Effect of Dates (Phoenix dactylifera L.) on Liver of Broiler Chicks Infected with Infectious Bursal Disease Virus. Biochemical and Histological Study

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Abstract

A number of (80) birds (Ross 308) at one day old were obtained from a local hatchery in the area. The birds were allotted into four equal groups (no.=20) and were supplied with basal scientific diet. Palm date (PD) was supplied to G3 and the birds were challenged with virulent Infectious Bursal Disease Virus (IBDV). G4 was treated with palm date 3 days prior to challenge age. G2 was challenged without supplementation of PD. G1 was neither given PD nor challenged. Inclusion rate of PD was 10%. The challenged age was at 33 days old. Blood serum was assayed for evaluation of transaminases and lipid profile 10 days post infection period (PIP). The result showed that histopathological investigation is more accurate in determining the health state of the birds and the palm date have to be supplied in more than 10% to give its significant effect.

***Key words**: Date palm, GPT, GOT, HDL, ALP, Cholesterol, Triglycerid, HDL, IBDV, Histology, Broiler Chicks.*

Introduction

Cereals are an important sources of food for human and animals alike. Because of the massive population growth, the world has been faced with a gap between providing food needs and maintaining the livestock and their needs for feedstuffs. Tropical and semi-tropical regions are popular in their cultivation of palm date, which is a major source of energy. The Middle East is considered one of the world's leading producers of dates, accounting for nearly 70% of the world's production [(1)]. Poultry industry is regarded as the main tool for supplying human demands of animal protein. To fulfill this requirements, grains have to be supplied and this will have negative impact on the level of human requirements. Because of dates and dates fruits are rich sources of protein and energy, the later can be used as an alternate of cereal in order to save human demands for their consumption, in addition to lower the cost of feedstuffs supplied in poultry industry [(2)]. Many researches have shown that inclusion of palm date kernel (PDK) or seed was considerably valuable in improving productive performance , feed cost and feed utilization when added to poultry feed [(2,3)]. The palm date has been proved to be an efficient diet during food shortage or during crises because of high nutritive value. It is rich in calcium, zinc, potassium, selenium, sulphur, chlorine, iron and manganese [(4,5)].

Medically, palm date have been proved in preventing liver against toxic damage produced by various toxic metabolites because of its anti-oxidant property due to high availability of various tocols as well as to its phenolic compound [(6)]. Liver is the largest organ in the body that may show lesions represented by swelling and infarct [(7)] as a result of infection with infectious bursal disease virus (IBDV) and this may have a negative impact on liver function. PDK is nontoxic and had been used against hepatotoxic and mutagenic effects, hyperlipidemia, hyperglycemic inflammation, microbial infection, oxidation and immunosuppressive effect of IBDV. [(8,9,10,11)].

Recently , poultry industry have adopted a new strategy in poultry rearing which aimed to reduce the

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marketing age through genetic selection breeding with high feed conversion ratio (FCR) and high body weight gain, palm date is said to have this property in enhancing productive performance [10,12]. For all the above mentioned reasons our study have been planned to study the effect of palm date as a medical agent in preventing liver damage caused by IBDV as well as for commercial purposes to have high productive performance.

Materials and Method

A total number (80) of chicks type (Ross 308) at the age of one day old were purchased from local hatchery in the area. They were divided into four sub-groups. All birds were supplied with basal scientific ration in addition to 10% whole grinded dry palm date type Zahdi (Phoenix dactylifera L.), the later was supplied at the day of challenge with exception of G4 which had been supplied with palm date three days before challenge (Table -1).

The birds were exposed to oral infection with highly virulent Infectious Bursal Disease Virus (IBDV) at the age of 33 day old. Infection was done by collection of grossly representative infected bursae with local field strain. The collected samples were homogenized, tissue suspension was centrifuged for a period of ten minutes at a rate of 2500 revolution per minute, supernatant fluid was aspirated, penicillin and streptomycin were added at a rate of two thousands IU and two mg per 1 ml respectively. Five birds from each group were sacrificed 10 days post infection period (PIP) to monitor any differences between treated and non-treated groups to evaluate blood serum related to different groups. The parameters adopted in evaluation were alkaline phosphatase (ALP), glutamic oxaloacetic acid transaminase (GOT), glutamic pyruvic transaminase (GPT), lipid profile which include cholesterol, high density lipoprotein (HDL), triglyceride (TG) and cholesterol. The technique followed was according to kits manufacturers.

- ALP kit from Biolabo SAS, France.
- GPT and GOT kits from Randox laboratories Inc. UK.
- TG and cholesterol kits from LINEAR CHEMICALS .L.U. Barcelona, Spain.
- HDL kit from Bio System S.A. Barcelona, Spain.

Histopathological study of liver was done according to Luna 1968 [13] in order to follow the differences between treated and non-treated groups histologically.

Distribution of chicks with their treatment.

Distribution of chicks with their treatment.

Groups	Treatment	Challenge
G1	-	+
G2	-	+
G3	+	+
G4	+(3 days prior to challenge)	+

- : Not given palm date
+ : Treated with 10% palm date

Statistical Analysis

This is done according to [14]

Results and Discussion

Table-2 showed that G1 (negative control) have no significant difference in GOT level when compared to G2 (171.1±34.974) which was not treated but was challenged with virulent IBDV. G2 showed high significant difference ($p<0.05$) as compared to G3 and G4 (110.6±4.751 , 108.4± 6.293) respectively. The result shown above indicated that IBDV increases the level of GOT in blood serum of IBDV infected chicks. At the same time G3 and G4 decrease significantly as compared to G1 and G2. Although G4 do not differ significantly from G3 but GOT level is lower than G3 and this may be due to reduced effect of virulent IBDV as a result of pre medication with palm date 3 days before challenge (Table-1). The study do not agree with Nadia K. J. Al-Dawah [17] who mentioned that the level of GOT serum showed no significant differences as compared to negative control group.

GPT : G1 showed no significant difference from G2 and G3 (18.25 ± 0.491, 18.00 ± 1.416) respectively ,on the other hand the pre-medicated group (G4) (17.29±1.960) differ significantly from G1 at $p<0.05$ level. This indicate that palm date have no effect on GPT level. The result remains questionable due to the absence of studies that support or oppose our study (Table-1).

ALP : The study showed that there were no significant differences between treated group and non-treated one or challenged or non-challenged (G1,G2 and G4) which may give an expression that ALP is non-indicative for the health status in poultry (Table-1). The result is in agreement with [18,19] who mentioned that the highest level of ALP in chicken occur in the first two

weeks of life then decrease significantly with age. ALP level in G3 remains unexplained and available study to be compared.

Table-1. 10 days PI GOT, GPT and ALP

Group	GOT Mean± SD	GPT Mean± SD	ALP Mean± SD
1	160.3±26.068 A	21.64 ± 2.732 A	52.82 ±3.700 A
2	171.1± 34.974 A	18.25 ± 0.491 AB	55.19± 0.277 A
3	110.6± 4.751 B	18.00 ± 1.416 AB	10.86 ± 2.721 B
4	108.4 ± 6.293 B	17.29 ± 1.960 B	39.71± 22.028 A

Note: Different capital letters refer to significant differences between different means at 95% confidence interval.

Cholesterol : Table-2 showed no significant differences in the level of serum cholesterol in G1 and G4. While G2 do not differ from G3. The low level in G1 may be related to absence of challenge. Elevation of cholesterol level in the other groups could be due to the liver damage created by IBDV. Pre-treatment with PD in G4 resulted in lower cholesterol level in comparison with other infected groups (G2 and G3). This is in agreement with [20,21] who mentioned that the polyunsaturated fatty acids that compose the vegetable oils supplemented in the diet reduce both egg and blood cholesterol levels.

HDL : The study showed no significant differences between G1 and G2 (26.49 ± 0.899, 26.30 ± 1.349) , (non-treated groups) on the other hand G3 and G4 (treated groups) (13.22 ± 5.709, 14.52 ± 9.980) do not differ from each others , this reflect the effect of addition of palm date to the diet, at the same time the result on HDL run in a parallel way to that effect on cholesterol (Table-2). G1 and G2 showed higher significant level than the treated groups (G3 and G4). The result agreed with [22] who mentioned that total cholesterol (TCH) level is positively correlated with HDL and LDL.

TG : The study showed no significant differences in different groups, treated or non-treated or challenged and non-challenged groups (Table-2**).** This is in agreement with [23] who mentioned that triglyceride and cholesterol concentrations were positively correlated with each other.

Table-2. 10 days PI. Cholesterol, **HDL and TG**

Group	Cholestrol Mean± SD	HDL Mean± SD	TG Mean± SD
1	57.81 ± 42.61 C	26.49 ± 0.899 A	154.2 ± 64.08 A
2	410.42 ±156.96 AB	26.30 ± 1.349 A	114.0 ± 78.25 A
3	578.47 ± 15.07 A	13.22 ± 5.709 B	156.0 ± 14.76 A
4	209.17 ± 171.33 BC	14.52 ± 9.980 B	190 ± 12.34 A

Note: Different capital letters refer to significant differences between different means at 95% confidence interval.

Pathological Study

The study revealed that IBDV have deteriorative effect on liver parenchyma and palm date have a great impact in reducing this effect. Transaminases are usually used to test for cellular necrosis and their increase in serum concentration may indicate liver malfunction [24]. Liver is regarded as the site of amino acids metabolism, any elevation in blood serum may give indication that there is damage to the liver as a consequence to harmful agent that causes leakage of these amino acids to the blood stream. This in agreement with [25].

G2 showed mild inflammatory cells infiltrations with congestion of sinusoid (Figure 2) as compared to control group (Figure 1). G3 showed milder pathological lesion as compared to G2 and more sever pathological lesion as compared to G4, this might be due to previous medication with palm date(Figures 3 and 4).

The study revealed that there is no correlation between serological tests and histological findings. G1 and G2 showed lower significant level of GPT in spite of sever pathological lesions in G3 and G4 as compared to the control group. The result remains unexplained because of no available comparative study [15,16]. Most high triglycerides are associated with an increase in LDL cholesterol and decrease in HDL cholesterol also there are many factors that estimate the level of lipid profile as the sex, age, abdominal fat[22]. This support our study that histological investigation is more indicative to determine the health status of the bird**.**

The study showed that IBDV have no effect in the level of TG and ALP , this is because of the positive relation-ship between triglyceride and cholesterol concentrations which are positively correlated with each other[19].

The study concluded that palm date may reduce the harmful effect of infectious agent as IBDV due to its high component of active anti-oxidants that playing an important role in neutralization of free radicals and decomposition of peroxides[26].

Conclusion: that the addition of 10% palm date may give no indicative serological parameters for the health status of the bird; Hisopathological investigation still remains the only way for evaluation the health status of birds.

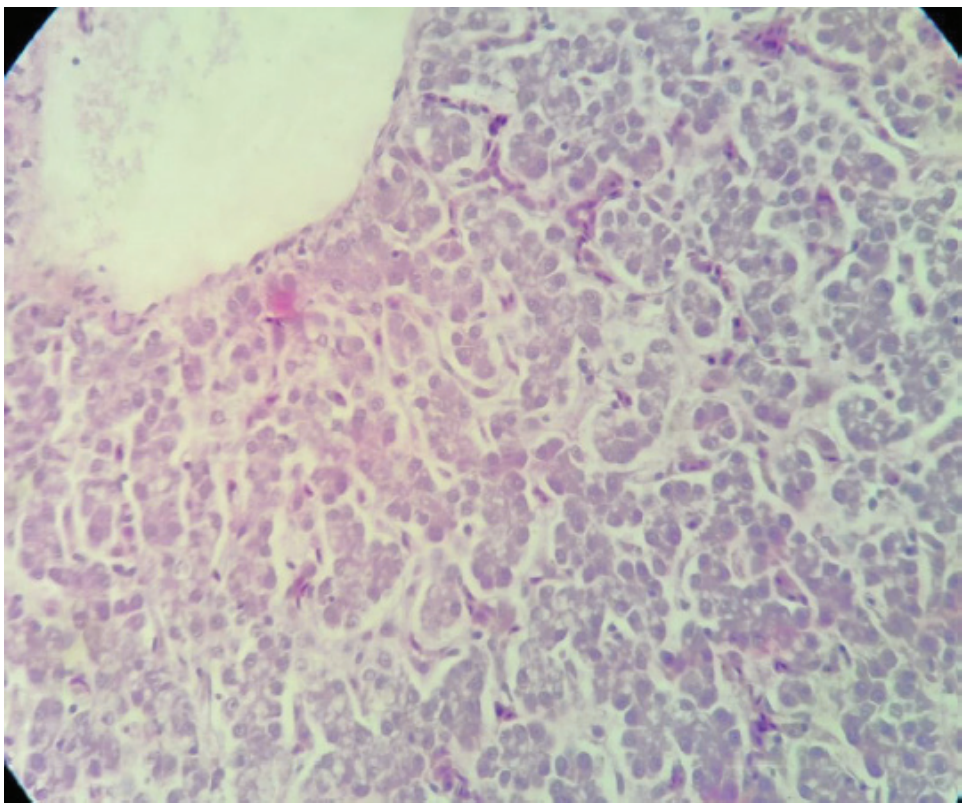

Figure :G 1. Liver. No Treatment , no Challenge. Normal histological appearance. H and E 100X.

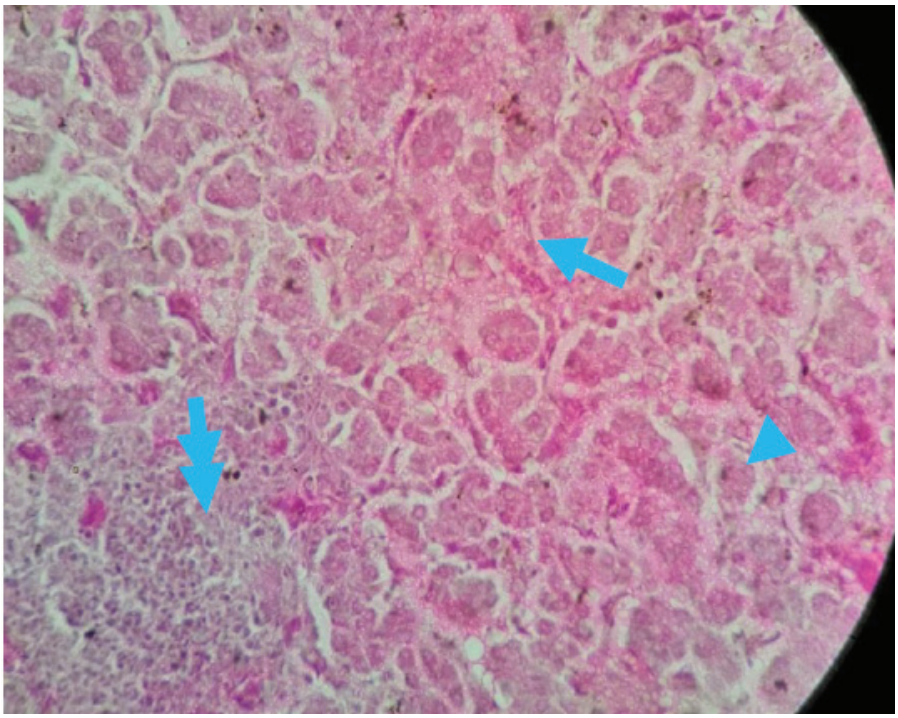

Figure 2: G2. liver showed fatty degeneration with coagulative necrosis of hepatocytes and inflammatory cell infiltration H and E 400X

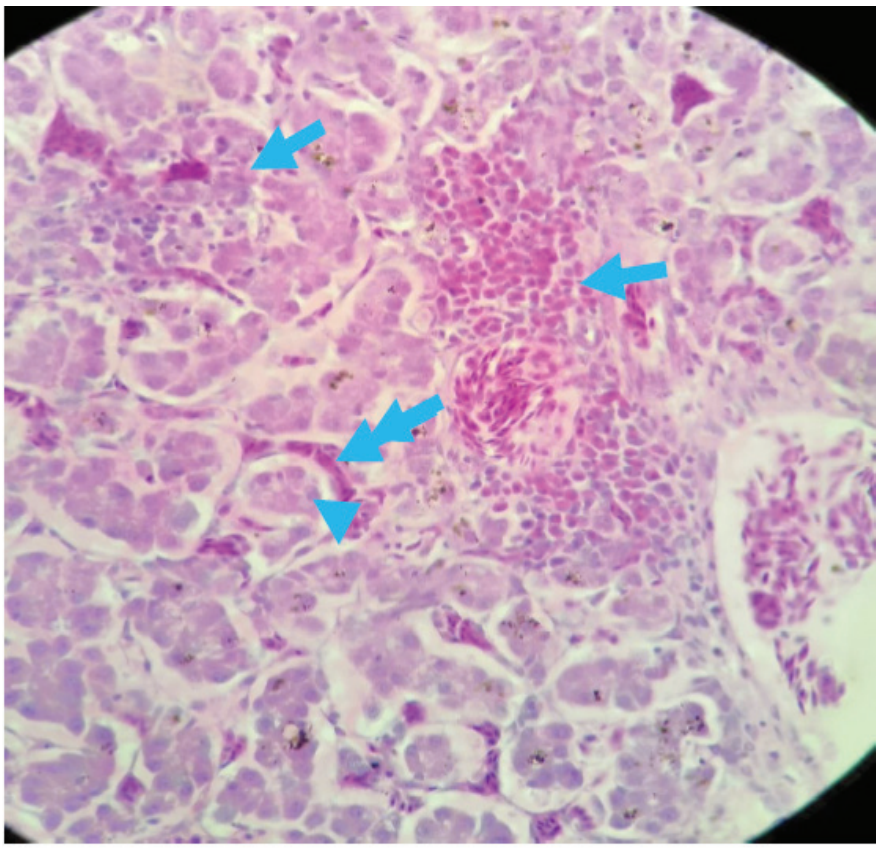

Figure 3 . G 3. Treatment after challenge. Inflammatory cell infiltration) and hepatocyte atrophy with sinusoid congestion .H and E 400X.

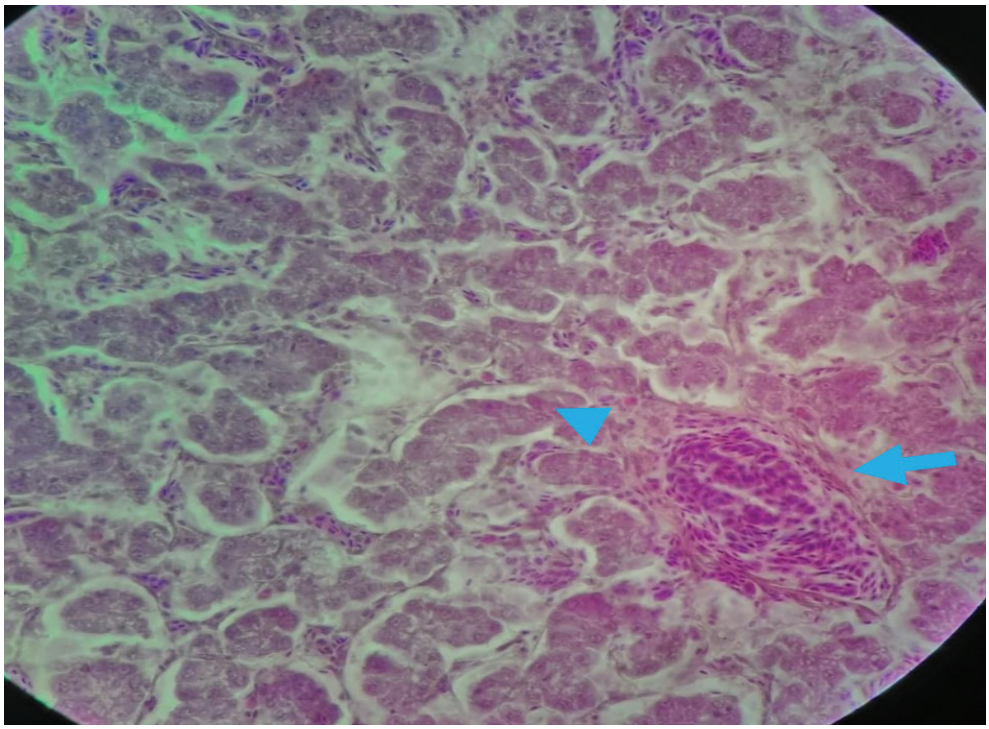

Figure 4.G4.Treated before + Challenge(liver showed congested blood vessel (arrow) with moderate hepatocyte atrophy(arrow head) H and E 400X

Ethical Clearance: The Research Ethical Committee at scientific research by ethical approval of both environmental and health and higher education and scientific research ministries in Iraq

Conflict of Interest: The authors declare that they have no conflict of interest.

Funding: Self-funding

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