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Safety evaluation of phytophosholipid complexes of quercetin in *Gallus gallus domesticus*

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Abstract
Quercetin is a plant pigment, that belongs to flavonoid category of phytochemicals. Quercetin has an antioxidant property nonetheless with less water solubility and little bioavailability. In current study quercetin was complexed with phytophospolipid to form phytophospolipid complexes (phytosomes) and current study aims to investigate the its safety, in *Gallus gallus domesticus* (chicken). The safety study was carried out in healthy broiler birds, as per protocol designed for safety evaluation of compounds and formulations as per OECD guidelines. At the end of study, all the birds were scarified humanely. Necropsy procedure was performed in treatment and control birds to note any gross changes in visceral organs and tissues from these organs were collected for histopathological studies. This study concludes the safety of phytophospolipid complexes in broiler birds.

Keywords: Quercetin, safety, chicken, phytosomes

Introduction
Quercetin {2-(3,4-dihydroxyphenyl)-3,5,7-trihydroxy-4H-chromen-4-one} is a significant plant-derived phytochemical with characteristic yellow colour that displays pharmacologically diverse activities. It is a distinctive flavonoid of subclass of flavonol group. Flavanols, encompassing naturally occurring polyphenols such as flavonoids, possess a variety of beneficial properties such as antimicrobial, immunomodulatory, antiviral and anti-inflammatory. Quercetin is present in different plants, namely in apples, berries, broccoli, cherries, kale, onions, red grapes, tea and red wine. (Almeida *et al.*, 2018; Anand *et al.*, 2016; Liu *et al.*, 2014) [2, 3, 11]. Several studies proved quercetin's role in the prevention of various illnesses, counting osteoporosis, different forms of tumors, cancers, and cardiovascular diseases. The antioxidant effects of quercetin play a noteworthy part in the preclusion and treatment of these illnesses (Boots *et al.*, 2008) [4].
Chemically quercetin holds bulky volume of hydroxyl group (-OH) along with a central C-ring and conjugated π orbitals that makes this flavonoid an important robust reducing agent (Rice-Evance *et al.*, 1996; Zhang and Kim 2020) [13, 17].
Quercetin is not only used in humans but also in animals as dietary supplement. In broiler birds quercetin is fed for its antioxidant effect and to enhance meat (Zhang & Kim 2020) [17] and egg quality through feed in layer birds (Liu *et al.*, 2014;) [11]. Further it enhances the immune responses in broiler chickens and regulate feed intake through dietary supplementation.
Because of the meagre water solubility and little bioavailability of quercetin (Cai *et al.*, 2013; Wang, *et al.*, 2016; Riva *et al.*, 2019) [5, 16, 14], numerous research has been executed to alter its structure to upsurge its water solubility and bioavailability, and therefore to augment its antioxidant activity (Chen *et al.*, 2005). It is recognized fact that phytophospolipid complexes (Phytosomes) are advanced drug delivery includes conjugation of phytochemical with lipid like phosphatidylcholine which improves absorption, stability, and bioavailability of phytochemicals (Susilawati *et al.*, 2021; Ghanbarzadeh *et al.*, 2016) [15, 9].
To upsurge the solubility and bioavailability of quercetin, we have formulated quercetin in phytophospolipid complexes form. Before using it in broiler birds for different studies it is obligatory to evaluate the safety of quercetin phytosomes, henceforth in present study we prearranged its safety evaluation in broiler birds as described below.

Materials and Methods

Materials: Quercetin was procured from Sigma Aldrich®, (Germany), Phytophospolipid was provided by Lipoid as gratuitous sample, phosphate buffer saline, and ethanol (pro analytical grade) were purchased from Himedia.

Method: Quercetin phytophospolipid complexes were prepared using thin film hydration techniques as described by Abd El-Fattah *et al.,* (2017) [1] with slight modifications.

Experimental Birds: A total of 12 Broiler Day old '*Vencob 400'* chicks with a body weight an average of 43 g were purchased from a certified hatchery nearby Nagpur. Birds were fed with standard commercial poultry feed as per their respective age group and allowed to have ad libitum access to potable drinking water. Birds were provided with all standard managemental practices under the cage-rearing system housed individually. No medication was given to control and treatment birds during the entire duration of study including the observation period. Regular prescribed vaccination schedule was followed for all experimental birds.

Dosing Protocol: As per the OECD guideline 223 (OECD, 2016) [12] limit dose test, on 21st day each bird from the treatment group was orally gavaged with quercetin phytosomes at a selected limit dose of 2000 mg/Kg body weight as a single dose. The total volume of quercetin phytosomes was measured carefully not to exceed above 5mL per bird. Birds from the treatment and control groups were fasted overnight before the dosing. In control group, each bird was orally gavaged with water at a dose of 5 mL per bird.

Body weight and feed consumption: Day old chick's body weight was taken on digital weighing balance individually. Thereafter body weights of all twelve birds from control and treatment groups on 7th, 14th, 21st, 28th, and on 35th day were recorded individually. Also feed consumption was calculated on weekly basis till end of experiment. Data for body weight and feed consumption was analysed by two sample 't- test' as shown in Table No. 1 and Table No. 2 respectively.

Observations of birds: After the administration of quercetin phytosomes in treatment group and sham dosing (water) in control group, all the experimental birds were kept under observation for an initial period of two hours uninterruptedly for any sign of regurgitation and other clinical signs of toxicity. Thereafter birds were observed, at an interval of two hours for the next 24 hours. From the subsequent day till 14 days broiler birds were observed daily twice in daylight for any clinical symptoms of toxicity. As per the protocol designated in the guideline birds were weighed before dosing (i.e. on 21st day) and thereafter on the 1st, 2nd, 3rd, 7th, and 14th day of dosing.

Necropsy procedure and collection of tissue samples: At the end of the study i.e. after the 14th day of dosing, all the experimental birds from control and treatment groups were sacrificed humanely to note any gross postmortem changes. Representative tissue samples of the lung, liver, kidney, spleen, and intestine from treatment and control group of birds were collected instantly in 10% formalin. The collected tissues were subsequently subjected to histological study. Briefly, the following technique was adopted to prepare the histological slides; tissues gained from broiler birds were fixed in 10% formalin for 24 hours and then underwent consecutive passes through the numerous compartments; dehydrated in rising concentration of ethanol, then cleared in xylene and lastly drenched in paraffin.

Results and Discussion

The limit dose test is the favored assessment when toxicity is anticipated to be little and lethality is improbable at the limit dose in targeted species. Accordingly, in present study, preferred limit dose study of quercetin phytosomes was 2000 mg/Kg body weight. Quercetin with high-purity is generally recognized as safe for use as an ingredient in various specified food categories at levels up to 500 mg per serving (U. S. FDA 2010) [8]. Nevertheless, limited studies (Okamoto 2005) [18] reported its toxic effects counting its mitochondrial toxicity, pro-oxidant activity, mutagenicity, and inhibition of certain enzymes complex in hormone metabolism. Feed safety is an indispensable criterion for producing and delivering safe animal products for consumers, animals, and the environment (den Hartog, 2003) [7]. There is constantly a need of natural and harmless feed additives that can alleviate broiler health, performance, and immune function.

To study the safety of any new formulation is an imperative part of drug discovery and development process. In the present study newly formulated quercetin in phytophospolipid complexes form, were therefore screened for its safety in broiler chicken for further studies. The study was intended with an objective to provide a knowledge of safety of quercetin phytosomes. In the current study safety was evaluated by OECD guideline 223 by limit test protocol at 2000 mg/Kg body weight in broiler birds.

Clinical symptoms and mortality: All the birds from control group were healthy. Whereas one bird from treatment group showed mild sign of diarrhoea after 12 hours of dosing of quercetin phytosomes, this might be due to handling stress or managemental reasons**.** After 24 hours of dosing signs of diarrhoea subsided in the same bird without any medical intervention. All other birds in both the groups were healthy. After 24 hours of observations all birds very healthy with no signs of dullness, depression or any other clinical symptoms in treatment and control group of broiler birds. There was no mortality reported in treated group and control group.

The body weight (BW), body weight gain (BWG), feed intake (FI), feed efficiency (FE, BWG/ FI), and feed conversion ratio (FCR, FI/BWG) are sensitive and useful indicators of overall animal health (Kim, 2023) [10]. Dietary supplementation of quercetin favors weight gain in humans and animals. In the present study, though there was increase in body weight of treatment group birds noticed after administration of quercetin phytosome on 7th and 14th day but it was non-significant.

In the present study, feed consumption of broiler birds on 7th, 14th, 21st, 28th and 35th day (age) from treatment and control groups were calculated. It was observed that there was no significant difference in feed consumption in control and treatment group birds. However, on day 23rd that is 2nd day after doing of quercetin phytosomes there was non-

significant decrease in feed consumption in treatment group birds. This might be due to mild diarrhoeal symptoms in a bird of treatment group and thereby low feed consumption in same bird. This might be resulted in a reduced amount of feed consumption as altogether effect was noticed as reduced feed intake of treatment group.

Table 1: Body weight (g) of broiler birds of control and treatment group from day one till end of study.

Sr. No.	Days of body weight recording	Body weight (g)	
		Control (n=6)	Treatment (n=6)
1.	1 day (age)	42.66 ± 0.24	42.58 ± 0.20
2.	7 days (age)	185.33 ± 0.33	185.16 ± 0.40
3.	14 days (age)	440.5 ± 0.25	440.83 ± 0.9
4.	21days (prior dosing)	839.83 ± 0.94	840.16 ± 0.83
5.	23 Day (3rd after dosing)	1002.66 ± 0.49	1000.5 ± 0.9
6.	28 Day (7th after dosing)	1360.16 ± 0.70	1363.66 ± 0.81
7.	35 Day (14th after dosing)	1931.16 ± 0.86	1934.33 ± 0.71

Values showed in table are given as mean ± SE of six birds and are statistically non-significant different from one another at 5% level of significance.

Table 2: Feed consumption (g) of broiler birds of control and treatment group from day one till end of study.

Sr. No.	Days	Feed consumption (g)	
		Control group	Treatment group
1.	7	175±0.93	175.16 ±0.6
2.	7-14	542.83 ±0.7	543.33 ±0.75
3.	14-21	1116.66±0.76	1113.66 ±0.66
4.	21-23	1366 ±0.79	1370±0.57
5.	23-28	1954.5±0.79	1956.5 ± 0.44
6.	28-35	2773.33 ±0.88	2774.66 ±0.75

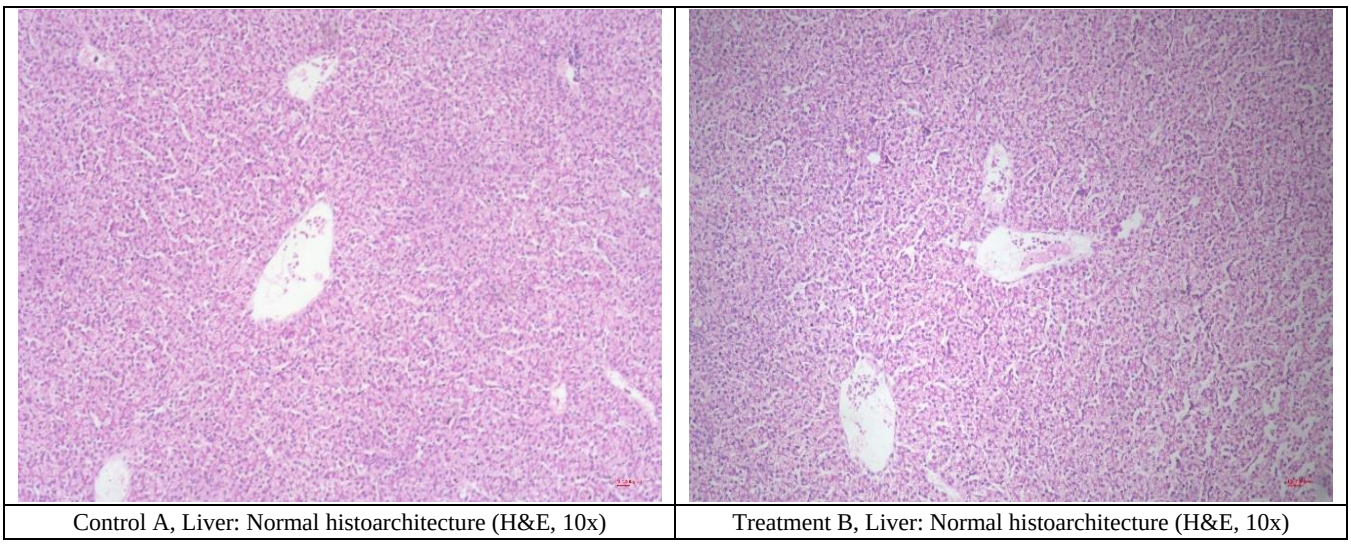
Values showed in table are given as mean ± SE of six birds and are statistically non-significant different from one another at 5% level of significance.

Gross observation after necropsy of birds

After humane sacrifice of all birds' visceral organs of control and treatment group bird's were collected and grossly observed for any abnormal changes. All the visceral organs of control and treatment group broiler birds appeared normal without any alteration in physiological aspects of birds.

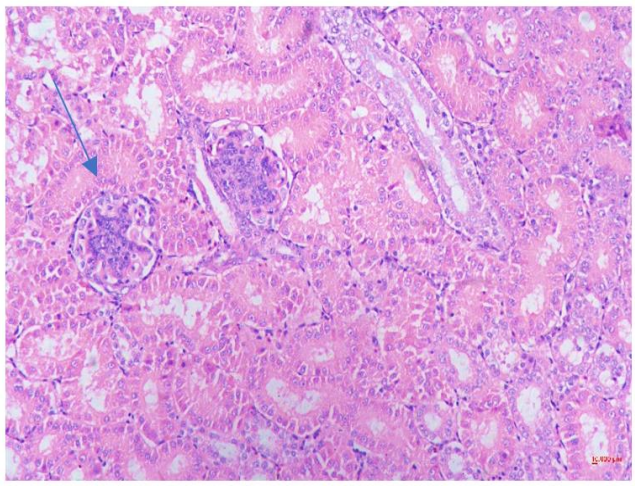
Histological observations: Histological sections of liver from treatment and control group revealed hepatocytes arranged in plates around the central vein. Each portal tract consisted of large interlobular branch of the portal vein, interlobular branches of the hepatic artery, and bile ductuli (Fig. 1). Kidney revealed normal histoarchitecture of renal lobule with cortical region and medullary cone (Fig. 2). Lungs of control and treatment group showed normal histoarchitecture. The respiratory lobules consisting of a parabronchus, atria, infundibula, and air-blood capillary bed separated by thin interstitial tissue (Fig. 3). Microscopic section of spleen from control and treatment group showed normal red pulp, and the ellipsoids and peri- ellipsoidal white pulp (Fig. 4). Microscopic section of intestine from control and treatment group showed normal mucosal architecture of intestine, villi (arrows), crypts, glands and intact submucosa (Fig. 5).

Abdel-Latif *et al.*, 2021 [19] reported that with the supplementation of quercetin in broiler birds' intestinal height of villi increased as compared to control group. In present study we have noted that there was normal histological architecture of intestine.

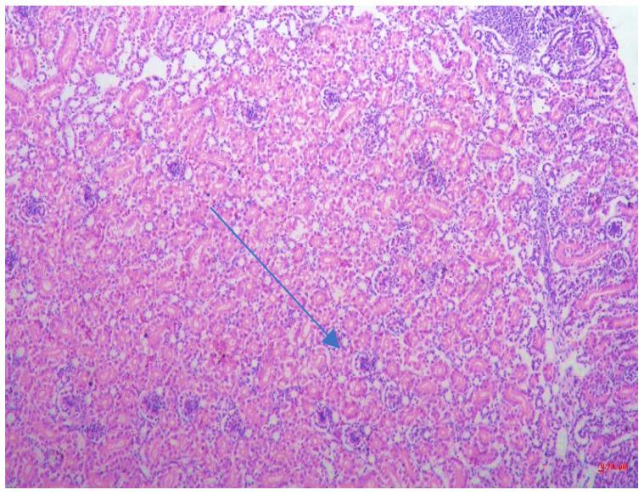


Control A, Liver: Normal histoarchitecture (H&E, 10x) | Treatment B, Liver: Normal histoarchitecture (H&E, 10x)

Fig 1: Microscopic Section of liver of control (A) and treatment (B) broiler birds

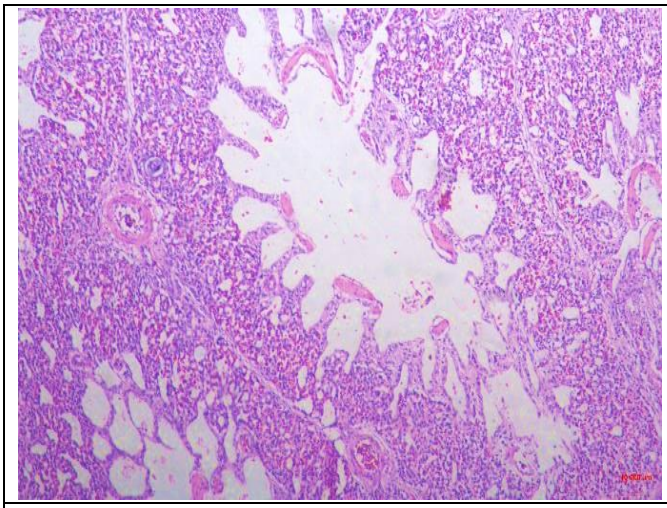


Control A, Kidney: Normal histoarchitecture (H&E, 20x)

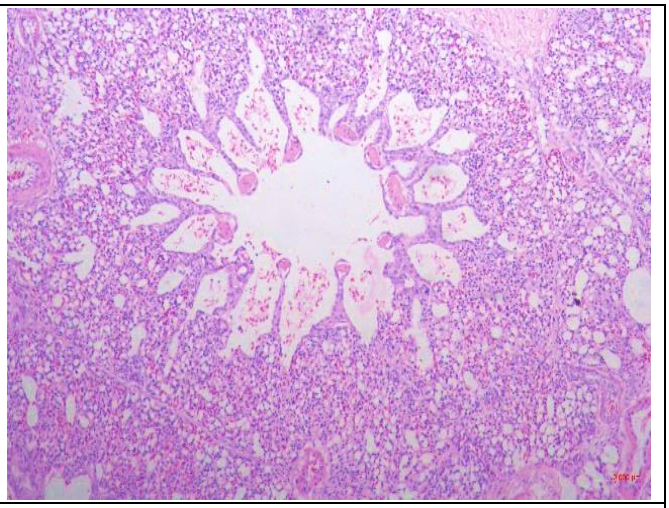


Treatment B, Liver: Normal histoarchitecture (H&E, 10x)

Fig 2: Microscopic section kidneys of control (A) and treatment (B) broiler birds

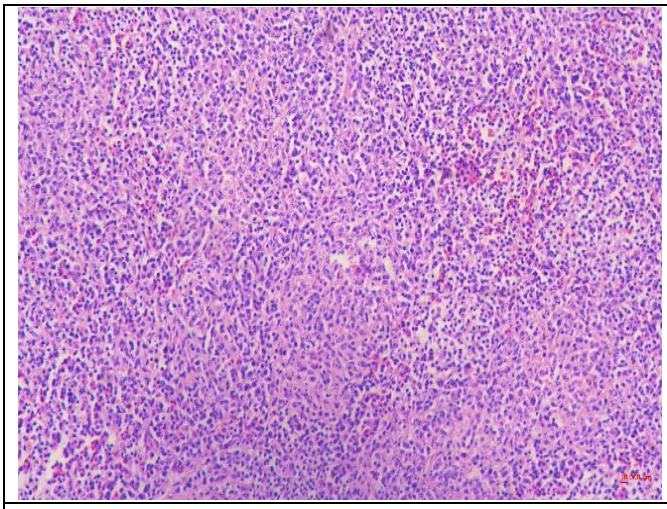


Control A, Lungs: Normal histoarchitecture (H&E, 10x)

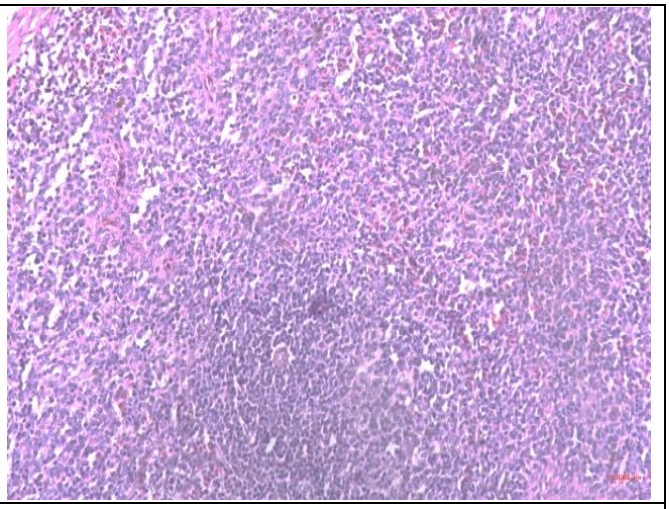


Treatment B, Lungs: Normal histoarchitecture (H&E, 10x)

Fig 3: Microscopic section of lungs tissue of control (A) and treatment (B) broiler birds

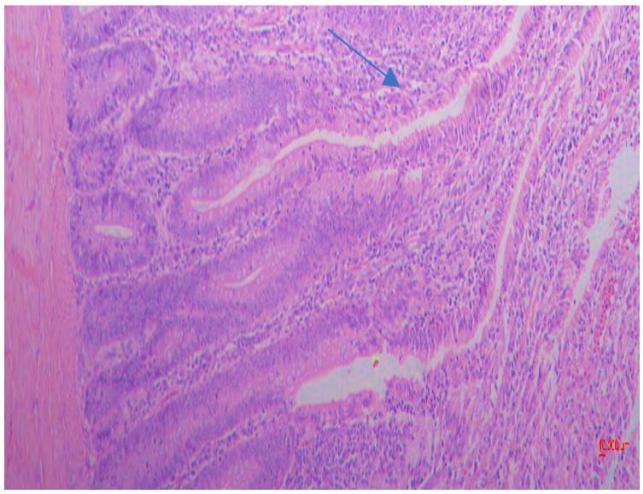


Control A, Spleen: Normal histoarchitecture (H&E, 10x)

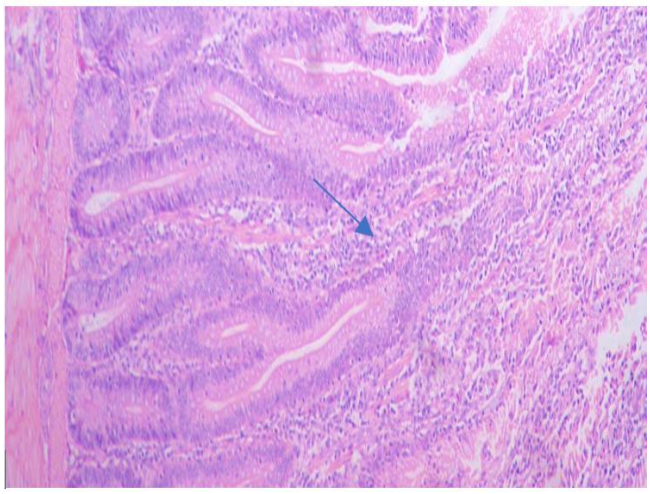


Treatment B, Normal histoarchitecture (H&E, 10x)

Fig 4: Microscopic section of spleen of control (A) and treatment (B) broiler birds (H&E staining)



Control A, Intestine: Normal histoarchitecture (H&E, 10x)



Treatment (B), Intestine Normal histoarchitecture (H&E, 10x)

Fig 5: Microscopic section of intestine of control (a) and treatment (b) broiler birds

Conclusions

Food safety is an indispensable obligation for protecting one health. Organizations of many nations are accountable for shielding and encouraging public health through animal feed safety protocols. As chicken is one of the important sources for lean meat, it is significantly important to determine the safety of nutraceuticals or herbal supplements used in poultry. So present study testified the safety of quercetin phytosomes for broiler birds without any mortality or other clinical symptoms of toxicity. Hence, it is concluded that quercetin phytosomes are safe at dose of 2000 mg/Kg body weight in broiler birds.

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Conflict of Interests

There is no conflict of interest.

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