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Methodology Article

The Curative Effect of Olive Oil and Ephedra Extract on Some Biochemical and Haematological Parameters of Domestic Male Rabbits After Administration of Nitrosamine

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Abstract: The aim of this study was to determine the level of toxicity of nitrosamine on some biochemical and hematological parameters and the curative effect of olive oil and ephedra extract on domestic 24 male rabbits divided into four groups, each with different doses which were applied for 33 days. In the present study, olive oil and ephedra administration with nitrosamine reduced its induced harm in a significant way. These finding suggest that olive oil, effectively, reduces nitrosamine-induced toxicity more than ephedra, and generally, their use concomitantly with nitrosamine have an extremely beneficial role in overcoming the adverse effects occurring due to chronic ingestion of nitrosamines.

Keywords: Nitrosamine, Olive Oil, Ephedra, Biochemical and Hematological

1. Introduction

N-Nitrosamines are mutagenic and carcinogenic chemicals and are present in large quantities in tobacco smokes [1] pacifiers and baby bottle nipples [2], and cured meats and smoked fish [3]. In addition, N-nitrosamines can be formed endogenously from interaction of nitrate, nitrite with secondary or tertiary amines and amides in human stomach [4]. Moreover, a variety of over-the-counter drugs, food additives, cosmetics, and many agricultural chemicals have been identified as having secondary or tertiary amine or amide groups in their structure that can react with nitrite to form nitrosamines and nitrosamides from simulating human gastric conditions [5–10]. Furthermore, nitrosation of drugs with tertiary amines or amides resulted in the production of known carcinogens [11]. Recently, it has been found that prenatal exposure to nitrosatable drugs may be associated with several congenital malformations, especially with higher nitrite intake [12]. Various N-nitrosamines have been observed to cause abnormal development through DNA alkylation of target genes [13]. A frog embryo exposed to N-nitrosamines was found to develop severe heart defects [14]. In rats, maternal exposure to such compounds resulted in increased incidence of limb malformations, neural tube defects, microcephalus, and hydrocephalus. Several patho-physiological mechanisms involved in the development of atherosclerosis are well known as one of these mechanism it has been proposed that reactive oxygen species or free radical-induced oxidation of lipoproteins may be an important event in this process [15].

Ephedra's (Ephedra sinica Stapf., Ephedra intermedia Schrenk et Meyer, or Ephedra equisetina Bge) active constituents are strong central nervous system stimulants, more powerful than caffeine but less potent than amphetamine [16]. Ephedrine itself opens the bronchial passages, thus

acting as a bronchodilator, stimulates the heart, and increases blood pressure, metabolic rate, and perspiration and urine production. It also reduces the secretion of both saliva and gastric acids [17]. Traditional Zen monks used ephedra to promote calm concentration during meditation. In China, ephedra is popular for chills and fevers, coughs and wheezing, and in combination with rehmannia is given to restores kidney yin and helps in its normal functioning [18]. For asthma use with almond; for "wind-cold" injury use with cinnamon; for allergic skin reaction use with mint and cicada molting. Ephedra is used principally in current Western herbal medicine as a treatment for asthma and hay fever, and for the acute onset of colds and flu. It also helps to raise blood pressure, cool fevers, and alleviate rheumatism [19]. The whole plant contains many compounds, some active, some inert, which in combination seems to act synergistically. The whole plant can be used at a much lower dosage than isolated constituents and it has significant therapeutic effects, including dilating the bronchial airways and increasing blood flow to the skin. Unlike ephedrine, the whole plant rarely gives rise to side effects. Ephedrine causes uterine contractions in laboratory animals. Pregnant women should not use it. Other women may try it to initiate menstruation [17, 19]. As a weight loss agent, ephedra has been commonly combined with caffeine; however, more recently the ephedra component has been replaced with bitter orange in US dietary supplements. Toxicological data on ephedra are limited. While ephedra extracts are cytotoxic to cultivated cells, the cytotoxicity is not primarily caused by ephedrine [20].

Olive oil is rich in monounsaturated fat and antioxidants like chlorophyll, carotenoids and vitamin E. Scientists have identified a compound in olive oil called oleuropein which prevents the LDL cholesterol from oxidizing. It is the oxidized cholesterol that sticks to the walls of the arteries and forms plaque [21]. It was identified that oleic acid, a monounsaturated fatty acid found in olive oil, as having the ability to reduce the affect of an oncogene (a gene that will turn a host cell into a cancer cell). This particular oncogene is associated with the rapid growth of breast cancer tumors. The conclusion of the researchers was that oleic acid when combined with drug therapy encouraged the self-destruction of aggressive, treatment-resistant cancer cells thus destroying the cancer. Olive oil has been positively indicated in studies on prostate and endometrial cancers as well [22].

Unlike other fats, which are associated with a higher risk of colon cancer, olive oil helps protect the cells of the colon from carcinogens. The antioxidants in olive oil reduce the amount of carcinogens formed when meat is cooked. Olive oil is also linked to lower triglyceride levels. Olive oil is very high in vitamin E, but other than that, most of its antioxidant properties come from its phenolic components [23]. Phenolic compounds such as flavonoids are widespread in many plant foods and influence the quality, palatability, and stability of foods by acting as flavourants, colourants, and antioxidants. When consumed, certain phenolic compounds are known to exhibit pharmacological effects on the body, such as anti-carcinogenic (anti-cancer), anti-inflammatory, anti-oxidant, anti-atherogenic effects etc. [21]. Laboratory investigations have found that the phenolic constituents of olive oil can significantly inhibit the oxidation of LDL “bad” cholesterol [22, 23]. Animal experiments have revealed that greater reductions in LDL oxidation occurred in rats, rabbits and hamsters when fed olive oil than when fed oils similar to olive oil other than its phenolic components. Human investigations have revealed greater reductions in LDL oxidation when given extra-virgin olive oil or virgin olive oil with a higher phenolic content than when given other olive oils. These studies suggest that the reduction in LDL oxidation that occurs after consumption of extra-virgin olive oil may be due to its phenolic constituents rather than just its monounsaturated oleic acid and vitamin E content.

The present investigation was aimed to study some biochemical and hematological parameters of adult male rabbits treated with nitrosamine and to compare the therapeutic actions of ephedra extract and olive oil.

2. Materials and Methods

2.1. Experimental Animals and Dosing

Twenty four adult rabbits were used in the present study weighing 1.0 - 1.5 kg. They were purchased from local markets. Rabbits were left in the animals house for 1 week before experimentation to adapt to laboratory condition. They were kept in plastic cages with wire mesh covers and maintained under the following conditions: temperature (20°C–21°C), relative humidity (40% - 60%) and a light /dark cycle of 14 and 10 hours. The cages were freshly spread by wood saw to absorb urine of animals. Rabbits were given free access to commercial balanced diet and water ad libitum all over the experimental period. Animals were divided into four groups comprising six rabbits each: the first group was kept as a control, the second group received nitrosamine at a dose of 5 ml / kg b.wt. once/day (2000 ppm dibutylamine + 4000 ppm nitrate as sodium salt dissolved in distilled water), as recommended by [40], the third group received 5% ephedra extract (5ml / kg b.wt. once/day) and 5ml nitrosamine, while the fourth group received olive oil at a dose of (1 ml / kg b.wt. once/day) and 5ml nitrosamine [39]. All doses were applied for 33 days. All chemicals used were of analytical grade and were bought from Sigma Chemical Company Germany.

2.2. Blood Sampling and Processing

Control and treated rabbits were decapitated at the end of the 33rd day. Blood was collected in dry centrifuge tubes. Sera were separated and kept at -20°C until analysis. However, determinations of enzyme activities were carried out on fresh serum samples, On the other hand, about 2 ml of blood samples were collected in a tube containing dipotasium ethylene diamine tetra acetate (EDTA) for the hematological tests.

2.3. Measurement of Biochemical and Blood Indices

Serum glucose, triacylglycerol and total cholesterol were determined using the method described by Trinder (1969);

Fossati et al., 1980 and Allain et al., 1974. Serum urea measurement was based on the cleavage of urea with urease (Berthelot's reaction) as described by Faweet and Scott (1960), serum uric acid was determined following the method described by Fossati and Prencip (1982). Serum creatinine was measured without protein precipitation according to Bartels and Bohner (1972), serum total protein was described by Biuret reaction as designed by Armstrong and Carr, 1964. The kits were purchased from Biotech laboratories, UK. Serum albumin was determined using RANDOX reagent kits, following their instruction manual according to the method described by Doumas et al., 1971. The concentrations of globulins (g/dL) were equal to total protein – albumin. The activities of serum AST and ALT were determined according to the method described by Gloiser and Mager, 1972. The measurement of serum ALP activity was based on the method of Bessey et al., 1946 and Perry et al., 1983. Estimation of serum Na, K and P were conducted by flame photometric method (Tietz, 1990), estimation of serum Ca was done by Gitelman method (Gitelman, 1967).

2.4. Haematological Parameters

Determination of hematological parameters were carried out using an 18 automated parameter hematology analyzer. ABX Micros 60 from Horiba ABX. France.

2.5. Data Analysis

Data were computer analyzed using SPSS version 13.0 for windows (Statistical Package for the Social Sciences lnc. Chicago, Illinois, USA). Means were compared by independent-samples test followed by Duncan's multiple range test (DMRT), $p<0.05$ were considered as significant. Percentage change was also calculated.

3. Results

Serum glucose, cholesterol, triglycerides, urea, uric acid, creatinine, total protein, albumin and globulin mean values of rabbits affected by nitrosamine (5ml/kg/day), nitrosamine with ephedra (5ml /kg/day) and nitrosamine with olive oil (1ml /kg/day) administration during 33 days were summarized in table 1. Data revealed that administration of nitrosamine with ephedra and nitrosamine with olive oil compared to nitrosamine alone decrease serum glucose level from 16.65% to 10.99% and 10.04% respectively as compared to the control level. Cholesterol was highly significant decreased from 37.38% to 15.59% and 15.35%. While triacylglycerol decreased significantly from 78.02% to 50.33% and 34.71% respectively as compared to the control level. Urea also increased significantly high from 5.69%when nitrosamine administered only to 50.51% when administered with ephedra and 38.57% when administered with olive oil. Results shows uric acid increasing significantly in the same way from 52.78% to 111.11% and 93.89% respectively. Also creatinine increased significantly high from 68.68% to 92.77% and decreased significantly from 68.68% to 62.65% respectively, total protein decreased significantly from -7.90% for nitrosamine alone to -17.92% and -28.04% for nitrosamine with ephedra and with olive oil respectively, same significant decrease was shown in albumin from -11.96% to - 30.51% and -37.46%, while there was a non-significant increase in globulin rate from -2.01% when nitrosamine administered only to 0.75% when administered with ephedra while a significant decrease of -14.07% when administered with olive oil, meanwhile, bilirubin had shown a significant increase when rabbits treated with nitrosamine alone compared to control, there was a clear decrement by applying ephedra as compared to to nitrosamine from 250% to 172.73%, whereas treatment of olive oil significantly increased the levels of bilirubin by 263.64% as compared to control. In the above studied parameters, a high significant decrease in the values was seen when rabbits were treated by nitrosamine with olive oil, as compared to the values when rabbits were treated by nitrosamine with ephedra extract.

Table 1. *Effect of Nitrosamine, Ephedra and Olive Oil Administration on Some Biochemical Parameters of Rabbits.*

Parameters	Experimental Drug			
	Control n=6	Nitrosamine n=6	Nitrosamine with Ephedra n=6	Nitrosamine with Olive oil n=6
Glucose (mg/dl)		105.10±0.30	100.0±0.26	99.15±0.23
% change	90.10±0.3125	16.65	10.99	10.04
P value		P<0.01	P<0.05	P<0.05
Cholesterol (mg/dl)		250.30±0.29	210.60±0.33	210.15±0.25
% change	182.20±0.21	37.38	15.59	15.35
P value		P<0.01	P<0.01	P<0.01
Triacylglycerol (mg/dl)		190.30±0.41	160.70±0.31	144.0±0.17
% change	109.60±0.22	78.02	50.33	34.71
P value		P<0.01	P<0.01	P<0.01
Urea (mg/dl)		30.95±0.23	44.10±0.21	40.60±0.22
% change	29.30±0.20	5.69	50.51	38.57
P value		P>0.05	P<0.01	P<0.01
Uric acid (mg/dl)		5.50±0.25	7.60±0.24	6.98±0.20
% change	3.60±0.28	52.78	111.11	93.89
P value		P<0.01	P<0.01	P<0.01

Parameters	Experimental Drug			
	Control n=6	Nitrosamine n=6	Nitrosamine with Ephedra n=6	Nitrosamine with Olive oil n=6
Creatinine (mg/dl)		1.40±0.06	1.60±0.05	1.35±0.03
% change	0.83±0.04	68.68	92.77	62.65
P value		P<0.01	P<0.01	P<0.01
Total Protein (gm/dl)		9.10±0.24	8.11±0.34	7.11±0.22
% change	9.88±0.36	-7.90	-17.92	-28.04
P value		P>0.05	P<0.01	P<0.01
Albumin (gm/dl)		5.20±0.23	4.10±0.20	3.69±0.26
% change	5.9/±0.25	-11.96	-30.51	-37.46
P value		P<0.05	P<0.01	P<0.01
Globulin (gm/dl)		3.90±0.19	4.01±0.137	3.42±0.14
% change	3.98±0.21	-2.01	0.75	-14.07
P value		P>0.05	P>0.05	P<0.05
Bilirubin (mg/dl)		0.77±0.05	0.66±0.03	0.80±0.04
% change	0.22±0.06	250	172.73	263.64
P value		P<0.01	P<0.01	P<0.01

All values were expressed as mean ± S.E; P<0.05 significant; P<0.01 highly significant.

Enzymes activities and electrolytes mean values of rabbits affected by nitrosamine (5ml/kg/day), nitrosamine with ephedra (5ml /kg/day) and nitrosamine with olive oil (1ml /kg/day) administration during 33 days were summarized in table 2. Results indicated that administration of nitrosamine significantly increased the percentage of sodium and potassium level by 10.99% and 23.42% respectively but decreased calcium level to -13.15%, however when ephedra was administered with nitrosamine, the increment was not significant for sodium (3%) and potassium (5.11%), but highly significant decrement in calcium -21.74% was observed. While treatment of nitrosamine with olive oil increased sodium non-significantly by 0.36% but significantly for potassium and calcium by 17.42% and 17.50% respectively. Enzymes Activities results showed that administration of nitrosamine increased in a highly significant way the activities of ALP, ALT and AST by 23.32%, 31.96 and 28.62% respectively, but when ephedra administered with nitrosamine the increments were significant 11.01%, 13.91% and highly significant at 25.79%, respectively as compared to control level. On the other hand, it was found that administration of nitrosamine with olive oil increased ALT in a high significant way by 27.07%, and showed a non-significant increments for AST and ALP by 6.20% and 4.68% respectively.

Table 2. *Effect of Nitrosamine, Ephedra and Olive Oil Administration on Enzymes Activities and Electrolytes of Rabbits.*

Parameters	Experimental Drug			
	Control n=6	Nitrosamine n=6	Nitrosamine with Ephedra n=6	Nitrosamine with Olive oil n=6
Sodium meq/l		155.50±0.29	144.30±0.27	139.60±0.16
% change	140.10±0.33	10.99	3.00	0.36
P value		P<0.05	P>0.05	P>0.05
Potassium meq/l		4.11±0.14	3.50±0.12	3.91±0.15
% change	3.33±0.18	23.42	5.11	17.42
P value		P<0.01	P>0.05	P<0.01
Calcium mg/dl	9.20±0.15	7.99±0.17	7.20±0.21	10.81±0.18
% change		-13.15	-21.74	17.50
P value		P<0.05	P<0.01	P<0.01
ALT (U/L)		35.10±0.29	30.30±0.31	33.80±0.16
% change	26.60±0.22	31.96	13.91	27.07
P value		P<0.01	P<0.05	P<0.01
AST (U/L)		40.90±0.27	40.0±0.28	33.77±0.21
% change	31.80±0.30	28.62	25.79	6.20
P value		P<0.01	P<0.01	P>0.05
ALP (U/L)		98.90±0.35	98.10±0.34	83.95±0.24
% change	80.20±0.36	23.32	11.01	4.68
P value		P<0.01	P<0.05	P>0.05

All values were expressed as mean ± S.E; P<0.05 significant; P<0.01 highly significant.

Change in blood indices after administration of nitrosamine (5ml/kg/day), nitrosamine with ephedra (5ml/kg/day) and nitrosamine with olive oil (1ml/kg/day) administration during 33 days were summarized in table 3. Data indicated that administration of nitrosamine alone increased in a highly significant way the counts of WBC, MCV and MCH by 58.67%, 24.64% and 14.73% respectively. Whereas RBC and platelets counts have shown a highly significant decrease by -23% and - 44.61% respectively. Hb show a significant decrease of -11.69%, but MCHC and HCT were shown a

non-significant decrease by -8.01% and -4% respectively. While treatment with nitrosamine and ephedra showed a highly significant increase in WBC, MCV and MCH, by 53.33%, 35.54%, 19.30% respectively, but also a high significant decrease in RBC, MCHC and platelets by -27.43%, -20.78% and -39.32% respectively. Also a significant and non significant decrease have shown in counts of Hb and HCT at -13.37% and -1.6% respectively. But when olive oil was applied with nitrosamine the increments of WBC, MCV and MCH were highly significant at 14.14%%, 96.96% and% 71.34 respectively, while a non-significant result was shown in an increment in MCHC by 0.73% and a decrement in Hb and HCT at -3.28% and -3.2% respectively. Data have shown also a highly significant decrease in counts of RBC and platelets by -43.54% and -36.71%.

Table 3. *Effect of Nitrosamine, Ephedra and Olive Oil Administration on Total Blood Counts of Rabbits.*

Parameters	Experimental Drug			
	Control n=6	Nitrosamine n=6	Nitrosamine with Ephedra n=6	Nitrosamine with Olive oil n=6
WBC count (x 10^3cell/ul)		11.90±0.13	11.50±0.11	14.14±0.18
% change	7.49±0.16	58.67	53.33	88.53
P value		P<0.01	P<0.01	P<0.01
RBC count (x 10^6 cell/ul)		4.35±0.22	4.10±0.21	3.19±0.14
% change	5.65±0.30	-23	-27.43	-43.54
P value		P<0.01	P<0.01	P<0.01
Hb (g/dl)		10.50±0.18	10.30±0.16	11.50±0.19
% change	11.95±0.169	-11.69	-13.37	-3.28
P value		P<0.05	P<0.05	P>0.05
Hematocrit (%)		36.00±0.33	36.90±0.28	36.30±0.29
% change	37.50±0.15	-4	-1.60	-3.20
P value		P>0.05	P>0.05	P>0.05
MCV (fi)		82.76±0.29	90.0±0.26	112.85±0.31
% change	66.40±0.33	24.64	35.54	96.96
P value		P<0.01	P<0.01	P<0.01
MCH (pg)		24.14±0.17	25.12±0.16	36.05±0.22
% change	21.04±0.20	14.73	19.39	71.34
P value		P<0.05	P<0.01	P<0.01
MCHC (g/dl)		29.17±0.26	25.12±0.21	31.94±0.27
% change	31.71±0.28	-8.01	-20.78	0.73
P value		P>0.05	P<0.01	P>0.05
Platelets (x 10^3cell/ul)		211.0±26.6	230.60±22.19	240.50±33.30
% change	380.0±28.80	-44.61	-39.32	-36.71
P value		P<0.01	P<0.01	P<0.01

All values were expressed as mean ± S.E; P<0.05 significant; P<0.01 highly significant.

4. Discussion

The present study is a comparative one, which performed to assess the protective effect of ephedra and olive oil on nitrosamine-induced toxicity in blood parameters of twenty four domestic rabbits. The $NaNO_2$ and other additives may react with amines of the foods in the stomach and produce nitrosamines and free radicals. Such products may increase lipid peroxidation, which can be harmful to different organs including liver and kidney [24]. On the other hand, these free radicals, known to cause oxidative stress, can be prevented or reduced by dietary natural antioxidants through their capacity to scavenge these products [25]. The present study was undertaken to determine whether ephedra and olive oil can prevent and/or reduce nitrosamine-induced oxidative stress by examining different biochemical parameters of oxidative damage in the serum, the liver and the kidney of rabbits.

Our results clearly showed that there was a significant increase in serum glucose concentration which indirectly shows decrease in liver glycogen content of nitrosamine treated rabbits. The findings suggest nitrite-stimulation of gluconeogenesis [26], and glucose shift from tissue to blood or an impairment of glucose mobilization. Furthermore, nitroso-compounds can alter the antioxidant system causing disturbance in the metabolic processes leading to hyperglycemia [27]. However, serum glucose and liver glycogen levels were ameliorated upon ephedra and olive oil supplementation. The hypoglycemic effect of olive oil was more than that of ephedra which might be due to their abilities to enhance insulin secretion [28, 29].

In response to nitrosamine treatment, urea, uric acid and creatinine were increased in the serum, suggesting an impairment of kidney functions. These effects could also be attributed to the changes in the threshold of tubular re-absorption, renal blood flow and glomerular filtration rate [30]. Olive oil showed a clear improvement in kidney functions than ephedra (even though they are still more on their values than the control values) perhaps due to the antioxidant properties of olive in scavenging free radicals leading to reduced levels of nitric oxide and lipid peroxidation. The increment observed in serum cholesterol and triacylglycerol contents in response to treatment by nitrosamine support the finding of [31].

Our results also indicate an inhibitory effect of nitrosamine on the biosynthesis of protein, which was more restored by olive oil supplementation than by ephedra extract. These data

suggest a stimulation of the thyroid and the adrenal glands by nitrosamine which can lead to a blockade in protein synthesis, fast breakdown, increased levels of free amino acids, and decreased protein turnover [32]. The present data of liver enzymes activities also show the protective effect of ephedra and olive oil over nitrosamine hepatotoxicity. There were a significant increase of ALT, AST and ALP values after administration of nitrosamine alone. These alterations were less severe in rabbits receiving nitrosamine-olive oil than that receiving nitrosamine-ephdra. In addition, nitrite interactions results into nitric oxide release, which can inhibit total protein synthesis [33]. However, the increase in bilirubin concentration as well as the activity of AST, ALT and ALP enzymes in the serum of nitrosamine-treated rabbits could be attributed to the toxic effect of nitroso-compounds, formed in the acidic environment of the stomach, in causing severe hepatic necrosis [34]. These abnormalities were prevented by supplementation of olive oil more as compared than supplementation by ephedra, (perhaps due to its role in stabilizing the cell membrane and protect the liver from free radical-mediated liver cell toxicity) [35].

The increments of Na+ and K+ and the decrement of Ca++concentrations was very obvious in this study, which was consistent with others, who suggest that nitrosamine caused an increase in fractional excretion of Na+, K+ and a decrease in urine osmolality, free-water re-absorption, and urine to plasma creatinine ratio, these results are also in agreement with [36, 37], who suggest that treatment with ephedra prevented the hypomagnesemic and the nephrotoxic effects of nitrosamine and can be of clinical significance.

The reported results indicate that exposure to nitrosamine-olive oil and nitrosamine-ephdra combinations, produces a clear increment of WBCs, MCV and MCH values after a equivalent increase when the animals injected with nitrosamine alone. This highly significant increase in WBCs count indicated the activation of defense mechanism and immune system of rabbit. This induction of white blood cells is a positive response for survival due to cell mediated immune response of animals which was more pronounced on olive oil than ephedra treatment [38]. Leukocytosis was manifested by lymphocytosis, which was the main feature of the differential leukocytic count.

It was found that exposure to nitrosamine/olive oil and nitrosamine/ephdra combination produced a significant decrease in the red blood cells RBCs, Hb, MCHC, HCT and platelets counts which was also observed more clearly by administration of nitrosamine alone. This finding may be explained on the basis of inhibitory effect of these drugs on histogenesis. The decreased in RBC count and hemoglobin (Hb) lowered the oxygen supply to different tissues thus resulting in low energy production. Decrease in Hb contained MCHC can be explained due to decreased in size of RBCs or impaired biosynthesis of heme in bone marrow [38, 39].

5. Conclusion

From the results achieved it can be concluded that the administration of olive oil and ephedra extract have an extremely beneficial role in overcoming the occurred adverse effects of chronic ingestion of nitrosamine, which is probably through their excellent antioxidant properties and highly nutritional values. It was also very clear from most hematological and biochemical studies, that applying olive oil with nitrosamine have ameliorative effect of nitrosamine induced side effects more than that of ephedra–nitrosamine combination.

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