The Pharma Innovation Journal 2022; SP-11(6): 147-152

www.ThePharmaJournal.com

The Pharma Innovation

ISSN (E): 2277-7695
ISSN (P): 2349-8242
NAAS Rating: 5.23
TPI 2022; SP-11(6): 147-152

www.thepharmajournal.com
Received: 12-03-2022
Accepted: 15-04-2022

Bawanthade UR
M.V.Sc. Scholar, Department of Veterinary Pathology, College of Veterinary and Animal Sciences, Parbhani, MS, MAFSU, Nagpur, India

Moregaonkar SD
Professor and Head, Department of Veterinary Pathology, College of Veterinary and Animal Sciences, Parbhani, MS, MAFSU, Nagpur, India

Gangane GR
Associate Professor, Department of Veterinary Pathology, College of Veterinary and Animal Sciences, Parbhani, MS, MAFSU, Nagpur, India

Dr. Kondre BM
Assistant Professor, Department of Veterinary Pathology, College of Veterinary and Animal Sciences, Parbhani, MS, MAFSU, Nagpur, India

Chavan RS
M.V.Sc. Scholar, Department of Veterinary Pathology, College of Veterinary and Animal Sciences, Parbhani, MS, MAFSU, Nagpur, India

Kachave PB
M.V.Sc. Scholar, Department of Veterinary Pathology, College of Veterinary and Animal Sciences, Parbhani, MS, MAFSU, Nagpur, India

Swami SB
M.V.Sc. Scholar, Department of Veterinary Pathology, College of Veterinary and Animal Sciences, Parbhani, MS, MAFSU, Nagpur, India

Pathade DS
M.V.Sc. Scholar, Department of Veterinary Pathology, College of Veterinary and Animal Sciences, Parbhani, MS, MAFSU, Nagpur, India

Corresponding Author
Bawanthade UR
M.V.Sc. Scholar, Department of Veterinary Pathology, College of Veterinary and Animal Sciences, Parbhani, MS, MAFSU, Nagpur, India

Dimethoate induced haemato-biochemical alterations and their amelioration by leaves powder of *Hibiscus rosa sinensis* in Kaveri birds

Bawanthade UR, Moregaonkar SD, Gangane GR, Dr. Kondre BM, Chavan RS, Kachave PB, Swami SB and Pathade DS

Abstract
The present work was undertaken to investigate the haemato-biochemical changes in dimethoate (an organophosphate) fed Kaveri birds. In addition, their amelioration by leaves powder of *Hibiscus rosa sinensis* was assessed. Hundred newly hatched day old, unsexed Kaveri birds were procured for the study. All birds were acclimatized for a period of seven days and then evenly split into four groups of 25 birds each. The group-I served as healthy control and was given standard feed and water *ad libitum*. The birds of groups II were toxicated daily with a solution of dimethoate @ 5.5 mg/kg [1/10th of $LD_{50\%}$] bodyweight through oral gavage. The birds of group III were treated as a plant control group and fed with a *Hibiscus rosa sinensis* leaves powder @ 1 % of feed daily. The birds in group IV were treated with dimethoate @ 5.5 mg/kg body weight daily through oral gavage + *Hibiscus rosa sinensis* leaves powder @ 1% of feed daily. The present investigation last for the period of 28 days excluding acclimatization period. The blood and serum samples were drawn from wing vein on 14th day and 28th day from the birds in each group for the estimation of haemato-biochemical indices. Significantly decreased hematological parameters i.e., Hb, PCV, TEC, lymphocytes and heterophil (%) counts whereas TLC and blood clotting time (Sec.) were significantly increased. The biochemical parameters such as indicators of liver health (i.e., Mean of AST, ALT and ALP values) and indicators of the kidney (i.e., Mean SUA, Mean BUN) of birds of this group were significantly increased. However, mean serum TP, albumin, globulin and AchE values were significantly decreased in dimethoate fed Kaveri birds in comparison to other heathy control and plant control group birds. The feeding of *Hibiscus rosa Sinensis* leaves powder against sub-acute dimethoate toxicity showed partial ameliorative effects on haemato-biochemical parameters.

Keywords: Dimethoate, kaveri birds, organophosphate, haemato-biochemical indices, *Hibiscus rosa sinensis*

Introduction
Poultry is one of the fastest-growing segments of the agricultural sector in India with around eight percent growth rate per annum [10]. India today is one of the largest manufacturers of eggs and broiler meat, India ranks third in egg production and fifth in chicken meat production. Currently, the total poultry population in our country is 851.81 million (as per 20th Livestock Census 2019) [1] and egg production is around 103.32 billion during 2018-19. The per capita availability (2018-19) is around 79 eggs per annum. The poultry meat production is estimated to be 4.06 million tonnes. Growth rate in 2018-19 was 7.8 % over the previous year.
Although the production is mainly achieved through commercial means, the rural backyard poultry sector also contributes significantly to the Indian poultry industry [28].
Backyard poultry is a manageable and encouraging enterprise to improve the socio-economic and nutritional status of rural people, especially landless or poor families with low initial investment and high economic return [8, 26]. Further, they assessed that in India about 15% of total poultry output is derived from backyard poultry production. Backyard poultry is a potent tool for upliftment of poor because it requires hardly any infrastructure set-up. Besides income generation and poverty reduction, rural backyard poultry can provide nutrition supplementation in the form of valuable animal protein [32]. Backyard poultry birds convert waste material like home kitchen waste, vegetable waste, green grass, leftover grains and cereals etc., into high-quality eggs and meat for human consumption. The importance of poultry can be highlighted through generation of income and employment along with its role in family nutrition.
Similarly, majority of the population in India is engaged in agriculture and is therefore highly exposed to the pesticides used in agriculture sector.

Indiscriminate and repeated application of pesticides leads to loss of biodiversity, pest-resistance and other ecological imbalance [25]. The backyard poultry birds get direct or indirect exposure to these pesticides through grains, contaminated fodder/feed and container of insecticides thrown in field.

Organophosphates (OPs) are a class of insecticides, several of which are highly toxic. Organophosphates poison insects and other animals, including birds, amphibians and mammals, primarily by phosphorylation of the acetylcholinesterase enzyme (AChE) at nerve endings. The result is a loss of available AChE so that the effector organ becomes overstimulated by the excess acetylcholine (ACh, the impulse-transmitting substance) in the nerve ending. Among these OP compounds Dimethoate is one of them extensively used as a crop protectant in the field, especially in and around Parbhani, hence this compound was taken for the study.

Now, a day's wide variety of plants and herbal preparations were utilized for the purpose of treatment of many diseases or disorders, certain toxicities. And the many people were shifting toward the herbal medicines. These various herbs and plants contain wide nutritional, medicinal, pharmaceutical, and phytochemical properties that aid in reducing the effects of any of irritant.

Hibiscus rosa sinensis possess multiple biological activities including antioxidant, antispermatogenic, androgenic, antitumor and anticonvulsant suggesting the hepatoprotective effect of *Hibiscus rosa sinensis* may be due to its antioxidant activity [3]. Hence, this plant was selected to conduct the experimental trial.

Materials and methods

This study was performed in poultry shed of College of Veterinary and Animal Sciences, Parbhani (MS).

Experimental Animals

One hundred healthy day old 'Kaveri' chicks were purchased from an authorized hatchery for this investigation. These birds were acclimatized for a period of seven days afterwards, all of the birds were randomly separated into four groups and kept in those groups for the duration of the entire experiment, which was last for 28 days (excluding 7 days of acclimatization period).

Chemical preparation and administration of birds

The dimethoate pesticide (30% EC) was procured from local fertilizer shop of Parbhani.

Dimethoate of 30% EC was diluted in distilled water to make a suitable dose of 5.5 mg/ kg Bwt, which was administrated by oral gavaging.

Leaves powder of *Hibiscus rosa sinensis*

Plant leaves of *Hibiscus rosa sinensis* were obtained from a nearby area of Parbhani (MS) which were dried, grind and powder was used throughout the experiment.

Experimental design

The group-I served as healthy control and was given standard feed and water *ad libitum*. The birds of groups II were toxicated daily with a solution of dimethoate @ 5.5 mg/kg [1/10th of $LD_{50\%}$] bodyweight through oral gavage. The birds of group III were treated as a plant control group and fed with a *Hibiscus rosa sinensis* leaves powder @ 1 % of feed daily. The birds in group IV were treated with dimethoate @ 5.5 mg/kg body weight daily through oral gavage + *Hibiscus rosa sinensis* leaves powder @ 1% of feed daily. The present investigation last for the period of 28 days excluding acclimatization period. The experiment was performed after approval of IAEC, and proper care was given to the chickens used in this experiment.

Haematology

Blood samples were collected from six birds from each group from wing vein in EDTA vails on 0, 14th and 28th day of the trial. Hematological studies included the determination of haemoglobin (Hb) by acid haematin method, packed cell volume (PCV) by micro haematocrit method described by Jain (1986) [19], total erythrocyte counts and total leukocytes counts by haemocytometer method described by Sastry (1989) [36], differential leucocyte counts by method described by Weiss and Wardrop (2010) [43] and clotting time by capillary method described Benjamin (1985) [5].

Serum biochemistry

Blood samples collected from six birds in each group on 0, 14th and 28th day of trial were allowed to clot in clot activator vial and centrifuged at 1000-1500 rpm for 20 minutes to separate and collected in serum collection vials.

Serum biochemicals estimations included Serum Total Protein (gm/dL), serum albumin (gm/dl) by biuret method described by Varley (2005) [41], serum glucose (mg/dl) by GOD POD method described by Trinder (1969) [40], serum AST (IU/L) and serum ALT (IU/L) by UK kinetic method described by Teitz (1976) [38], serum alkaline phosphatase (IU/L) by kinetic rate method described by Robison (1923) [35], serum urea nitrogen (mg/dl) by Berthelot method suggested by Chaney and Marbach (1962) [9], serum uric acid (mg/dl) by POD method described by Fossati *et al*., 1980 [13] and serum AchE (U/L) by Butyrylthicholine method described by Henry Hallett Dale (1915) [17].

Statistical Analysis

The resulting data generated from various parameters will be statistically analyzed by using WASP (Anonyms, 2018 WASP version 2.0

http: //www.ccari.res.in/wasp2.0/index.php.). Data were shown as mean± standard error.

Results

Hematological parameters

The result of haemoglobin, packed cell volume, TEC and lymphocyte count (%) indicated significant reduction in birds of group II and IV as compared to birds of group I and III. While, TLC, Heterophil Count (%) and Blood Clotting time (Sec.) values of birds of these groups showed significant elevation when compared with them on both 14th and 28th day of study. There was no significant variation in all parameters between the birds of all groups on 0 day of study. Haematological parameters of birds in group IV showed mild improvement over birds in group II. The results of hematological studies have been presented in Table 1.

Table 1: Effect of acute oral administration of dimethoate on various haematological parameters in Kaveri birds on 0, 14th and 28th day of study and protective effect of *H. sinensis* leaves powder.

Parameters/ study intervals	Groups				*P* value
	Group I (Healthy control)	Group II (Dimethoate 5.5mg/kg)	Group III (*H. sinensis* plant control)	Group IV (Dimethoate +*H. sinensis)*	
Haemoglobin (Hb) gm/dl					
0	10.72 ±0.21	10.90 ±0.18	10.26 ±0.33	10.86 ±0.15	NS
14th	10.70a±0.36	09.46b ±0.19	10.93a±0.31	09.80b±0.26	HS
28th	10.60a ±0.21	09.13c ±0.36	10.30ab ±0.35	09.56bc ±0.25	HS
Packed Cell Volume					
0	32.70 ±1.46	31.56 ±1.16	31.73 ±1.01	32.13 ±1.16	NS
14th	32.84a ±1.01	28.33c ±0.95	32.50ab±0.88	30.17bc ±0.60	HS
28th	33.00a ±0.96	27.67b ±1.05	32.17a ±1.17	30.00ab±0.89	HS
Total Erythrocyte Count (10^6/mm^3)					
0	3.22 ±0.05	3.15 ±0.02	3.19 ±0.03	3.22 ±0.02	NS
14th	3.05a ±0.07	2.71b ±0.08	2.99a ±0.06	2.86ab ±0.05	S
28th	3.26a ±0.09	2.63b±0.08	3.09a ±0.17	2.88ab ±0.17	S
Total Leucocyte Count (10^3 /mm^3)					
0	23.13 ±0.32	22.31 ±0.12	22.59 ±0.15	23.00 ±0.18	NS
14th	23.29c ±0.40	25.98a ±0.20	23.89bc ±0.50	24.48b±0.28	HS
28th	24.70bc ±0.97	27.55a ±0.68	23.70c ±0.41	26.46ab ±1.00	S
Lymphocyte count (%)					
0	66.16 ±0.87	67.17 ±1.01	67.33 ±0.71	68.00 ±0.37	NS
14th	64.83a ±0.83	58.50b ±0.96	65.66a ±0.92	59.33b±1.08	HS
28th	66.16a±0.60	58.66c ±0.55	63.66b±0.62	60.33c ±0.84	HS
Heterophil Count (%)					
0	24.83 ±0.54	25.17 ±1.08	25.00 ±0.73	24.83 ±0.75	NS
14th	27.17b ±0.87	31.83a ±1.01	26.00b ±0.57	30.17a±0.79	HS
28th	25.17b ±1.01	31.67a±0.61	26.67b ±0.71	30.17a±0.79	HS
Blood Clotting time (Sec.)					
0	46.83 ± 3.48	44.17 ±4.72	49.33 ±2.79	46.67 ±3.80	NS
14th	46.83b±1.90	56.67a±3.16	48.17b±1.74	52.50ab ±2.92	S
28th	50.50bc ±2.92	63.67b ±2.02	48.50c ±3.25	57.00ab ±1.29	HS

Values indicate mean ± S.E

Non-significant (NS)=P>0.05, Significant (S)= P<0.05 and Highly significant (HS)= P<0.01

Biochemical parameters

Investigation for biochemical parameters of birds of group II revealed that, serum glucose, indicators of liver health (i.e., Mean of AST, ALT and ALP values) and indicators of the kidney (i.e., Mean SUA, Mean BUN) of birds of this group were significantly increased. Whereas, mean serum TP, albumin, globulin and AchE values were significantly decreased when compared with values of birds in the healthy control and plant control group. The results of biochemical studies have been summarized in Table 2. Almost in all biochemical indices the birds in group IV showed improvement

Table 2: Effect of acute oral administration of dimethoate on various biochemical parameters in Kaveri birds on 0, 14th and 28th day of study and protective effect of *H. sinensis* leaves powder.

Parameters/study intervals	Groups				*P* value
	Group I (Healthy control)	Group II (Dimethoate 5.5mg/kg)	Group III (*H. sinensis* plant control)	Group IV (Dimethoate + *H. sinensis)*	
Serum glucose (mg/dl)					
0	255.65 ±7.50	262.73 ±4.80	257.20 ±4.92	249.00 ±6.28	NS
14th	253.76b ±6.44	277.91a ±5.90	259.73b ±6.48	265.03b±5.40	S
28th	247.95c ±3.37	266.56a ±2.82	252.92bc ±2.36	260.46ab ±2.45	S
Packed Cell Volume					
0	4.70 ±0.17	4.73 ±0.25	4.78 ±0.14	4.68 ±0.16	NS
14th	4.83a ±0.14	4.18b ±0.18	4.78a ±0.13	4.28b ±0.09	HS
28th	4.61a ±0.23	4.15b ±0.19	4.73a ±0.14	4.29ab ±0.04	S
Serum Albumin (gm/dl)					
0	2.56 ±0.09	2.53 ±0.09	2.60 ±0.11	2.59 ±0.09	NS
14th	2.64a ±0.12	2.36b ±0.06	2.65a ±0.07	2.37b ±0.03	S
28th	2.61a ±0.14	2.21c ±0.05	2.55ab ±0.08	2.31bc ±0.06	S
Serum globulin (gm/dl)					
0	2.14 ±0.10	2.20 ±0.19	2.18 ±0.07	2.10 ±0.09	NS
14th	2.19a ±0.04	1.83b ±0.19	2.13a±0.09	1.92ab ±0.08	S
28th	2.01 ±0.15	1.95 ±0.15	2.17 ±0.08	1.97 ±0.04	NS
Serum aspartate transaminase (AST, IU/L)					
0	123.13 ±3.15	127.45 ±3.54	124.80 ±1.23	125.98 ±5.67	NS

14th	127.52b±2.71	143.47a ±1.84	126.45b±3.65	136.70a ±2.69	HS
28th	128.25b ±3.72	142.51a±3.10	126.55b ±3.78	134.22ab±1.95	HS
Serum alanine transaminase (ALT, IU/L)					
0	11.05 ±0.47	11.34 ±0.27	11.15 ±0.48	11.00 ±0.46	NS
14th	11.13b±0.41	12.75a ±0.19	11.02b ±0.46	11.50b ±0.27	HS
28th	11.27b ±0.42	12.67a± 0.22	10.99b ±0.34	11.83ab± 0.16	HS
Serum Alkaline Phosphatase (ALP, IU/L)					
0	1043.30 ±17.63	1054.50 ±14.52	1046.50 ±18.69	1057.80 ±33.43	NS
14th	1076.50c ±34.81	1316.70a ±27.12	1075.80c ±26.92	1209.00b ±35.68	HS
28th	1060.7b ±39.02	1302.70a±44.12	1087.3b ±36.85	1178.0ab ±60.62	HS
Serum Uric Acid (SUA, mg/dl)					
0	4.24 ±0.32	4.70 ±0.28	5.24 ±0.16	4.69 ±0.18	NS
14th	4.12b±0.64	6.22a±0.15	4.07b±0.70	5.88a±0.09	HS
28th	4.55b±0.64	8.06a±0.48	4.97b±0.70	6.94a ±0.43	HS
Blood urea nitrogen (BUN, mg/dl)					
0	5.83 ±0.37	5.78 ±0.21	5.89 ±0.19	5.93 ±0.30	NS
14th	6.17b ±0.30	7.47a ±0.27	6.16b±0.21	6.75ab± 0.23	HS
28th	6.47b±0.14	7.94a ±0.36	6.53b ±0.18	7.38a ±0.25	HS
Serum Acetylcholine Esterase (AChE, U/L)					
0	3198.50 ±54.84	3175.50 ±70.39	3217.80 ±56.86	3152.00±33.65	NS
14th	3067.0a ±80.59	2440.5b ±68.70	3042.0a ±97.01	2583.2b ±90.52	HS
28th	2943.5a ±59.60	2431.2c±53.20	2921.3a ±68.30	2707.3b±76.26	HS

Values indicate mean ± S.E
Non-significant (NS)=P>0.05, Significant (S)= P<0.05 and Highly significant (HS)= P<0.01

Discussion

The Hb concentration, PCV value and total erythrocyte count in OP intoxicated group i.e., group II was significantly reduced at both intervals of study (14th and 28th day). This reduction in haematological parameters might be due to decrease or impaired haemoglobin synthesis or binding of OP compound to iron resulting into decrease size of RBCs and lowered biosynthesis of heme in bone marrow [16]. Further, it might be due to the distractive action of insecticides on erythropoietic tissue resulting in lysis of RBCs or impairment in Hb synthesis [33] Or it might be attributed due to intravascular haemolysis, anemia or depression of the hemopoiesis [30]. The increase in total leucocyte counts of dimethoate insecticide toxicated birds might be due to toxin elicited inflammatory response induced due to raised oxidative stress because of tissue injuries caused by OP insecticide [18] or it might be raised due to stimulation of leucocyte production as a result of the action of OP insecticide as a chemical stressor leads to an increase in adrenaline level and results in lymphatic leucocytosis as stated by [20].

The lowered lymphocyte counts at both 14th and 28th study interval, might be because of immunosuppressive effect of dimethoate OP compound [15] resulting in decrease in the production of lymphoid tissue, as the lymphocytes are the main indicator for the normal functioning of the immune system, which was further supported by decreased weight of lymphoid organs [27]. The toxicated birds showed heterophilia throughout the trial might be because of toxic effects of dimethoate [21]. Elevated blood clotting time in toxicated birds, because of reduction in platelet count [11].

Partial improvement in all haematological parameters of group IV, might have been due to antioxidant properties due to different phytochemicals such as flavonoids, triterpenoids, alkaloids, tannins etc. present in the plant leaves [31], which reduces the oxidative stress [29]. The partial reversion of total leucocyte count towards normal range might be due to the anti-inflammatory actions of *Hibiscus rosa sinensis* [39].

Elevated serum glucose level (hyperglycemia) in dimethoate treated birds could be due to hyperaesthesia, intermittent spasms, muscular tremors and convulsions. This involuntary energy demanding activity triggers the release of glucose by glycogenolysis of the liver [15] or it could be because of a severe disturbance in carbohydrate, lipid, and protein metabolism. It may be due to increased activity of the enzymes involved in gluconeogenesis, resulting in the formation of glucose from non-carbohydrate sources, combined with inhibition or stimulation of liver [2].

A significant decrease in serum total protein, albumin and globulin in birds of group II might be due to changes in protein and free amino acid metabolism and their synthesis in the liver. It could also be due to protein loss caused by a decrease in protein synthesis, increased proteolytic activity, or its degradation [4]. Additionally, it might be attributed to reduced feed consumption and also due to hepatic damage (which was target organ of organophosphate insecticide), since the liver is a major organ for protein synthesis [23]. It was further supported by elevated serum levels of AST, ALT and ALP observed in this study.

The increase in AST, ALT and ALP level in toxicated group might be attributed to parenchymatous degeneration of vital organs resulting in the leakage of enzymes from the cells [22]. Since the liver architecture of birds of group II revealed pathological alterations, hence this might be the reason for this elevation in these hepatic indicators in birds of this group. Or it could be attributed because of peroxidative damage to liver due to oxidative free radicles damaging mechanism [6, 37].

The significant elevation of serum uric acid values in organophosphate dimethoate compound toxicated groups was might be due to renal tissue damage [12]. Also, Urea is considered as the major end product of protein metabolism, their level in serum depends mostly upon the renal functioning. This elevated blood urea level in present study suggested the damage to renal functioning [37].

Significant reduced level of AchE was observed in dimethoate administrated birds at both 14th and 28th day study interval. Acetylcholine is found throughout the mammalian nervous system, including at cholinergic synapses in the central nervous system, the junction of postganglionic parasympathetic neurons in exocrine glands and smooth and

cardiac muscles, at pre- and post-ganglionic neurons in the autonomic nervous system, at neuromuscular junctions of the somatic nervous system [46, 47]. The AChE inhibition is the characteristic property of OP insecticides, AChE inhibition leads to development of nicotinic, muscarinic and central nervous effects [13].

Generally, acetylcholine a neurotransmitter hydrolysed rapidly as they serve their function by acetylcholine esterase (AChE), the inhibition of AChE activity resulting into the accumulation of acetylcholine at synaptic end, leads to overstimulation of nervous system, also the dysfunctioning in the transmission of nerve impulse in both central and peripheral nervous system [24].

Non-significant partial reversal in serum glucose level in birds of group IV than the birds of group II was, because of hypoglycaemic properties and antioxidant properties possess by the leaves of *Hibiscus rosa sinensis* [45]. Mild non-significant improvement in serum total protein, albumin and globulin in birds of group IV over dimethoate toxicated birds indicates partial amelioration by leaves powder of *Hibiscus rosa sinensis.* This beneficial effect might be because of various phytochemicals and nutritional properties of plant *Hibiscus rosa sinensis.*

The partial reduction in elevated activities of serum AST, ALT and ALP levels of birds in group IV, indicating that this attenuation effects of *Hibiscus rosa sinensis* could be attributed to its phytochemical phenolic compounds, predominantly flavonoids, triterpenoid and tannins, these compounds reduce the risk of hepatotoxicity by acting against free radical mediated damages to restore the functional integrity of the membrane of hepatocytes [29]. Similarly, partial restoration of serum uric acid and blood urea nitrogen levels were because of protection against renal damage caused by dimethoate insecticide. AchE activity was numerically improved might have been due to the prevention of oxidative damaged induced by free radicals because of antioxidant properties [14].

Conclusions

1. Administration of Dimethoate @5.5 mg/kg body weight through oral gavage daily for 28 days induced toxicity, which was evidenced by haemato-biochemical alterations in kaveri birds.
2. The feeding of *Hibiscus rosa sinensis* leaves powder through feed daily for 28 days could be partially beneficial against the administrated organophosphate compound.

References

1. 20th livestock census. Department of Animal Husbandry and Dairying, Government of India Animal Husbandry Statistics Division. Livestock Census. 20th Livestock Census 2019-all India Report. Ministry of Fisheries, Animal Husbandry and Dairying, 2019. Available from: http://dahd.nic.in/animal-husbandry-statistics. Accessed 20th July 2021.
2. Al-Attar AM. Effect of grapeseed oil on diazinon-induced physiological and histopathological alterations in rats. Saudi Journal of Biological Sciences. 2015;22(3):284-292.
3. Al-Snafi AE. Chemical constituents, pharmacological effects and therapeutic importance of *Hibiscus rosa-sinensis*: A review. Journal of Pharmacy. 2018;8(7):101-119.
4. Attia AM. Dimethoate-induced changes in biochemical parameters of experimental rat serum and its neutralization by black seed (*Nigella sativa* L.) oil. Slovak Journal of Animal Science (Slovak Republic), 2009.
5. Benjamin MM. Outline of Veterinary Clinical Pathology. 3rd Edn, Lowa State University Press, Ames, Lowa, USA, 1978, 29-35.
6. Bharathi P, Reddy AG, Reddy AR, Alpharaj M. A study of certain herbs against chlorpyrifos-induced changes in lipid and protein profile in poultry. Toxicology International. 2011;18(1):44.
7. Blodgett DJ, Peterson ME, Talcott PA. (Eds.), Organophosphate and Carbamate Insecticides: Small Animal Toxicology, second ed., Elsevier Saunders, St. Louis, MO, 2006, 941-947.
8. Chakrabarti AA, Dey Barari SK. Backyard poultry farming- a source of better livelihood for rural farmers. Krishisewa, 2014. (http://www.krishisewa.com/articles/livestock/410 backyardpoultryfarming.html/12.04.2019).
9. Chaney AL, Marbach EP, Modified reagents for determination of urea and ammonia. Clinical chemistry. 1962;8(2):130-132.
10. Chatterjee RN, Rajkumar U. An overview of poultry production in India. Indian Journal of Animal Health. 2015;54(2):89-108.
11. El-Deeb AEA, Abd El-Aleem IM, Sherin SG. Harmful effect of some insecticides on vital parameters of albino rats. J. Egypt. Soc. Toxicol. 2007;36:53-60.
12. EL-Nahhal Y, Lubbad R. Acute and single repeated dose effects of low concentrations of chlorpyrifos, diuron, and their combination on chicken. Environmental Science and Pollution Research. 2018;25(11):10837-10847.
13. Fossati P, Prencipe L, Berti G. Use of 3, 5-Dichloro-2-Hydroxybenzenesulfonic Acid/4-Ami No-Phenazone Chromogenic System in Direct Enzymic Assay of Uric Acid in Serum and Urine. Clinical Chemistry. 1980;26:227-231.
14. Garg D, Shaikh A, Muley A, Marar T. *In-vitro* antioxidant activity and phytochemical analysis in extracts of *Hibiscus rosa-sinensis* stem and leaves. Free Radicals and Antioxidants. 2012;2(3):41-46.
15. Garg UK, Pal AK, Jha GJ, Jadhao SB. Pathophysiological effects of chronic toxicity with synthetic pyrethroid, organophosphate and chlorinated pesticides on bone health of broiler chicks. Toxicologic pathology. 2004;32(3):364-369.
16. Ghaffar AS, Ashraf R, Hussain T, Hussain M, Shafique S, Noreen, Aslam S. Clinico-hematological disparities induced by triazophos (Organophosphate) in Japanese quail. Pakistan Veterinary Journal. 2014;34(2):257-259.
17. Henry HD. A Potentiometric Sensor Based on Protoporphyrin Ix for The Assay of Acetylcholine. U.P.B. Sci. Bull., Series B. 1915;78(2).
18. Hussain R, Ali F, Javed MT, Jabeen G, Ghaffar A, Khan I, *et al*. Clinico hematological, serum biochemical, genotoxic and histopathological effects of trichlorfon in adult cockerels. Toxin Reviews, 2019. DOI: 10.1080/15569543.2019.1673422.
19. Jain NC. Schalm's Veterinary Haematology, 4th Edition. Lea and Febiger, Philaldelphia, 1986, 15-81, 356-404.
20. Jain N, Sharma P, Sharma N, Joshi SC. Haemato-biochemical profile following sub acute toxicity of

malathion in male albino rats. Avicenna J. Phytomed. 2009;2:500-506.
21. Kafle A, Roy DC, Sarma J, Upadhyaya TN, Mahanta JD, Gogoi R. Histopathological Changes and Tissue Residue Deposition in Broiler Birds Following Profenofos Administration. International Journal Curr. Microbiol. App. Science. 2018;7(01):206-213.
22. Kammon AM, Brar RS, Banga HS, Sodhi S. Patho-biochemical studies on hepatotoxicity and nephrotoxicity on exposure to chlorpyrifos and imidacloprid in layer chickens. Veterinarskiarhiv. 2010;80(5):663-672.
23. Krishnamoorthy P, Vairamuthu S, Balachandran C, Muralimanohar B. Pathology of chlorpyriphos and T-2 toxin on broiler chicken. Veterinarskiarhiv. 2007;77(1):47-57.
24. Kumar SV, Md. Fareedullah Y, Sudhakar Venkateswarlu B, Kumar EA. Current review on organophosphorus poisoning. Archives of Applied Science Research. 2010;2(4):199-215
25. Kumar S, Sharma AK, Rawat SS, Jain DK, Ghosh S. Use of pesticides in agriculture and livestock animals and its impact on environment of India. Asian Journal of Environmental Science. 2013;8(1):51-57.
26. Landes MS, Persaud SC, Dyck J. India's poultry sector: development and prospects. Economics Research Service: Agricultural and Trade Report WRS-04-03. Washington DC: United States Department of Agriculture, 2004.
27. Malik G, Gera S, Dahiya JP, Kadian SK, Agarwal VK. Biochemical and immunological alterations induced by chlorpyriphos intoxication in broiler chickens. Indian J. Anim. Sci. 2005;75(6):668-671.
28. Mitra A. Poultry Farming: Prospects and Impediments in India. Saudi J. Humanities Soc Sci. 2021;6(6):193-198.
29. Mubarak OB, Morsi NF, Hamama M. Roles of *H. rosa sinensis* extract in the treatment of liver against CCl4-induced hepatic oxidative stress and antioxidants disorder in male albino rats. Int. J Med. Plants Res. 2016;5:209-220.
30. Obaineh M, Matthew O. Toxicological effects of chlorpyrifos and methidathion in young chickens. African journal of biochemistry Research. 2009;3(3):048-051.
31. Rao KNV, Geetha K, Alagar Raja M. David Banji. Quality control study and standardization of *Hibiscus rosa sinensis* L. Flowers and leaves as per WHO guidelines, Journal of Pharmacognosy and Phytochemistry. 2014; 3(4):29-37.
32. Rath PK, Mandal KD, Panda P. Backyard poultry farming in India: a call for skill upliftment. Research Journal of Recent Sciences. 2015;4:1-5.
33. Rathod P, Hajare SW, Jamgade S, Ingole RS, Ingawale MV, Pooja P, *et al*. Ameliorative effect of *Tribulus terrestris* on haematological parameters against acephate induced subacute toxicity in Wistar rats, The Pharma Innovation Journal. 2020;9(2):23-26.
34. Reigart JR, Roberts JR. Organophosphate Insecticides: Recognition and Management of Pesticide Poisonings, fifth ed., U.S Environmental Protection Agency, Office of Prevention, Pesticides and Toxic Substances, Office of Pesticide Programs, U.S. Government Printing Office, Washington, DC, 1999.
35. Robison R. The possible significance of hexosephosphoric esters in ossification. Biochem J. 1923;17:286-293.
36. Sastry GA. Veterinary Clinical Pathology, 2nd Edition., Cbc Publisher's and Distributor's, New Delhi, 1989, 12-13.
37. Singh R, Srivastava AK, Gangwar NK, Giri DK, Singh R, Kumar R. Pathology of sub-chronic cadmium and chlorpyrifos toxicity in broilers, 2016.
38. Teitz NW. Expert Panel on Enzyme of the IFSC, Clin. Chem. Acta, 70, PM Practical Clinical Biochemistry 4th Edition CBS Publisher And Distributors Pvt. Ltd., New Delhi India, 1976, 236-242.
39. Tomar Vivek, Kannojia P, Jain KN, Dubey KS. Antinoceceptive and antiinflammatory activity of leaves of *Hibiscus rosa sinensis*. International Journal of Research in Ayurveda and Pharmacy. 2010;1(1):201-205.
40. Trinder P. Determination of glucose in blood using glucose oxidase with an alternative oxygen acceptor. Annals of Clinical Biochemistry. 1969;6:24-27. (Http://Dx.Doi.Org/10.1177/000456326900600108)
41. Varley H. Practical Clinical Biochemical. 4th Edition., CBS Publishers And Distributers Pvt. Ltd., New Delhi, India, 236-242. Agent--Experimental and Clinical Studies. J. Postguard Med. 2005;42(4):105-108.
42. WASP version 2.0 http: //www.ccari.res.in/wasp2.0/index.php.
43. Weiss DJ, Wardrop JK. Schalms Veterinary Hematology, 6th Edition, Blackwell Publishing Ltd., Iowa, U.S.A, 2010, 964-967.
44. Wilson BW, Arrieta DE, Henderson JD. Monitoring cholinesterases to detect pesticides exposure, Chem. Biol. Interact. 2005;157:253-256.
45. Zaki LH, Mohamed SM, Bashandy SA, Morsy FA, Tawfik KM, Shahat AA. Hypoglycemic and antioxidant effects of *Hibiscus rosa-sinensis* L. leaves extract on liver and kidney damage in streptozotocin induced diabetic rats. African Journal of Pharmacy and Pharmacology. 2017;11(13):161-169.