/g	X S.E P	14 0.70	23.2 2 normal value	14.4 0.20 normal value	14.2 0.50	19 0.40 normal value	15 0.30 normal value
Liver ALT u/g	X S.E P	16.4 0.50	24.2 2 normal value	15.4 0.40 normal value	15.2 0.60 normal value	21.2 0.60 normal value	14.8 0.30 normal value
Muscle ALT u/g	X S.E P	18.6 0.20	24.2 1.1 normal value	16.6 0.60 normal value	16.4 1.2 normal value	21.4 0.40 normal value	16.5 0.60 normal value
Kidney ALT u/g	X S.E P	16 0.70	20.6 0.40 normal value.	15.2 0.90 normal value	15.2 0.90 normal value	17.4 0.90 normal value	15.4 0.90 normal value

Heart ALT u/g	X S.E P	18.8 0.60	21.4 0.80	13.2 0.40	16.4 1.2	18.6 0.50	14.5 0.90
			normal value.	normal value	normal value	normal value	normal value

Table (7): Some serum enzyme activities in rats treated with food additives and royal-jelly and their control after both treated and recovery periods.

Parameter		Treated period (1 month)			Recovery period (15 days)		
		Control	NaNO ₃ + S.S.Y	NaNO ₃ + S.S.Y and royal jelly	Control	NaNO ₃ + S.S.Y	NaNO ₃ + S.S.Y + Royal jelly
γ - GT u/L	X S.E P	16.8 0.70	37.2 0.90 <.01	18.4 0.80 -	16.8 0.50	31 0.40 <.01	18.4 0.60 -
LDH u/L	X S.E P	245 2.2	266 2.4 <.01	250 2.2 -	244 2.4	244 2.4 -	239 1.8 -
CPK u/L	X S.E P	55 2.2	74 2.4 <.01	54 2.4 -	54 1.8	63 2 <.01	54.2 1.9 -
Alk ph/ u/L	X S.E P	96 0.60	106 1.8 <.01	93 2.2 -	91 0.90	115 2.2 <.01	94 2.4 -
Acid ph. u/L	X S.E P	9 0.40	10.6 0.40 -	10 0.30 -	10.36 0.20	11.3 0.30 -	10.6 0.20 -

Table (8): Protein contents of serum and some organs in rats treated with food additives or food additives and royal jelly and their control after treated and recovery periods.

Parameter		Treated period (30 days)			Recovery period (15 days)		
		Control	Treated	Treated + R.J	Control	Treated	Treated + R.J
Serum Protein g/dL	X S.E P	8.34 0.30	6.48 .030 <.01	7.8 0.20 -	7.9 0.16	7.78 0.19 -	7.7 0.15 -
Brain Protein mg/g	X S.E P	110.4 0.60	110 0.70 -	110.6 0.20 -	110 0.70	110.5 0.80 -	110.4 0.40 -
Liver Protein mg/g	X S.E P	56 1.8	55 2.2 -	57 3 -	50 3.5	53.6 2.4 -	55.2 1.5 -
Muscle Protein mg/g	X S.E P	92 1.2	90.8 0.90	92.6 1.1 -	92.4 0.40	91.4 0.90 -	92.2 1.1 -
Kidney Protein mg/g	X S.E P	68.4 0.90	64.4 2.1 -	66.4 2.1 -	67.6 1	64.2 1.5 -	65.4 0.60 -
Heart Protein mg/g	X S.E P	88 0.90	85.2 1.5 -	85.2 1.4 -	87 1.1	88.6 0.40 -	85.4 0.50 -
Serum Albumin g/dL	X S.E P	5.54 0.10	4.22 0.10 <.01	5.76 14 -	4.9 0.14	4.84 0.09 -	5.06 0.09 -

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Serum Globulin g/dL	X S.E P	2.71 0.13 -	2.41 0.17 -	1.84 0.10 -	3.1 0.10	2.6 0.18 <. 05	2.04 0.10 <. 05
A/G	X S.E P	2.04 0.10	1.98 0.18 -	3.3 0.12 <. 05	1.58 0.10	1.76 0.22 -	2.66 0.10 <. 01

Table (9): Total lipids level of serum and some organs in rats treated with food additives or food additives and royal jelly and their control after treated and recovery periods.

Parameter		Treated period (30 days)			Recovery period (15days)		
		Control	NaNO ₃ + S.S.Y	NaNO ₃ + S.S.Y and royal jelly	Control	NaNO ₃ + S.S.Y	NaNO ₃ + S.S.Y + Royal jelly
Serum Total lipids g/dL	X S. E P	388 4.8	390 5.1 -	382 4.8 -	382 7.3	388 7.3 -	368 5.8 -
Brain Total lipids Mg/g	X S. E P	49 1.8	58 4.1 -	53 2.2 -	51.6 2.6	52 2.5 -	49.4 0.40 -
Liver Total lipids mg/g	X S. E P	92.6 2.8	99 2.4 -	95.6 1.7 -	92 2.5	96 1.4 -	96.8 0.60 -
Muscle Total lipids mg/g	X S. E P	46.4 2.7	56.2 4.1 -	47 3 -	45 2.8	47 1.2 -	43.4 1.8 -
Kidney Total lipids mg/g	X S. E P	44.4 1.5	53.2 3.8 -	44 0.60 -	45 1.4	48.2 1.3 -	42.8 0.60 -
Heart Total lipids mg/g	X S. E P	53.6 1.8	55.8 2.3 -	50.2 0.50 -	49.6 2.2	44.8 5.2 -	45.4 1.6 -

Table (10): Cholesterol level of serum and some organs in rats treated with food additives or food additives and royal jelly and their control after treated and recovery periods.

Parameter		Treated period (30 days)			Recovery period (15days)		
		Control	NaNO ₃ + S.S.Y	NaNO ₃ + S.S.Y and royal jelly	Control	NaNO ₃ + S.S.Y	NaNO ₃ + S.S.Y + Royal jelly
Serum Cholesterol g/dL	X S.E P	131 4.5	150 4 <. 05	120 5.8 -	129 7.8	136 4 -	128 3.6 -
Brain Cholesterol mg/g	X S.E P	34.4 1.6	46 2.1 <. 01	35.6 1.2 -	33.8 1.2	37.4 2.2 -	32 0.9 -
Liver Cholesterol mg/g	X S.E P	28 0.90	39.2 1.6 <. 01	28.6 0.90 -	30.8 1.8	26.4 1.5 -	28.8 0.80 -
Muscle Cholesterol mg/g	X S.E P	17.2 0.90	21.8 2.5 -	16.6 0.70 -	14.2 0.90	15.2 1.1 -	13.6 0.50 -

Kidney Cholesterol mg/g	X S.E P	14.4 0.70	16.4 0.70	14.8 0.40	13 0.90	17.6 2.1	14 1.3
Heart Cholesterol mg/g	X S.E P	18.4 0.80	23.8 1.2 <.01	16.6 0.40	17 0.90	21 1.8	14.6 0.60
Serum Triglycerides mg/dL	X S.E P	98 3.7	118 5.8	96 2.4	90.8 1.7	99 3.5	95 1.8

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CPK, LDH, γ -GT

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