Journal of Pharmacology and Toxicology

ISSN 1816-496X

Academic Journals Inc.

www.academicjournals.com

Journal of Pharmacology and Toxicology 5 (8): 450-459, 2010
ISSN 1816-496X

Evaluation of Safety of Naturaliv™ Liquid and Zist® Liquid on Acute Oral Exposure in Rats

V. Vijayabalaji, J. Joshua Allan, G. Pavan Kumar, B. Murali,
M.L. Suryanarayana and A. Amit
R and D Centre, Natural Remedies Pvt. Ltd., Bangalore-560 100, India

Abstract: The objective of the current study was to assess the acute oral toxicity of some herbal veterinary preparations in female albino Wistar rats as per the OECD guidelines for testing of chemicals, Acute oral toxicity-Fixed dose procedure (Test guideline 420). The investigational substances, Naturaliv™ liquid and Zist® liquid are used in poultry for hepatoprotective and antioxidant properties, respectively. Naturaliv™ liquid was tested at 5000 mg kg^{-1} b.wt., whereas Zist® liquid was evaluated in sequential manner to one animal each at 2000 and 5000 mg kg^{-1} b.wt. in the sighting study, followed by four animals at 5000 mg kg^{-1} b.wt. in the main study. The treated animals were observed for mortality (twice daily), adverse clinical signs (once daily), changes in weekly body weight gain and gross necropsy findings. All the treated animals survived throughout the study period. Treatment with Naturaliv™ liquid did not reveal any abnormal clinical signs at the tested dose level while Zist® liquid treated rats exhibited few clinical signs for a transient period on the day of dosing. The overall percent body weight gain in rats was found to be normal during the 14 day observation period. The terminal sacrifice revealed no gross pathological lesions that can be attributable to treatment. Based on this, it can be stated that Naturaliv™ liquid and Zist® liquid, on single oral administration to female Wistar rats, were found to be safe up to 5000 mg kg^{-1} b.wt.

Key words: Polyherbal formulations, toxicity, median lethal dose, poultry, hepatoprotective, anti-oxidant

INTRODUCTION

The global poultry industry forms one of the most dynamic aspects of world agribusiness trade. Despite the fast growth of commercial chicken production during 1990's, it is perceived that, in the first decade of the 21st century, the world may experience continued increase in chicken meat and egg production but not at the same rapid pace (Aho, 2002). The present poultry industry faces a variety of managemental and economical barriers. To optimize production levels for combating financial crises and also to protect consumers safety, various strategies are explored.

The use of herbal feed supplements for poultry is popular worldwide. Herbal preparations composed of single or multiple plant ingredients are used in poultry for various indications (Ranade and Desai, 2005; Waghmare *et al*., 2006; Ramnath *et al*., 2008;

Corresponding Author: J. Joshua Allan, Division of Pharmacology and Toxicology, R and D Centre, Natural Remedies Pvt. Ltd., # 5B, Veerasandra Industrial Area, 19th K.M. Stone, Hosur Road, Bangalore-560 100, India Tel: +91-80-4020 9824 Fax: +91-80-4020 9817

Jadhav *et al*., 2009). Many of the herbal supplements are based on the earlier compilations of various traditional medicine systems and are used for medicinal and non medicinal properties (Okitoi *et al*., 2007). As observed in current animal healthcare systems, the cost spent for commercial medicines at times outstrip the value of animals, especially in case of smaller stocks like poultry (Lans *et al*., 2007). Since, the conventional therapeutic agents being expensive and the availability of professional veterinary services are inadequate particularly in the developing countries, use of herbs and herbal preparations seems to be popular alternative largely due to the accessibility of cost effective animal healthcare services from familiar, local traditional healers.

On the other side, certain limitations are also reported in the published literature regarding the use of botanical preparations; one of the critical claims being lack of scientifically validated safety information of the herbal formulations. Attempts to collate toxicity data on poultry herbal supplements revealed not only the scanty information available in this aspect but also the fact that the scientific investigations were not carried out uniformly throughout the world (Oyagbemi *et al*., 2008).

With the advancement of standardised procedures for toxicity characterization of test substances, safety evaluation of veterinary formulations by approved regulatory protocols is being warranted for universal validation and acceptance. Various guidelines were made available for assessment of safety of test compounds by different international, federal and national agencies (WHO, 1993; OECD, 1993; FDA, 1993; EMEA, 1994). Studies conducted in compliance to the regulatory guidelines are considered imperative and as essential prerequisite for registration of products internationally.

Based on the above considerations, the acute oral toxicity studies of two polyherbal formulations viz., Naturaliv™ liquid and Zist® liquid were performed in accordance with the OECD guidelines for testing of chemicals, test guideline 420, acute oral toxicity-fixed dose procedure (OECD, 2001a). Naturaliv™ liquid, a combination of selected medicinal herbs, is recommended for broiler and layer birds for optimizing liver functions, utilization of nutrients, better body weight gain, feed conversion and livability. Zist® liquid, also an herbal supplement, possesses adaptogenic, antistress and immunomodulating activities. The liquid formulation, in poultry birds, helps to adjust and increase the threshold against stressful conditions, regularizes plasma cortisol levels and improves weight gain, feed conversion and livability. Zist® also optimizes egg production and hatchability in breeder birds. Generally herbal formulations are regarded as safe. However, considering the lack of adequate, scientifically validated safety data for veterinary herbal formulations in general and also taking into account the presence of several herbal ingredients in the liquid formulations, testing at an additional upper dose level of 5000 mg kg^{-1} was considered since the outcomes of such a test will provide a direct relevance for protecting target species health (OECD, 2001a). The findings of study will be useful for selection of doses for repeated dose toxicity studies and may also provide preliminary information on the target organ toxicity on acute exposure, if any. The outcome of the study observations categorizes the test substances and safety of use can be ascertained.

MATERIALS AND METHODS

Test Substances

The investigational substances viz., Naturaliv™ liquid and Zist® liquid are polyherbal preparations used in poultry for hepatoprotective and antioxidant properties were developed by M/s Natural Remedies Pvt. Ltd., Bangalore, India.

Naturaliv™ Liquid

Naturaliv™ liquid consists majorly of aqueous extracts of *Andrographis paniculata*, *Terminalia chebula*, *Eclipta alba*, *Boerhaavia diffusa*, *Mangifera indica* and *Terminalia arjuna*. The plant materials used were analyzed for respective marker compounds by High Performance Liquid Chromatography (HPLC) method (Indian Pharmacopoeia, 2007).

Zist® Liquid

Zist® liquid contains majorly aqueous extracts of *Emblica officinalis*, *Withania somnifera* and *Ocimum sanctum*. The plant materials used were analyzed for respective marker compounds by HPLC method (Indian Pharmacopoeia, 2007).

Standardization of Naturaliv™ Liquid and Zist® Liquid

The crude powders obtained from the plant materials, after verifying the content of marker compounds, were mixed in appropriate proportions and extracted to prepare Naturaliv™ liquid and Zist® liquid. The Thin Layer Chromatography (TLC) profile of product was compared with the reference material using High Performance Thin Layer Chromatography (HPTLC). The product and respective reference standard measuring 20 mL was dried separately on a water bath to dryness. The residue was extracted with 50 mL of methanol on a water bath for 30 min and then filtered after cooling. The filtrate was concentrated to 25 mL. Equal volumes (15 μL) of sample and reference standard were spotted on Silica gel 60 F_{254} plate of 0.2 mm thickness as bands. The plate was developed in a mobile phase consisting of chloroform:methanol:acetic acid (90:10:2). The dried plate was scanned at 254 and 366 nm. The plate was sprayed with anisaldehyde sulphuric acid reagent and dried in oven at 100°C. The fingerprint of the product sample was compared with reference standard. The reference standard was prepared in the laboratory by mixing the herbs as per the approved formulation and extracting with water.

Experimental Animals

Female rats of albino Wistar strain (8 to 12 weeks) were chosen for the study. The animals were received from Central Animal Facility, R and D Centre, Natural Remedies Pvt. Ltd., Bangalore, India. The animals were housed in individual polypropylene cages provided with clean bedding of paddy husk. The rats were maintained under standard housing conditions (Temperature: 25±2°C, relative humidity between 30 and 70%, with optimal air changes per hour and 12:12 h dark and light cycle) with one week acclimatization period before treatment with test substances. The animals were provided with standard pelleted rodent feed (M/s Gold Mohur Foods and Feeds Ltd., Bangalore, India) and UV treated water *ad libitum*. The project was conducted during 2008 in the R and D Centre, Natural Remedies Pvt. Ltd., Bangalore-560 100, India.

Study Design

The study was conducted in accordance with the OECD guidelines for testing of chemicals, test guideline 420, acute oral toxicity-fixed dose procedure. Healthy adult female rats were randomly allotted to the cages and each animal was identified by individual cage card number and picric acid marking on fur. The females were nulliparous and non-pregnant. Naturaliv™ liquid was tested in five animals (one animal for sighting study and four animals for main study) at 5000 mg kg^{-1}. Zist® liquid was evaluated in one animal each at the dose levels of 2000 and 5000 mg kg^{-1} b.wt. in the sighting study and in four animals at 5000 mg kg^{-1} b.wt. in main study. The rats were deprived of feed overnight before and 3 h

after the administration of the test substances. Water was not withheld during this period. The herbal formulations were administered by oral gavage to rats using an intubation needle of appropriate size fitted on to a syringe so as to deliver the dose levels of 2000 or 5000 mg kg^{-1} b.wt. Naturaliv™ liquid was diluted with demineralised water to obtain 500 mg mL^{-1} strength to administer the dose of 5000 mg kg^{-1} body weight (10 mL kg^{-1} b.wt.) whereas Zist® liquid was administered as such at 20 and 50 mL kg^{-1} b.wt. in two equally divided doses at 3 h apart so as to deliver the dose levels of 2000 and 5000 mg kg^{-1} b.wt. of plant powder, respectively.

Cage Side Observations

The treated animals were observed for mortality and clinical signs to record the onset, duration and reversal (if any) of toxic effects at various time intervals (i.e., 10 min, 30 min, 1 h, 2 h, 4 h and 6 h after the administration of the formulations). The procedure was continued thereafter for 14 days for observation of mortality (twice daily) and abnormal clinical signs (once daily). The routine cage side observations included changes in skin and fur, eyes and mucous membrane and also functions of respiratory, autonomic and central nervous systems and somatomotor activity and behaviour pattern. Clinical observations of tremors, convulsions, salivation, diarrhoea, lethargy, sleep and coma, if any, were also given particular attention.

Body Weight

Body weight of individual animals was recorded on the day of dosing and at termination on day 15 and also at weekly intervals. Weekly changes in body weight gain were calculated and recorded.

Gross Pathology and Histopathology

All the rats in the study, dying during the observation period, sacrificed moribund for humane reasons or sacrificed terminally were subjected to a complete necropsy and examined for the post mortem findings. Histopathology of organs and tissues was considered only in case of evidence of any gross pathological lesions.

RESULTS

The herbal formulations were analyzed for respective marker compounds. The marker compounds in Naturaliv™ liquid were andrographolide (*Andrographis paniculata*), chebulagic acid and chebulinic acid (*Terminalia chebula*), wedelolactone (*Eclipta alba*), boeravinone B (*Boerhaavia diffusa*), mangiferin (*Mangifera indica*) and arjungenin (*Terminalia arjuna*). The marker compounds of Zist® liquid include gallic acid (*Emblica officinalis*), withanolide A and withaferin A (*Withania somnifera*) and eugenol (*Ocimum santum*) (Table 1). The HPTLC profile of Naturaliv™ liquid and Zist® liquid were found to be similar to that of respective reference standards (Fig. 1a, b, 2a, b).

Naturaliv™ Liquid

Animals treated up to the dose level of 5000 mg kg^{-1} b.wt. survived till end of the study period and did not show any toxic clinical signs following dosing and during the observation period of 14 days, post treatment. The animal in the sighting study showed reduced body weight gain during the second week of 14 day observation period as compared to first week. However, the overall body weight gain was found to be normal at the end of 14 day study

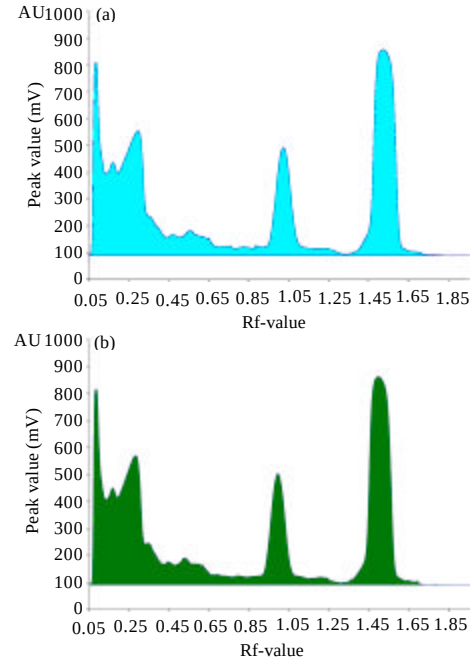

Fig. 1: HPTLC finger print pattern of standard mix (a) and sample (b) of Naturaliv™ liquid

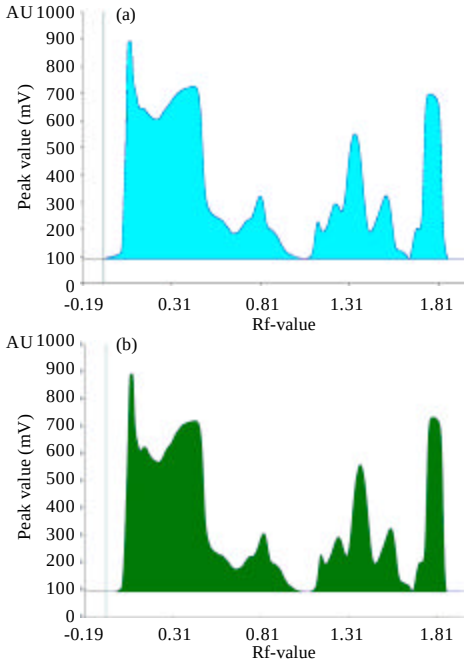

Fig. 2: HPTLC finger print pattern of standard mix (a) and sample (b) of Zist® liquid

Table 1: List of major plants with proportions used in formulations of Naturaliv™ liquid and Zist® liquid with respective marker compounds

Botanical name	% Added	Marker compounds
Naturaliv™ liquid		
Andrographis paniculata (Burm f.) Wall ex Nees.	7	Andrographolide
Terminalia chebula Retz.	6	Chebulagic acid and Chebulinic acid
Eclipta alba (L.) Hassk	9	Wedelolactone
Boerhaavia diffusa Linn.	8	Boeravinone B
Mangifera indica L.	3	Mangiferin
Terminalia arjuna (Roxb.) Wight and Arn.	6	Arjungenin
Zist® liquid		
Emblica officinalis Gaertn.	16.6	Gallic acid
Withania somnifera Dunal.	27.7	Withanolide A and Withaferin A
Ocimum sanctum Linn.	46.2	Eugenol

Table 2: Effect of herbal preparations on body weight and percent body weight gain in rats (sighting study)

		Body weight (g)			Body weight gain (%)		
Herbal preparations	Dose (mg kg^{-1})	Day 0	Day 7	Day 14	Day 0-7	Day 7-14	Day 0-14
Naturaliv™ liquid	5000	161	184	201	14.29	9.24	24.84
Zist® liquid	2000	163	201	215	23.31	6.97	31.90
	5000	160	188	207	17.50	10.11	29.38

Table 3: Effect of herbal preparations on body weight and percent body weight gain in rats (main study)

		Body weight (g)			Body weight gain (%)		
Herbal preparations	Dose (mg kg^{-1})	Day 0	Day 7	Day 14	Day 0-7	Day 7-14	Day 0-14
Naturaliv™ liquid	5000	158	188	192	18.99	2.13	21.52
		159	191	199	20.13	4.19	25.16
		155	188	192	21.29	2.13	23.87
		160	193	208	20.63	7.77	30.00
Zist® liquid	5000	160	198	205	23.75	3.54	28.13
		165	190	206	15.15	8.42	24.85
		167	198	216	18.56	9.09	29.34
		157	190	210	21.02	10.53	33.76

period (Table 2). Similarly, animals of the main study showed decreased body weight gain during the second week when compared to first week. But, the overall body weight gain was found to be normal at the end of 14 day observation period (Table 3). Macroscopic examination of animals sacrificed at termination revealed no abnormalities.

Zist® Liquid

Zist® liquid treated rats survived throughout the study period and did not exhibit any major adverse clinical signs immediately following dosing and during the observation period of 14 days except for the animal treated at the dose level of 5000 mg kg^{-1} in the sighting study exhibited abdominal twitches, shivering and vocalization at 1 h 5 min post treatment for 20 sec and rapid jaw movements and grinding of teeth at 2 h post treatment for 45 sec while two animals in the main study at 5000 mg kg^{-1} showed rapid jaw movements and grinding of teeth at 10 min post treatment for 10 sec only on the day of dosing. In the sighting study, though the weight gain of female rat was found to be less in second week of experimental period as compared to first week, the overall weight gain was found to be normal at the end of 14 day observation period (Table 2). Likewise, the rats of main study exhibited reduced body weight gain during the 2nd week of 14 day observation period in comparison to 1st week. But, the overall body weight gain was found to be normal at the end of two weeks of study period (Table 3). On necropsy, no gross pathological changes were observed in any of the treated rats.

DISCUSSION

From the long-term traditional use, herbs and herbal formulations are generally regarded as safe (Bhattacharjee, 1998). However, scientific research on a number of medicinal plants has documented general and systemic adverse effects on acute/chronic exposure (Francis, 2000). Establishing safety profiles of herbal extracts/formulations is now gaining importance in lieu of regulatory needs across the world (Schilter *et al*., 2003; Igboasoiyi *et al*., 2007; Abukakar *et al*., 2008; Younis and Adam, 2008).

Safety characterization of any substance involves study of effects in biological systems either in target species or in suitable laboratory animals for specific period through different routes of exposure (EMEA, 1994). In toxicological screening programme of a test substance, acute oral toxicity testing is considered to be the preliminary step (OECD, 1987; EPA, 1998; Walum, 1998; FDA, 2000). The objectives of acute toxicity study are to provide information on the biologic activity of a substance and gain insight into its mechanism of action (Walum, 1998). Furthermore, it also helps in establishing a dosage regimen for subchronic and other safety studies (OECD, 1987).

Different approaches are recommended by various regulatory authorities for acute oral toxicity testing (EMEA, 1987; EPA, 1998; OECD, 2001a, b, 2008). Published reviews and revised guidelines indicate that the current approaches seem to emphasize more on characterization of toxic nature of a test substance based on the specific data requirements rather than solely depending on death as the end point. Also, decreasing the number of animals used for testing forms one of the important considerations of selection of appropriate testing method (OECD, 2001c). All the treated rats survived the complete 14 day study period indicating the relatively safe nature of test formulations. Treatment with Naturaliv® liquid did not exhibit any adverse clinical signs immediately following dosing and during the 14 day observation period where as symptoms such as abdominal twitches, shivering and vocalization, rapid jaw movements and grinding of teeth were observed shortly after administration with Zist® liquid. As mentioned elsewhere, the reported clinical signs were noticed only in few treated animals and the incidence was only once in each animal which could largely be due to sudden exposure of excessive amount of xenobiotic material. Also, the fact that the symptoms were noticed for transient period of time only on the day of test substance administration and did not recur at any point during the 14 day observation period indicates the transient and harmless nature of side effects.

In toxicological studies, observation on changes in body weight or growth rate is considered to be a critical endpoint (EPA, 1998; OECD, 2001a; Wang *et al*., 2009). Any substance that causes adverse effects on weight gain or growth rate of the experimental animals are considered having some toxic potential (Rodríguez-Burford *et al*., 1999). From earlier research works, it can be observed that body weight of treated animals, depending on the nature of test substances, was reported to be progressively decreased with the time course after exposure, or remained transiently affected before returning to normalcy (Joshua *et al*., 2008). The findings of the current study indicated that the experimental animals have shown increase in overall body weight gain during the 14 day observation period though the percent gain was comparably less during second week of study period in some animals compared to first week.

Gross necropsy findings provide evidences of treatment related target organ toxicity. Substances with toxic characteristics result in pathological lesions or changes in organ weights. Therefore, it is commonly recommended by the regulatory guidelines that all the treated animals (including those that die during the study or are removed from the study for

animal welfare reasons or sacrificed at the end of the study) should be subjected to postmortem examination and for individual animals all gross pathological changes should be recorded (EMEA, 1987; OECD, 2001a, b, 2008). In the present study, no gross pathological changes were observed in any of the treated rats that can be attributable to treatment.

A critical review on the safety studies on medicinal plants of the tested herbal preparations indicates the relatively safe nature of the herbal ingredients on acute exposure. In an acute oral toxicity study, female rats were treated with extract of *Andrographis paniculata* and observed for signs of toxicity for 14 days. The extract did not show any treatment-related toxic effects in rats up to the dose level of 5000 mg kg^{-1}. Also, the percent weight gain in all the treated animals during the 14 day study period was found to be normal (Chandrasekaran *et al*., 2009). The aqueous extract of fruits of *Terminalia chebula* was reported to exhibit high margin of safety since the median lethal dose was found to be above 3 g kg^{-1} and there was no mortality (Murali *et al*., 2007). Ethanolic extract of *Eclipta alba* did not show any signs of toxicity and the minimum lethal dose was found to be more than 2 g kg^{-1} in mice on oral administration (Singh *et al*., 1993). The juice of leaves of *Boerhaavia diffusa* and aqueous decoction of *Mangifera indica* on oral administration did not cause any toxic effects in mice up to 5000 mg kg^{-1} (Hiruma-Lima *et al*., 2000; Severi *et al*., 2009). An acute oral toxicity evaluation reported that *Ocimum sanctum* leaf extract was not toxic up to 15 g kg^{-1} in Sprague-Dawley rats (Khumphan and Lawso, 2002). In another study, the ethanolic extract of *Ocimum sanctum* leaves did not cause any mortality or toxicity up to the dose level of 4 g kg^{-1} in albino rats (Udupa *et al*., 2006). Hence, the available, published literature on the safety characteristics of plant ingredients authenticate the contribution of the medicinal plants to the overall safety of Naturaliv™ liquid and Zist® liquid.

In conclusion, the findings of the present study indicate that Naturaliv™ liquid and Zist® liquid after oral administration as a single dose to female albino Wistar rats were found to be safe up to 5000 mg kg^{-1} b.wt. Based on this, the herbal preparations can be categorized as 'unclassified' according to Globally Harmonised System (GHS) and can be considered as safe.

ACKNOWLEDGMENTS

The authors are thankful to Sri. R.K. Agarwal, Chairman, M/s Natural Remedies Pvt. Ltd., Bangalore, India for his constant encouragement and support in completing this work successfully.

REFERENCES

Abukakar, M.G., A.N. Ukwuani, R.A. Shehu, 2008. An evaluation of the toxic effects of *Tamaridus indica* pulp extract in albino rats. J. Pharmacol. Toxicol., 3: 111-118.

Aho, P.W., 2002. The World's Commercial Chicken Meat and Egg Industries. In: Commercial Chicken Meat and Egg Production, Bell, D.D. and W.D. Jr. Weaver (Eds.). 5th Edn., Kluwer Academic Publishers, Massachusetts, pp: 3-17.

Bhattacharjee, S.K., 1998. Suggestions for Improvement of Medicinal Crop Industry, Handbook of Medicinal Plants. 1st Edn., Pointer Publishers, Jaipur, ISBN: 81-7132-156-9, pp: 384.

Chandrasekaran, C.V., P. Thiyagarajan, K. Sundarajan, K.S. Goudar and M. Deepak *et al*., 2009. Evaluation of the genotoxic potential and acute oral toxicity of standardized extract of *Andrographis paniculata* (KalmCold™). Food Chem. Toxicol., 47: 1892-1902

EMEA, 1987. Single dose toxicity. http://www.ema.europa.eu/pdfs/human/swp/3bs1aen.pdf.

EMEA, 1994. Scientific guidelines for veterinary medicinal products: Evaluation of the safety of veterinary medicinal products for the target animals. http://www.ema.europa.eu/pdfs/vet/vetguidelines/7ae2a.pdf.

EPA, 1998. Health effects test guidelines, OPPTS 870. 1100, Acute oral toxicity. U.S. Government Printing Office, Washington DC. http://iccvam.niehs.nih.gov/methods/acutetox/invidocs/EPA_870_1100.pdf.

FDA, 1993. Toxicological principles for the safety assessment of direct food additives and color additives used in food. http://www.fda.gov/Food/ GuidanceComplianceRegulatory Information/GuidanceDocuments/FoodIngredientsandPackaging/Redbook/ucm078717.htm.

FDA, 2000. Guidance for industry and other stakeholders toxicological principles for the safety assessment of food ingredients. http://www.fda.gov/Food/GuidanceCompliance RegulatoryInformation/GuidanceDocuments/FoodIngredientsandPackaging/Redbook/default.htm.

Francis, B.N.D., 2000. The Toxicology of Botanical Medicines. 3rd Edn., Eclectic Medical Publ., Sandy, Oregon, pp: 11.

Hiruma-Lima, C.A., J.S. Gracioso, E.J.B. Bighetti, R.L. Germonsen and A.R. Souza Brito, 2000. The juice of fresh leaves of *Boerhaavia diffusa* L. (Nyctaginaceae) markedly reduces pain in mice. J. Ethnopharmacol., 71: 267-274.

Igboasoiyi, A.C., O.A. Eseyin, N.K. Ezenwa and H.O. Oladimeji, 2007. Studies on the toxicity of *Ageratum conizoides*. J. Pharmacol. Toxicol., 2: 743-747.

Indian Pharmacopoeia, 2007. Ministry of Health and Family Welfare. Government of India, Ghaziabad, India, pp: 2018-2070.

Jadhav, N., S. Maini and K. Ravikanth, 2009. Comparative efficacy studies of herbal and synthetic choline supplements on broiler growth and performance. Internet J. Vet. Med.

Joshua, A.J., K.S. Goudar, A. Damodaran, N. Sameera and A. Amit, 2008. Acute oral toxicity evaluation of some polyherbal formulations in albino wistar rats. Int. J. Pharmacol., 4: 388-392.

Khumphan, E and D.B. Lawso, 2002. Acute toxicity, mutagenicity and antimutagenicity of ethanol *Ocimum sanctum* leaf extract using rat bone marrow micronucleus assay. Int. Bibliographic Inform. Dietary Suppl. Database,

Lans, C., T.E. Khan, M.M. Curran and C.M. McCorkle, 2007. Ethnoveterinary Medicine: Potential Solutions for Large Scale Problems. In: Veterinary Herbal Medicine, Wynn, S.G. and B. Fougère (Eds.). Elsevier Health Sciences, USA., pp: 17-32.

Murali, Y.K., P. Anand, V. Tandon, R. Singh, R. Chandra and P.S. Murthy, 2007. Long-term effects of *Terminalia chebula* Retz. on hyperglycemia and associated hyperlipidemia, tissue glycogen content and *in vitro* release of insulin in streptozotocin induced diabetic rats. Exp. Clin. Endocrinol. Diabetes, 115: 641-646.

OECD, 1987. OECD guideline for testing of chemicals: Guideline 401. Acute Oral Toxicity. OECD, Paris, France. http://iccvam.niehs.nih.gov/docs/acutetox_docs/udpProc/udpfin01/append/AppI.pdf

OECD, 1993. Health Effects: OECD Guidelines for the Testing of Chemicals. Vol. I, OECD, Paris, France.

OECD, 2001a. OECD guideline for testing of chemicals: Guideline 420. Acute Oral Toxicity-Fixed Dose Procedure, OECD, Paris, France. http://www.oecd.org/dataoecd/17/51/1948378.pdf.

OECD, 2001b. OECD guideline for testing of chemicals: Guideline 423. Acute Oral Toxicity-Acute Toxic Class Method, OECD, Paris, France.

OECD, 2001c. Guidance document on acute oral toxicity. Environmental Health and Safety Monograph Series on Testing and Assessment No 24. ENV/JM/MONO(2001)4. OECD, Paris, France. http://www.olis.oecd.org/olis/2001doc.nsf/LinkTo/NT00004CE6/$FILE/JT00111082.PDF.

OECD, 2008. OECD guideline for testing of chemicals: Guideline 425. Acute Oral Toxicity-Up-and-Down Procedure, OECD, Paris, France.

Okitoi, L.O., H.O. Ondwasy, D.N. Siamba and D. Nkurumah, 2007. Traditional herbal preparations for indigenous poultry health management in Western Kenya. Livest. Res. Rural Dev. Vol. 19.

Oyagbemi, A.A., A.B. Saba and R.O.A. Arowolo, 2008. Safety evaluation of prolonged administration of Stresroak® in grower cockerels. Int. J. Poult. Sci., 7: 574-578.

Ramnath, V., P.S. Rekha and K.S. Sujatha, 2008. Amelioration of heat stress induced disturbances of antioxidant defense system in chicken by brahma rasayana. eCAM., 5: 77-84.

Ranade, A.S. and D.N. Desai, 2005. Natural products for enhanced poultry productivity. http://www.poulvet.com/poultry/articles/enhanced_productivity.php.

Rodriguez-Burford, C., R.A. Lubet, I. Eto, M.M. Juliana, G.J. Kelloff, C.J. Grubbs and V.E. Steele, 1999. Effect of reduced body weight gain on the evaluation of chemopreventive agents in the methylnitrosourea-induced mammary cancer model. Carcinogenesis, 20: 71-76.

Schilter, B., C. Andersson, R. Anton, A. Constable and J. Kleiner *et al*., 2003. Guidance for the safety assessment of botanicals and botanical preparations for use in food and food supplements. Food Chem. Toxicol., 41: 1625-1649.

Severi, J.A., Z.P. Lima, H. Kushima, A.R. Brito, L.C. Santos, W. Vilegas and C.A. Hiruma-Lima, 2009. Polyphenols with antiulcerogenic action from aqueous decoction of mango leaves (*Mangifera indica* L.). Molecules, 14: 1098-1110.

Singh, B., A.K. Saxena, B.K. Chandan, S.G. Agarwal, M.S. Bhatia and K.K. Anand, 1993. Hepatoprotective effect of ethanolic extract of *Eclipta alba* on experimental liver damage in rats and mice. Phytother. Res., 7: 154-158.

Udupa, S.L., S. Shetty, A.L. Udupa and S.N. Somayaji, 2006. Effect of *Ocimum sanctum* Linn. on normal and dexamethasone suppressed wound healing. Indian J. Exp. Biol., 44: 49-54.

Waghmare, D.L., A.S. Ranade, D.N. Desai, M.B. Patil and P.E. Avari *et al*., 2006. Evaluation of oil fortified with herbs on performance of broilers. J. Bombay Vet. Coll., 14: 1-2.

Walum, E., 1998. Acute oral toxicity. Environ. Health Perspect., 106: 497-503.

Wang, H., F. Feng, B.Y. Zhuang and Y. Sun, 2009. Evaluation of hepatoprotective effect of Zhi-Zi-Da-Huang decoction and its two fractions against acute alcohol-induced liver injury in rats. J. Ethnopharmacol., 126: 273-279.

WHO, 1993. Research Guidelines for Evaluating the Safety and Efficacy of Herbal Medicines. WHO, Manila, pp: 94.

Younis, S.I. and S.E.I. Adam, 2008. Evaluation of toxicity of *Rhanterium epapposum* in Wistar rats. J. Pharmacol. Toxicol., 3: 134-140.