# The hepatoprotective effect of dimethyl 4,4- dimethoxy 5,6,5,6- dimethylene dioxy-biphenyl- dicarboxylate (D.D.B.) on aflatoxin B1 induced liver injury

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**Abstract**

Seventy five samples of frozen meat, raw milk and poultry feed (25 samples each), were examined mycologically and for detection of aflatoxin $B_1$ ($AFB_1$). The results revealed that the isolated fungi represented 6 genera of moulds. The most prevalent fungi in these samples was the genus aspergillus (60%, 60% and 76%) with mean of count of ($1.6 \times 10^2 \pm 0.1$, $6.0 \times 10 \pm 0.23$ and $3 \times 10^2 \pm 1.0$), respectively, which was at the top of all isolated fungi . However, *A. flavus* was isolated form all kind of samples and that which isolated from feed produced aflatoxin $B_1$ with mean level of ($60 \pm 0.1$ ppb) followed by that isolated from frozen meat ($9.5 \pm 0.71$ ppb), but those isolated from milk had the lowest $AFB_1$ level ($1.0 \pm 0.1$ ppb). The effect of dimethyl 4, 4- dimethoxy 5, 6, 5, 6- dimethylene dioxybiphenyl 2, 2- dicarboxylate (D.D.B.) in degradation of AF was evaluated by intraperitoneal injection of 30 rats with 1.5 ppm of $AFB_1$ to evaluate their effect on haematological, biochemical and protein electrophoretic patterns of aflatocicated rats. The obtained results indicates an improvement in the haematological picture (Hb, RBCs and PCV) together with WBCs and differential leucocytic count of the treated rats compared with non treated ones. Also, biochemical analysis revealed significant changes in urea and creatinine levels; AST and ALT activities; total protein and protein electrophoretic patterns of treated rats. The administrated of DDB effectively improved haematological alterations and prevent serum biochemical changes, ameliorated, the toxic effect of aflatoxin B1 and had hepatoprotective effect on $AFB_1$ induced liver toxicity. [Life Science Journal 2010;7(3):148-153]. (ISSN: 1097-8135).

**Keywords:** frozen meat; raw milk; poultry feed; aflatoxin $B_1$ ($AFB_1$); genus aspergillus; toxicity

## INTRODUCTION

The increased population in the world requires a parallel raise in the production of food. Some countries as Egypt had to import many food and feeds. The recent researches reported that the majority of this food may carry the dangerous factors for human and animal health. Fungal contaminations and their toxins represents the most significant contaminant of these food **(Magnoli *et al.*, 1999)**. Aflatoxins are a group of secondary metabolites produced by *A. flavus* and *A. parasiticus* in food and feed commodities (**Oguz, 1997**). The consumption of food contaminated with mould and their toxins induced food poisoning, hemorrhages, hepatotoxicity, nephrotoxicity, neurotoxicity, dermatitis, carcinogenic, hormonal and immunosppression effects **(Hassan *et al.*, 2004 and 2005 )**. Therefore, the degradation of such fungi and their toxins become critical demand.

It was investigated that aflatoxin B1 is the most potent one of aflatoxins (**Hamdy et al., 1995**). The dimethyl diphenyl bicarboxylate (DDB) could directly protect hepotocyte DNA from oxidative damage and inhibit TNF- alpha mRNA expression in liver tissue which resulted in prevention of liver damage (**Gao *et al.*, 2005**). Also **Park *et al.* (2005)** demonstrated that DDB exerted protection of liver from chemical- induced injury potentiated by the condition of glutathione (GSH) deficiency and has additional advantages in lowering the plasma lipids.

Therefore, this study was undertaken to screen poultry feeds, frozen meal and raw milk for A. flavus and Aflatoxin $B_1$ production by isolated *A. flavus* and to evaluate the effect of DDB in recovering aflatoxicosis.

## MATERIALS AND METHODS

### 2.1. Material:

**2.1.1. Samples:** 75 samples of frozen meat , raw milk and poultry feed (25 of each ) were collected from markets at Cairo Governorate and poultry farms for investigation of fungal contamination and detection of aflatoxin contamination.

**2.1.2. Aflatoxin standard:** standard of aflatoxins $B_1$, was purchased from sigma chemical company (USA).

**2.1.3. Animals:** Thirty apparently healthy albino rats weighted (100-120 g) were housed under hygienic conventional conditions in suspended stainless steel cages. Prior to experiment rats fed on healthy basal diet free from any cause of disease. Drinking water was supplied in glass bottles, cleaned three times a week.

**2.1.4. Dimethyl diphenyl bicarboxylate (DDB):** It was imported by Al-Ahram Pharmaceutical and Medical Equipment Company, Egypt.

**2.1.5. Chemicals and reagents for using polyacrylamide gel electrophoresis :** They included acrylamide, bisacrlamide, 2,2,2,2-Tetramethylethylendiamine(TEMED), B-mercaptoethanol, 1.5 M Tris-cl p H 8,8, 10% SDS(sodium dodecyl sulphate), initiator(10% ammonium persulphate), buffers, comassie stain and destaining by methanol ands acetic acid solutions, and protein

molecular weight marker. These chemicals were purchased from sigma chemical company , USA.

**2.2. Methods:**

**2.2.1. Isolation and identification of moulds:** each feed samples was subjected for isolation and identification of fungi as recommended by (**Conner *et al.*, 1992**).

**2.2.2. Production and estimation of aflatoxins** (**Gabal *et al.*, 1994**).

**2.2.2.1. Cultivation and extraction of aflatoxins:**

Isolated strains of *Aspergillus flavus* were inoculated into flasks conaining 50 ml of sterile yeast extract solution 2% containing 20% sucrose (YES).

Inoculated flasks were incubated at 25ºC for 7-10 days . At the end of the incubation period, 50 ml chloroform were added and the mixture was thoroughly mixed for one minute in ultraurax apparatus, then centrifuged (3000 r.p.m.) for 10 minutes after which the chloroform layer decanted. The chloroform extraction was repeated for one more time.

One ml ethanol, 3 gm copper – (III)-hydroxide carbonate and 5 gm anhydrous sodium sulphate were added to the chloroform extract, mixed well and filtered.

The filtrate was then evaporated in a rotatory vacuum evaporator, the residue cooled and resuspended in exactly 5 ml of colorophorm.

**2.2.2.2. Thin layer Chromatographic analysis of choloroform extract (Scott, 1990);**

The concentrated extract was spotted onto activated thin layer chromatography plates coated with silica gel of 0.25 mm thickness. Standard solution of aflatoxins $B_1$, $B_2$, $G_1$and $G_2$ were spotted on the plate using 10-20 µl capacity pipette. The spotes were air dried and the TLC plates out in the developing tank containing the developing solvent system (5Toluene :4 ethyl acetate :1 of 90% formic acid ( V/V/V) or (chloroform: hexan: petroleum ether: benzene: acetone 6:1:1:1:1).

When the solvent travels about 12 cm front, the plates were removed from the tank, air dried and inspected under a ultraviolet light lamp for examining the tested and standerd spots and determining the rate of flow ( $R_f$[1]of the toxin) then the results recorded

$$R_f = \frac{\text{Distance traveled by unknown material}}{\text{Distance traveled by solvent front}}$$

Aflatoxin was calculated by the following equation or formula.

$$\mu g/kg = \frac{S \times Y \times V}{Z \times W}$$

S = µl of aflatoxin standard equal to unknown.
Y = Concentration of aflatoxin standard in µg/ml
V = µl of final dilution of sample extract
Z = µl of sample extract giving a spot fluorescent intensity equal to the standard (S)
W = Mass of sample, represented by the final extract in gm. Applied to Column =(100 g x filtrate volume)/ 200.

**2.2.3. Experimental design:** Thirty rats were divided into 3 equal groups. Rats of the first group were given normal feed (free from mycotoxins and without any treatment) and kept as a negative control. Rats of the other two groups were given single dose of $AFB_1$ intraperitonealy at the rate of 1.5 ppm **(Bao, 2002)**. Then on the second day rats of the third were dosed orally by 300 mg DDB for 3 weeks **(Bao, 2002)**, while those of the second group were left without any treatment to kept as positive control.

**2.2.4. Haematological and biochemical investigations:**

At the end of the experimental period, two blood samples were collected from each rat. The first portion was collected in small labeled dry and clean vials containing Na EDTA (1 mg/ 1ml fresh blood, **Schalm *et al.*, 1975)** as anticoagulant for haematological study according to routine methods described by **Jain (1986)**. While, the second portion was taken without anticoagulant in centrifuge tube, allowed to clot, then centrifuged at 3000 rpm for 10 minutes for separation of serum which used to assay the biochemical parameters. Serum analysis included estimation of serum aspartate aminotransferase (AST) and alanine aminotransferase (ALT) activities according to **Reitman and Frankel, (1957**), serum urea according to **Wybenga *et al.* (1971**), serum creatinine level according to **Henry (1974) and** total serum protein as described by **Pesce and Kaplan (1978**).

2.2.5. **Estimation of molecular weight of different plasma protein by using SDS-polyacrylamide gel electrophoresis as described by Laemmli (1970):** After the separating gel was prepared and pored into wells , rinse with distilled water and invert to drain the wells. 25 ul of serum sample underlay in each well and the upper buffer chamber putted in place . Then , contact sandwiches to the bottom of the upper chamber .The upper buffer chamber was placed on the heat exchanger in the lower buffer chamber . Fill the lower buffer chamber with tank buffer until the sandwiches are immersed in buffer and add a drop of 0.1 % phenol red to the upper buffer chamber (tracking dye). Alternatively, add the dye directly to the sample after it has been heat-treated. The upper buffer chamber was filled with tank buffer .After placing the lid on unit, the power supply was connected(PS 500XT). The cathode should be connected to the upper buffer chamber . Turn the power supply on and adjust the current 2 m. Amp. Per sample for 90 minutes, the voltage should start at about 70-80 V, but will increase during the run. When the dye reaches the bottom, turn the power supply off and disconnect the power cables. The gels sandwiches placed in stain and gently shake the gels for one hour on the PR 70 red rotor. Then put it in destaining solution and shake for one hour. Quantitation of different protein molecular weight were made by using densitometer G 700 (Bio-Rad, USA).

**2.2.6. Estimation of isoelectric focusing of plasma protein by using polyacrylamide gel electrophoresis as described by O'Farrell (1975):**

The isoelectric focusing is carried out with anode electrode solution (0.01 M 3PO4) and cathode electrode solution (0.02 M Na OH) which are laid along the long length of each side of the gel and a potential difference applied. Under the effect of this potential difference, the ampholytes from a PH gradient between the anode and cathode, (the gels are pre-run at 200 V for 15 minutes at 22 °C and 400 V for 30 min at 22 °C). Depending on which point on the PH gradient the sample has been loaded, protein that initially at a PH region below their isoelectric point will be positively charged and will initially migrate towards the cathode. As they proceed, however, the surrounding PH will be steadily increasing and therefore the positive charge on the protein will decrease correspondingly until eventually the protein arrives at a point zwitterions form with no net charge. Likewise substances that are initially at PH regions above their isoelectric points will be negatively charged and will migrate towards the anode until they reach their isoelectric point and become stationary. The gels are fixed in 50% v/v ethanol, 7% v/v acetic acid for 2 hours. The fixation gel must be done before staining because the ampholytes will stain too, giving a totally blue gel, the fixation will precipitate the proteins in gel and allows the much smaller ampholytes to be washed out. After staining and destaining of gels, the distance of each band from one electrode is measured and graph of distance for each protein is determined using densitometer G 700 (Bio-Rad, USA).

**2.3. Statistical analysis:** The obtained date were computerized and analyzed for significance. Calculation of standard error and variance according to (**SPSS 14, 2006**).

## RESULTS AND DISCUSSION

The current data in table (1) showed isolation of 6 genera of mould and one yeast species. The most prevalent fungi in frozen meat, raw milk and poultry feed was the genus aspergillus (60%, 60% and 76%) with mean of count of ($1.6 \times 10^2 \pm 0.1$, $6.0 \times 10 \pm 0.23$ and $3 \times 10^2 \pm 1.0$), respectively, which was at the top of all isolated fungi. Other genera of mould were recovered in different frequency. Whereas, the yeasts were isolated in higher frequency (44% with the mean count of ($3.5 \times 10 \pm 0.1$ in frozen meat) (40% with the mean count of $1.1 \times 10^2 \pm 0.25$ in raw milk) and (20% with mean count of $4 \times 10^2 \pm 0.3$) in poultry feed.

*Aspergillus flavus* was the most frequent mould of aspergillus species isolated from all tested samples of frozen meat, milk and poultry feed (44% with mean count of $1.6 \times 10 \pm 0.3$), (60% with mean count of $3.3 \times 10 \pm 0.3$) and (60% with mean count of $1.0 \times 10^2 \pm 0.2$) respectively. The similar results were previously reported by **Hassan *et al.* (1997); Wafia and Hassan (2000); Hassan and Hamad (2001); Hassan *et al.*, (2002, 2004 and 2005)** and **El- Ahl *et al.* (2006)**.

Other members of aspergillus were isolated in various frequency (Table, 2). The isolation of large numbers of fungi in present samples may be due to their exposure to environmental factors as high temperatures and humidity during harvesting, transportation, handling, processing and/or storage which help in all ways to fungal pollution by different genera of fungi. Significant levels of aflatoxin was produced by *A. flavus* isolated from present samples (Table, 3), where, the maximum levels of toxin was obtained from *A. flavus* isolated from poultry feed (80% of isolates produced mean level of 60 ± 0.1 ppb) followed by those isolated from frozen meat (50% with the mean level of 9.5 ± 0.7 ppb). However, *A. flavus* recovered from raw milk showed the lowest rat of toxin production (30%) with mean level of (1.0 ± 0.1 ppb).

Results of haematological study as presented in table (4) showed significant reduction in haemoglobin level (Hb), red blood cells (RBCs) and Packed cell value (PCV) of rats given aflatoxin only (group 2) indicating presence of anaemia. This anaemia may be due to the direct effect of the toxin on the haemobiotic system. Similar findings were reported by **Hassan and Mogda (2003)** and **Salem *et al.* (2007)**.

The treatment with DDB significantly improved the function of this system by degradation of aflatoxin and It improved concentration of Hb, RBCs count and haematocrit value**(Abdel- Hameid (2007)**.

Results presented in table (5) showed significant leucocytosis in groups 2, 3 compared to group 1, this recorded leucocytosis may be attributed to the toxic effect of $AFB_1$ on haemobiotic tissue as recorded by **Parent-Massin (2004)**. It could be noticed from the tabulated results in table (5), that treatment with DDB improved the total leucocytic count compared to the aflatoxicated rats toward the control –ve group (group, 1). The results in table (5) revealed a significant increase of segmented and staff cells percent while lymphocytes, monocyte, eosinophils and basophils were lowed in group 2 which received $AFB_1$ only without any treatment compared to the control –ve group. On the other hand treatment with DDB induced an improvement in segmented, lymphocytes, monocyte, basophils and staff percent compared to aflatoxicated group toward the control – ve group.

Concerning the effect of $AFB_1$ alone on the kidney and liver functions of rats, a significant elevation in levels of urea, creatinine, AST and ALT activity were observed. **Aravind *et al.* (2003)** reported that AFB1 caused an increase in AST and ALT activities, which indicated the liver damage. Also, **Bilgic and Yebyldere (1998)** reported that $AFB_1$ cause petecial haemorrhages in the kidney and liver due to the animals feeding on diets containing $AFB_1$. This findings was in agreement with results obtained by **Celyk *et al.* (1999)** and **Eraslan *et al.* (2006)**. The treatment with DDB leads to a decrease in AST and ALT activities due to protection of treated rats against $AFB_1$ hepatotoxicity by increasing the detoxifying metabolism of $AFB_1$ in the liver as recorded by **Lu and Li (2002)**. Similar results were reported by **Gao *et al.* (2005)** recording that DDB significantly inhibited hepatocyte nuclear DNA fragmentation and prevented the direct DNA damage, these results suggest that DDB could directly protect hepatocyte DNA from oxidative damage and inhibit tumor necrosis factor (TNF)- alpha

mRNA expression in liver tissue, which resulted in prevention of liver damage. Similarly, **Park *et al.* (2005); Sun and Lu (2006)** and **Jin *et al.* (2007)** recorded that DDB effectively prevented increases in plasma transferases. **Abdel- Salam *et al.* (2007)** and **Abdel-Hameid (2007)** reported that DDB has significantly prevented the occurrence of liver damage. On the other hand this findings disagreed with that reported by **Kin *et al.* (1999)** and **Nan *et al.* (2000)** who recorded that DDB did not improve AST and ALT activities caused by hepatotoxicity. Results shown in table (7) indicated that the effect of DDB on the total protein and its electrophoretic pattern in aflatoxicated rats showed a significant improvement in their serum levels. This elevation may be attributed to the improvement in hepatocytes as reported by **Lu and Li (2002)** leading to enhancement of protein synthesis which was impaired by aflatoxicosis. The reported impairment of protein synthesis due to aflatoxicosis was in agreement with that reported by **Raju and Devegawda (2000); Aravind *et al.* (2003); Don and Kaysen (2004)** and **Eraslan *et al.* (2006)**.

The presence of fungi and their toxins in feed and food reflected unhygienic measure during cultivation, irrigation harvesting transportation, handling, storage and processing of feed and food. Therefore, frequent testing programs of food during different steps of production must be monitored before given to animals or human for consumption. The fungal inhibitors may be added if the level of contamination over the limited level. Therefore, continuous investigations for finding new safe methods for controlling the growth of fungi and mycotoxins production to keep the human and animals consumer are critical demand. All ways for increasing the quality of human health and animals wealth.

Table (1): Mycoflora of frozen meat, raw milk and poultry food.

| Fungal genera | Prevalence of fungal genera | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | Frozen meat (25) | | | Raw milk (25) | | | Poultry feed (25) | | |
| | No. of +ve | % | Mean of count ± SE | No. of +ve | % | Mean of count ± SE | No. of +ve | % | Mean of count ± SE |
| Total fungi | 20/ 25 | 80 | $3.8 \times 10^2 \pm 2.0 \times 10$ | 17/ 25 | 75 | $6.1 \times 10 \pm 0.2$ | 22/ 25 | 88 | $3 \times 10^2 \pm 1.0 \times 10$ |
| *Aspergillus sp.* | 15/ 25 | 60 | $1.6 \times 10 \pm 0.1 \times 10$ | 15/ 25 | 60 | $6.0 \times 10 \pm 0.23$ | 19/ 25 | 76 | $3 \times 10^2 \pm 1.0 \times 10$ |
| *Penicillium sp.* | 8/ 25 | 32 | $1.1 \times 10 \pm 0.3 \times 10$ | 12/ 25 | 48 | $1.8 \times 10 \pm 0.25$ | 15/ 29 | 60 | $4x\ 10^1 \pm 0.7x10$ |
| *Fusarium sp.* | 5/ 25 | 20 | $1.5 \times 10 \pm 0.2 \times 10$ | 1/ 25 | 4 | $0.5 \times 10 \pm 0.0$ | 10/ 25 | 40 | $5 \times 10 \pm 2x10$ |
| *Cladosparium sp.* | 9/ 25 | 36 | $3.5 \times 10 \pm 0.1 \times 10$ | 10/ 25 | 40 | $1.1 \times 10 \pm 0.2$ | 25 | 20 | $4 \times 10^2 \pm 0.3x10$ |
| Mucor sp. | 6/ 25 | 24 | $2.1 \times 10 \pm 0.1 \times 10$ | 5/ 25 | 20 | $1 \times 10 \pm 0.12$ | 10 | 40 | $0.3 \times 10^1 \pm 2.0x10$ |
| Rhizop sp. | 4/ 25 | 16 | $1.0 \times 10 \pm 0.1 \times 10$ | 4/ 25 | 16 | $0.5 \times 10 \pm 0.2$ | 6 | 20 | $1x10^2 \pm 0.2x10$ |
| Yeast sp. | 11/ 25 | 44 | $2.0 \times 10 \pm 0.1$ | 5/ 25 | 20 | $1 \times 10 \pm 0.3$ | 5 | 20 | $10^1 \pm {}^{0.03x10-}$ |

25 samples were examined.

Table (2): prevalence of members of Aspergillus spp. in frozen meat, milk and poultry feed.

| Fungal genera | Prevalence of fungi | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | Frozen meat | | | Raw Milk | | | Poultry Feed | | |
| | +ve | % | Colony count ± SE | +ve | % | Colony count ± SE | +ve | % | Colony count ± SE |
| *A. flavus* | 11 | 44 | $1.6 \times 10 \pm 0.2x10$ | 15 | 60 | $3.3 \times 10 \pm 0.3x10$ | 15 | 60 | $1 \times 10^2 \pm 2x10$ |
| *A. niger* | 10 | 40 | $2.8 \times 10 \pm 0.3x10$ | 12 | 48 | $2.8x\ 10 \pm 0.2x10$ | 10 | 40 | $2 \times 10 \pm 1x10$ |
| *A. candidus* | 8 | 32 | $2.5\ x10 \pm 0.1\ x10$ | 3 | 12 | $1x10 \pm 0.2x10$ | 8 | 32 | $0.5x10^2 \pm 0.03$ |
| *A. fumigatus* | 6 | 24 | $1.0 \times 10 \pm 0.0$ | 8 | 32 | $1.6x10 \pm 0.2x10$ | 7 | 28 | $3x10 \pm 0.1x10$ |
| *A. ochraceus* | 3 | 12 | $0.7 \times 10 \pm 0.0$ | 4 | 16 | $1x10 \pm 0.1x10$ | 13 | 52 | $1x10^1 \pm 0.3x10$ |
| *A. terrus* | 3 | 12 | $1 \times 10 \pm 0.0$ | 2 | 8 | $1.3x10 \pm 0.1x10$ | 5 | 20 | $0.5x10 \pm 0.0$ |

25 samples were exam.

Table (3): Rates of aflatoxins production by *A. flavus* isolated from frozen meat, milk and poultry ration.

| Source of isolates | No. of isolates | + ve samples | | Mean of count | Levels of AF ppb | | |
|---|---|---|---|---|---|---|---|
| | | No. | % | | Max | Min | Mean ± SE |
| Frozen meat | 10 | 5 | 50 | 16 ± 2.0 | 14 | 5.5 | 9.5 ± 0.71 |
| Raw Milk | 10 | 3 | 30 | 10 ± 2.0 | 2.0 | 0.5 | 1.0 ± 0.1 |
| Poultry feed | 10 | 8 | 80 | 10 ± 0.042 | 1000 | 150 | 60 ± 0.1 |

Table (4): Haematological picture of aflatoxicated rats and those treated with DDB and control ones.

| | Hb | RBCs | PCV |
|---|---|---|---|
| Control 1 | $14.6 \pm 0.33^{A}$ | $5.57 \pm 0.17^{A}$ | $42.4 \pm 0.92^{A}$ |
| Group 2 | $12.6 \pm 0.11^{aB}$ | $4.88 \pm 0.058^{aB}$ | $38.2 \pm 0.374^{aB}$ |
| Group 3 | $15.12 \pm 0.16^{bc}$ | $5.65 \pm 0.183^{abcD}$ | $45.6 \pm 0.245^{abD}$ |
| F-calculated | 28.022# | 28.788# | 8.377# |
| LSD | 0.75333 | 1.8500 | 1.4333 |

# Significant at $P < 0.05$ using ANOVA test

Aa, Bb, Cc Significantly different between two comparison groups against capital litter at $P < 0.05$ using LSD.

Group 1: control –ve

Group 2: treated with aflatoxin

Group 4: treated with aflatoxin + DDB

Table (5): Total and differential leucocytic count of aflatoxicated rats and those treated with DDB and control ones.

| | WBCs X $10^3$/$mm^3$ | Differential lymphocytic count | | | | | |
|---|---|---|---|---|---|---|---|
| | | Segment | Lymphocytes | Monocyte | Eosinophils | Basophils | Staff |
| Control 1 | $11.02 \pm 0.32^{A}$ | $30.6 \pm 1.03^{A}$ | $61.2 \pm 0.73^{A}$ | $3.8 \pm 0.49$ | $3.4 \pm 0.25^{A}$ | $0.6 \pm 0.24$ | $0.4 \pm 0.24$ |
| Group 2 | $17.8 \pm 0.192^{aB}$ | $42.00 \pm 0.447^{aB}$ | $51.4 \pm 0.4^{B}$ | $3.2 \pm 0.374$ | $2.4 \pm 0.245^{a}$ | $0.40 \pm 0.245$ | $0.80 \pm 0.20^{B}$ |
| Group 3 | $14.78 \pm 0.107^{ab}$ | $32.20 \pm 0.20^{bD}$ | $61.2 \pm 0.489^{bD}$ | $2.60 \pm 0.245$ | $2.80 \pm 0.20^{c}$ | $0.60 \pm 0.245$ | $0.40 \pm 0.245$ |
| F-calculated | 8.377# | 111.558# | 130.729# | 2.174 | 4.529# | 0.207 | 3.500# |
| LSD | 0.68400 | 4.8500 | 5.5000 | -- | 0.750 | -- | 0.8000 |

# Significant at $P < 0.05$ using ANOVA test

Aa, Bb, Cc Significantly different between two comparison groups against capital litter at $P < 0.05$ using LSD.

Group 1: Control –ve Group 2: treated with aflatoxin Group 3: treated with aflatoxin + DDB

Table (6): The induced effect of DDB treatment on urea, creatinine and tarnsaminases activities in aflatoxicated rats and control ones.

| | Urea | Creatinine | ALT | AST |
|---|---|---|---|---|
| Gp 1 | $19.45 \pm 0.36^{A}$ | $0.56 \pm 0.003^{A}$ | $17.50 \pm 0.47^{A}$ | $56.0 \pm 0.31^{A}$ |
| Group 2 | $31.86 \pm 0.99^{aB}$ | $0.712 \pm 0.012^{aB}$ | $34.2 \pm 0.86^{aB}$ | $86.6 \pm 2.56^{aB}$ |
| Group 4 | $30.66 \pm 0.546^{cD}$ | $0.70 \pm 0.007^{acD}$ | $26.4 \pm 0.812^{abD}$ | $75.6 \pm 1.50^{abcD}$ |
| F-calculated | 74.981# | 70.949# | 98.615# | 118.355# |
| LSD | 3.091 | 0.024 | 6.916 | 8.266 |

# Significant at $P < 0.05$ using ANOVA test

Aa, Bb, Cc Significantly different between two comparison groups against capital litter at $P < 0.05$ using LSD.

Group 1: Control –ve Group 2: treated with aflatoxin

Group 3: treated with aflatoxin + DDB

Table (7): Serum protein electrophoretic pattern of toxicated rats and those treated with DDB and control ones.

| Groups | T.P. | Albumin | Alpha1 | Alpha2a | Alpha2b | Beta1 | Beta2 | Gama1 | Gama2a | Gama2b |
|---|---|---|---|---|---|---|---|---|---|---|
| Control | $7.64 \pm 0.64^{A}$ | $2.08 \pm 0.011^{A}$ | $0.523 \pm 0.016$ | $0.41 \pm 0.016^{A}$ | $0.646 \pm 0.018^{A}$ | $1.217 \pm 0.007^{A}$ | $0.533 \pm 0.006^{A}$ | $1.2 \pm 0.021^{A}$ | $0.67 \pm 0.019^{A}$ | $0.353 \pm 0.004^{A}$ |
| Group 2 | $7.11 \pm 0.046^{B}$ | $1.748 \pm 0.009^{aB}$ | $0.544 \pm 0.009$ | $0.398 \pm 0.008^{B}$ | $0.54 \pm 0.004^{aB}$ | $1.06 \pm 0.01^{aB}$ | $0.50 \pm 0.007^{aB}$ | $1.096 \pm 0.012^{a}$ | $0.834 \pm 0.014^{a}$ | $0.39 \pm 0.027^{B}$ |
| Group 4 | $7.86 \pm 0.059^{abcD}$ | $2.09 \pm 0.019^{bcD}$ | $0.526 \pm 0.009$ | $0.498 \pm 0.008^{abcD}$ | $0.644 \pm 0.008^{bcD}$ | $1.138 \pm 0.008^{abcD}$ | $0.59 \pm 0.008^{abD}$ | $1.118 \pm 0.0107^{aD}$ | $0.870 \pm 0.011^{ac}$ | $0.388 \pm 0.009^{cD}$ |
| F-calculated | 53.491# | 171.594# | 1.089 | 36.273# | 30.069# | 63.702# | 43.379# | 15.345# | 39.271# | 12.618# |
| LSD | 0.22133 | 0.08667 | -- | 0.5333 | 0.04583 | 0.0333 | 0.0333 | 0.08200 | 0.05333 | 0.04550 |

# Significant at $P < 0.05$ using ANOVA test

Aa, Bb, Cc Significantly different between two comparison groups against capital litter at $P < 0.05$ using LSD.

Evaluation of total proteins showed significant increase in group 4 which administrated aflatoxin and DDB together with salicylic acid.

Group 1: Control –ve - Group 2: treated with aflatoxin-Group 4: treated with aflatoxin + DDB

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