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Effect of Phytol in healthy rats following repeated Oral administration

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Abstract
Phytol is a diterpene member of the long-chain unsaturated acyclic alcohols and is a constituent of the chlorophyll molecule. Phytol and its derivatives, including phytanic acid, exert a wide range of biological effects. Phytol is a valuable essential oil component used for fragrance and it is a potential candidate for a broad range of applications in the pharmaceutical and biotechnological industry. There is ample evidence that phytanic acid may play a crucial role in the development of pathophysiological states. The two important metabolites of phytol are phytanic acid and pristanic acid. The main sources of phytanic acid in humans are dairy products and ruminant meat. Phytanic acid formed in the rumen is absorbed into adipose tissue and milk of ruminant. The literature search revealed that phytanic acid plays a possible role in the management of insulin resistance by increasing glucose uptake in hepatocytes without strongly promoting adipogenic differentiation. Besides, phytanic acid in a dose-dependent manner cause changes in various pathways in numerous cell types including pathways involved in carcinogenesis. The present study was undertaken to assess the effect of repeated administration of phytol in healthy rats. Phytol, suspended in one percent carboxy methyl cellulose was administered orally for seven consecutive days once daily at a dose rate of 100 mg/kg to the test group. All the animals under study showed normal behaviour and were healthy throughout the experiment. Phytol caused no significant increase in body weight. However, a significant decrease in the feed was noted on day three and an increase in water intake on day one. Gross pathological lesions were not observed in any of the organs examined.

Keywords: Phytol, consecutive, feed intake, water intake, relative organ weight

1. Introduction
Plants act as a phytochemical building block for the development of new drugs and play a key role in combating serious illnesses. Essential oil is a plant product that is an abundant resource of phytochemical constituents with various useful bioactivities. Among such constituents, terpenes play a major role. Terpenes are one of the broadly investigated essential oil components.

Phytol (3, 7, 11, 15- tetramethylhexadec-2-en-1-ol) is a diterpene abundantly present in nature as a part of the chlorophyll molecule. Structurally, phytol is a branched-chain unsaturated acyclic fatty alcohol with significant diverse bioactivities. Phytol and its metabolites have got numerous applications in both pharmaceutical and biotechnological fields. An extensive range of bioactivities was reported which included antioxidant, antianxiety, cytotoxic, metabolism-modulating, autophagy and apoptosis-inducing, anti-nociceptive, anti-inflammatory, immune-modulating, and antimicrobial effects (Islam *et al.,* 2018) [1]. In addition, phytol is primarily used as a fragrance constituent and as a food additive. *In-silico* docking tests have further revealed its potential to inhibit tumor proliferation factor and glucose -6- dehydrogenase phosphate (Thakor *et al.*, 2017) [2].

Phytol plays important roles in diverse metabolic processes and human diseases such as obesity, diabetes, fatty liver disease, and cardiovascular diseases by its interaction with gut microbiota. In this context, food constituents with phytol might have a variety of health and disease-related effects that might be associated with the effect on the human gut microbiota (Roca-Saavedra *et al.*, 2017]) [3]. Recently, phytol loaded on (polylactic-co-glycolic acid) (PLGA) nanoparticles showed enhanced cellular uptake of the compound and thus might result in higher effectiveness when tested *in vitro* in a cellular model with relevance for Alzheimer's disease (Sathya *et al*., 2017) [4].

Since phytol in the future can be used as a long-term therapeutic drug to treat metabolic disorders, the current study was undertaken to assess the effects in healthy rats following repeated dose administration once daily for seven consecutive days.

2. Materials and Methods

2.1 Chemicals

The chemicals and solvents used in the research work were procured from M/s Merck India Ltd., Mumbai and M/s Sigma-Aldrich India Ltd., Bengaluru. The pure standards phytol was purchased from M/s Sigma Aldrich India Ltd., Bengaluru and used without further purification. The purity of the standards were ≥ 97%. Glycerol formal (≥ 98%), carboxy methyl cellulose were also purchased from Merck Life Science Private Ltd, Mumbai.

2.2 Animal

The study was conducted on 12 healthy adult Wistar rats with an average body weight of 200-250 g. The rats were procured from Small Animal Breeding Station, College of Veterinary and Animal Science, Mannuthy. The experiment was approved in the Institutional Animal Ethics Committee (IAEC) of College of Veterinary and Animal Sciences, Wayanad (IAEC/COVAS/PKD/3/2020). All the rats were maintained in well ventilated cages (polypropylene rat cages) at 24ºC temperature and relative humidity ranging at 50-60% and fed on standard laboratory rat feed and had access to *ad libitum* water.

2.3 Experiment

The animals were divided into two groups comprising six animals each in the test and control groups. The animals of the test group were given oral administration at 100 mg/kg daily in 0.5 per cent sodium carboxy-methylcellulose for seven consecutive days and control rats were administered the vehicle only. Water was given *ad libitum* during the experiment. The quantity of feed and water intake of each rat was recorded daily. The body weight change was also noted for seven days. After seventh day of repeated oral administration of phytol, the rats from the test and control group were humanely sacrificed as per ethical guidelines and organs such as brain, heart, lungs, liver, spleen, kidney, and adrenal glands were collected immediately, observed for gross pathological lesions and organ weights were recorded.

2.4 Statistical analysis

Statistical analysis for the results were done by using SPSS software. One sample t-test was done for comparing the mean of different parameters of treated group with control. A value of $p < 0.05$ was considered significant.

3. Results

3.1 Mean change in the body weight of rats after repeated oral administration of phytol

Body weight of rats after repeated oral administration of phytol were measured daily for seven consecutive days and change in body weight was calculated and is depicted in table 1 and fig. 1. There was no significant difference in body weight of experimental rats when compared with the control rats.

Table 1: Mean change in body weight (g) of rats after repeated oral administration of phytol (@ 100 mg/kg) once daily for seven consecutive days (n=6).

Day	Treated group	Control	T-value (P-value)
Day 1	225.27 ± 6.97	215.5	1.402^{ns} (0.220)
Day 2	219.25 ± 8.46	215.91	0.395^{ns} (0.709)
Day 3	219.37 ± 8.68	218.24	0.130^{ns} (0.902)
Day 4	219.92 ± 9.18	219.68	0.026^{ns} (0.980)
Day 5	221.5 ± 9.86	219.53	0.200^{ns} (0.849)
Day 6	219.98 ± 12.46	217.37	0.210^{ns} (0.842)
Day 7	221.33 ± 12.2	220.81	0.043^{ns} (0.967)

NS non-significant

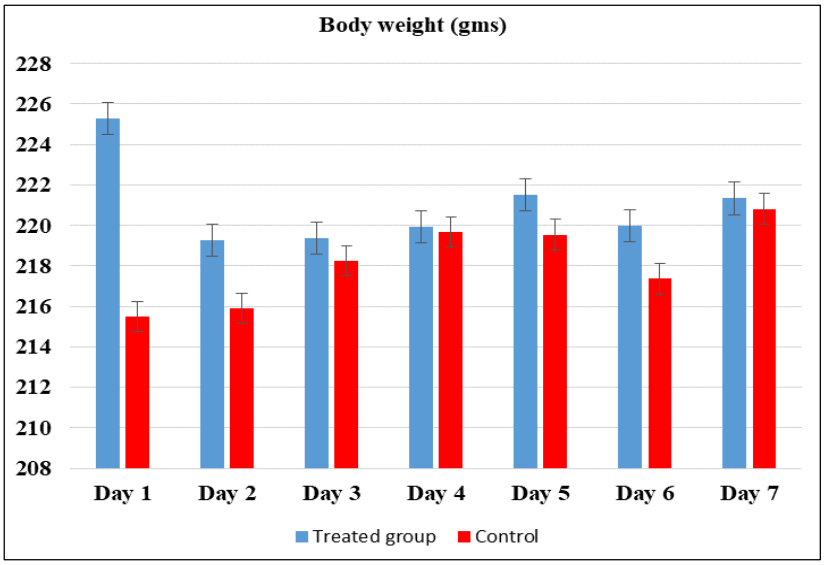

Fig 1: Change in body weight (Mean ±SEM) of rats after repeated oral administration of phytol (@ 100mg/kg) once daily for seven consecutive days (n=6).

3.2. Mean change in feed intake of rats after repeated administration of phytol

Feed intake of rats after repeated oral administration of phytol were measured and change in feed was calculated and is depicted in table 2 and fig. 2. There was no significant difference in feed intake of experimental rats when compared with the control rats except on day 3.

Table 2: Mean feed intake (g) of rats after repeated oral phytol administration of phytol (@ 100 mg/kg) once daily for seven consecutive days (n=6) (Mean ±SEM)

Day	Treated group	Control	T-value (P-value)
Day 1	25.82 ± 4.42	18.73	1.602^{ns} (0.170)
Day 2	21.15 ± 2.82	23.55	0.853^{ns} (0.433)
Day 3	17.98 ± 1.50	26.68	5.804** (0.002)
Day 4	21.72 ± 2.66	28.29	2.473^{ns} (0.056)
Day 5	19.25 ± 2.10	23.62	2.085^{ns} (0.091)
Day 6	21.8 ± 2.76	21.33	0.170^{ns} (0.170)
Day 7	19.38 ± 1.73	27.17	1.279^{ns} (0.257)

** Significant at 0.01 level; ns non-significant

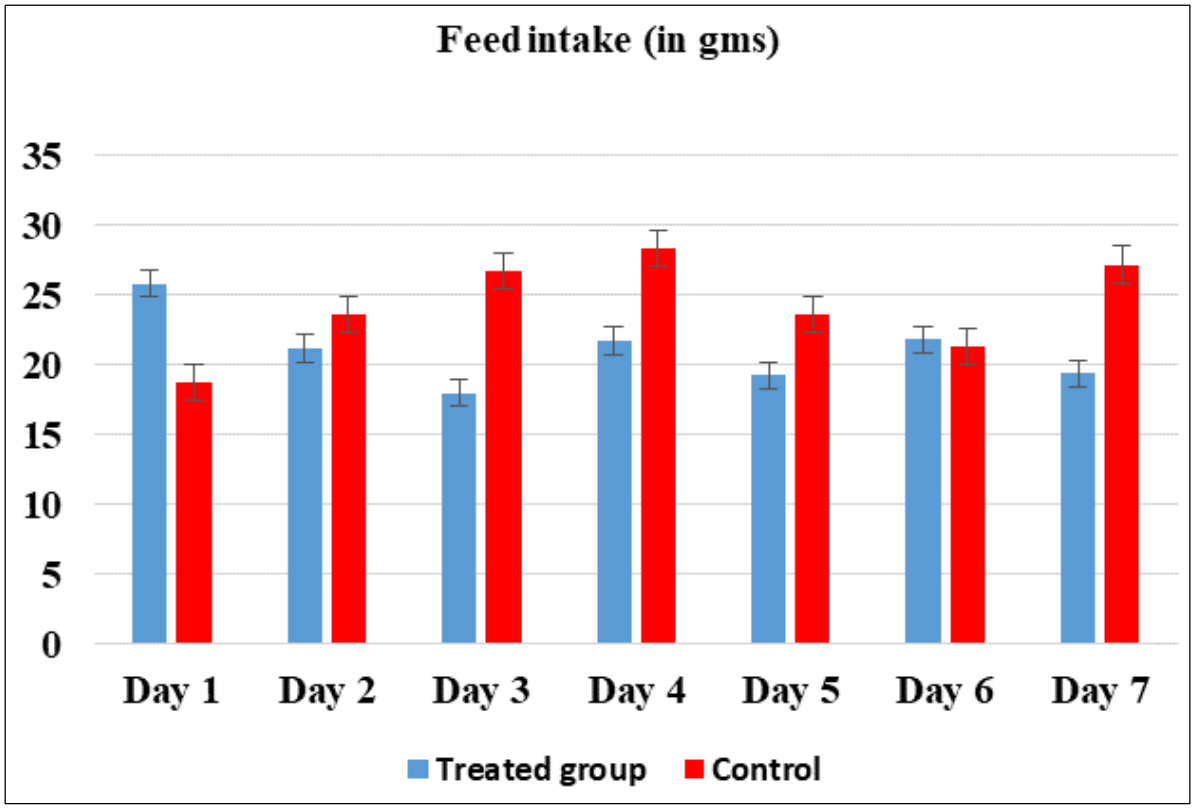

Fig 2: Mean (±SEM) feed intake of rats after repeated oral administration of phytol (@ 100mg/kg) once daily for seven consecutive days (n=6).

3.3. Mean change in the water intake of rats after repeated administration of phytol

Water intake of rats after repeated oral administration of phytol were measured and change in water intake was calculated and is depicted in table 3 and fig. 3. The quantity of water intake remained slightly high throughout the experiment. But there was no significant difference in water intake of experimental rats when compared with the control rats except on day one. On day one the intake was significantly increased.

Table 3: Mean water intake (g) of rats after repeated oral phytol administration of phytol (@ 100 mg/kg) once daily for seven consecutive days Mean ±SEM; n=6).

Day	Treated group	Control	T-value (P-value)
Day 1	34.87 ± 3.75	21	3.695* (0.014)
Day 2	21.5 ± 5.88	25	0.595^{ns} (0.578)
Day 3	22.17 ± 4.03	18	1.034^{ns} (0.348)
Day 4	24.67 ± 3.6	19	1.573^{ns} (0.177)
Day 5	20.50 ± 5.06	17	0.691^{ns} (0.520)
Day 6	19.10 ± 4.87	22	0.595^{ns} (0.578)
Day 7	21.33 ± 3.88	27	1.461^{ns} (0.204)

* Significant at 0.05 level; ns non-significant

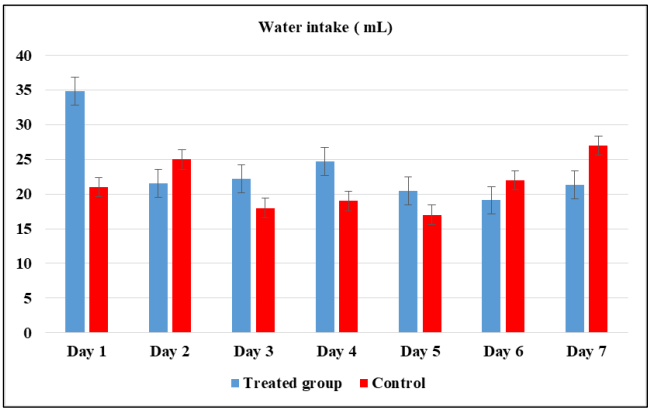

Fig 3: Mean (±SEM) water intake of rats after repeated oral administration of phytol (@ 100mg/kg) once daily for seven consecutive days Mean ±SEM; n=6).

3.4. Mean relative organ weight of rats after repeated oral administration of phytol

Relative organ weight of rats after repeated oral administration of phytol were measured after humane slaughter on day seven. The mean relative organ weight of

rats after repeated oral administration of phytol at a dose of 100 mg/kg is shown in table 4 and fig. 4. There was no significant difference in relative organ weight of experimental rats when compared with the control rats.

Table 4: Mean relative organ weight of rats after repeated oral administration of phytol at a dose of 100 mg/kg once daily for seven consecutive days (Mean ±SEM; n=6)

Organ	Control	7th day	t-value
Kidney	0.8 ± 0.01	0.77 ± 0.31	0.036^{ns} (0.389)
Spleen	0.28 ± 0.01	0.68 ± 0.28	0.544^{ns} (0.331)
Adrenal	0.03 ± 0.005	0.04 ± 0.02	0.224^{ns} (0.379)
Heart	0.34 ± 0.01	0.76 ± 0.31	0.512^{ns} (0.337)
Lung	0.71 ± 0.01	1.78 ± 0.73	0.556^{ns} (0.329)
Liver	2.79 ± 0.03	7.81 ± 3.19	0.595^{ns} (0.321)
Brain	0.75 ± 0.01	1.67 ± 0.68	0.510^{ns} (0.338)

Ns non-significant

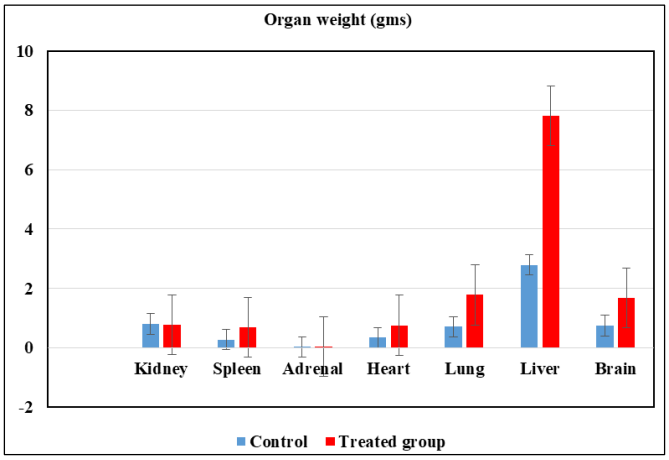

Fig 4: Mean relative organ weight of rats after repeated oral administration of phytol at a dose of 100 mg/kg once daily for seven consecutive days (Mean ±SEM; n=6)

4. Discussion

During the experiment no mortality and abnormal behavior of the rats were recorded. Steinberg *et al.*, (1966) [4], reported an inhibition of growth and mortality of mice within 1-4 weeks of exposure to phytol at a concentration of 5 % in feed. In the present study, there was no significant difference in the daily body weight of rats administered phytol orally once daily at a dose rate of 100 mg/kg for seven consecutive days. In addition, many authors have reported a significant decrease in the body weight of rats following dietary supplementation of phytol at different concentrations ranging from 0.1 to 5 % in diet for more than one week (Steinberg *et al.*, 1966, Wang *et al.*, 2017, Zhang *et al.*, 2018, Nakanishi *et al.*, 2020) [5, 6, 7, 8]. The change in body weight could not be appreciated in the present study as the rats were administered the drug once daily for seven days only. However, a single oral administration at 250mg/kg of phytol to rats significantly increased the weekly body weight of rats from third week (Saranya *et al.*, 2022) [9].

Likewise, there was also no significant difference in the feed intake of rats administered phytol once orally for seven consecutive days. Our findings are in consistent with the reports of Zhang *et al.* (2018) [7] wherein no apparent change in the average weekly food intake in the mice administered phytol at 500 mg/kg every alternate day for seven weeks was observed.

To evaluate the toxic effect of a drug, comparison of organ weights between treated and untreated groups of animals have conventionally been used (Peters and Boyd, 1966) [10].Organ weights are considered to be useful screening tool to characterize drug-related effects in general toxicity studies. However, the opinions varied widely as to which organ weights are most useful. In addition, that organ weight changes in and of themselves were not be necessarily toxic effects. Organ weight data should be assessed in the context of the entire study. Others factors such as body weight changes, pharmacologic action of drug, clinical pathology data, knowledge of the animal's fasted state or if exsanguinated, as well as macroscopic and microscopic findings should also be considered (Michael *et al.*, 2007) [11]. In the present study, the relative organ weight of rats sacrificed seventh day post repeated administration of phytol showed no significant differences when compared with that of control rats. However, a significant increase in the relative organ weights was reported following single oral dose of phytol at 250 mg/kg (Saranya, 2022) [12]. The results of the present study could be attributed to the lower concentration of phytol administered orally. Besides, our findings are contradictory to the reports of previous studies. Gloerich *et. al.* (2005; 2007) [13, 14] reported an increase in the protein level and activity of all of the peroxisomal-oxidation enzymes increased in mice after phytol feeding. Selkala *et al.* (2015) [15] also reported that the α-methylacyl-C_OA racemase (AMACR) deficient mice fed with 5% of phytol in diet for two weeks showed an increase in the relative organ weight of liver indicating hepatomegaly. Landrock *et al.* (2017) [16] reported a decrease in the relative organ weight of liver of *Fabp 1/Scp-2/Scp-x* gene ablated mouse fed with diet containing 0.5% phytol compared to the wild-type mouse. Similarly, no significant gross pathological changes were noted in the any of the organs examined.

5. Conclusion

The result of the current study reveals that repeated dose of phytol when given orally once daily for seven consecutive days caused no effect on the body weight and relative organ weight. Significant change in feed and water intake was noted on day three and day one respectively. However, detailed investigation has to be carried out to find the potency of the drug in causing pathological as well as physiological changes by altering the dosage as well as increasing the duration of the study.

6. Funding

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7. Conflicts of interest

The authors declare that there are not any conflicts of interests

8. References

1. Islam MT, Ali ES, Uddin SJ, Shaw S, Islam MA, Ahmed MI, *et al.*: A review of biomedical activities. Food and Chemical Toxicology. 2018;121:82-94.

2. Thakor P, Subramanian RB, Thakkar SS, Ray A, Thakkar VR. Phytol induces ROS mediated apoptosis by induction of caspase 9 and 3 through activation of TRAIL, FAS and TNF receptors and inhibits tumor progression factor Glucose 6 phosphate dehydrogenase in lung carcinoma cell line (A549). Biomedicine and Pharmacotherapy. 2017 Aug 1;92:491-500.
3. Roca-Saavedra P, Marino-Lorenzo, P, Miranda, JM, Porto-Arias JJ, Lamas A, Vazquez BI, *et al*. Phytanic acid consumption and human health, risks, benefits and future trends: A review. Food Chemistry. 2017; 221: 237-247.
4. Sathya S, Shanmuganathan B, Saranya S, Vaidevi S, Ruckmani K, Pandima Devi K. Phytol-loaded PLGA nanoparticle as a modulator of Alzheimer's toxic Aβ peptide aggregation and fibrillation associated with impaired neuronal cell function. Artificial Cells, Nano medicine and Biotechnology. 2018;46:1719-1730.
5. Steinberg D, Avigan J, Mize CE, Baxter JH, Cammermeyer J, Fales HM, Highet PF. Effects of Dietary Phytol and Phytanic Acid in Animals. Journal of Lipid Research. 1966;7:684-691.
6. Wang, J, Hu, X, Ai, W, Zhang, F, Yang, K, Wang, L, *et al*. Phytol increases adipocyte number and glucose tolerance through activation of PI3K/Aktsignaling pathway in mice fed high-fat and high-fructose diet. Biochemical and Biophysical Research Communications. 2017 Aug 5;489(4):432-438.
7. Zhang F, Ai W, Hu X, Meng Y, Yuan C, Su H, *et al*. Phytol Stimulates the Browning of White Adipocytes through the Activation of AMP-Activated Protein Kinase (AMPK) α in Mice Fed High-Fat Diet. Food and Function. 2018;9(4):2043-2050.
8. Nakanishi, T, Kagamizono K, Yokoyama, S, Suzuki, R, Sakakibara H, Erickson L, *et al*. Effects of dietary phytol on tissue accumulation of phytanic acid and pristanic acid and on the tissue lipid profiles in mice. Journal of Animal Science. 2020 Jan;91(1):13424.
9. Saranya C, Madhusudhanan M, John MS, Sanjay BM, Suraj SV, Ranjith D, *et al*. Effect of phytol following single dose oral and intravenous administration in healthy rats. The Pharma Innovation Journal; c2022.
10. Peters JM, Boyd EM. Organ weights and water levels of the rat following reduced food intake. The Journal of Nutrition. 1966 Dec 1;90(4):354-360.
11. Michael B, Yano B, Sellers RS, Perry R, Morton D, Roome N, *et al*. Evaluation of organ weights for rodent and non-rodent toxicity studies: a review of regulatory guidelines and a survey of current practices. Toxicologic pathology. 2007 Aug;35(5):742-750.
12. Saranya C. Pharmacokinetic evaluation of phytol in rats. M.V.Sc thesis, Kerala Veterinary and Animal Sciences University, Pookode; c2022. p. 82.
13. Gloerich J, van Vlies N, Jansen GA, Denis S, Ruiter JPN, Van Werkhoven MA, *et al*. A Phytol-Enriched Diet Induces Changes in Fatty Acid Metabolism in Mice Both via PPARα-Dependent and-Independent Pathways. Journal of Lipid Research. 2005 Apr 1;46(4):716-726.
14. Gloerich J, Van den Brink DM, Ruiter JPN, Van Vlies N, Vaz FM, Wanders RJA, *et al*. Metabolism of Phytol to Phytanic Acid in the Mouse, and the Role of PPARα in Its Regulation. Journal of Lipid Research. 2007 Jan 1;48(1):77-85.
15. Selkälä EM, Nair RR, Schmitz W, Kvist AP, Baes M, Hiltunen JK, *et al*. Phytol Is Lethal for Amacr-Deficient Mice. Biochimica Biophysica Acta (BBA)-Molecular and Cell Biology Lipids. 2015 Oct 1;1851(10):1394-1405.
16. Landrock D, Milligan S, Martin GG, McIntosh AL, Landrock KK, Schroeder F, *et al*. Effect of Fabp1/Scp-2/Scp-x Ablation on Whole Body and Hepatic Phenotype of Phytol-Fed Male Mice. Lipids. 2017 May;52(5):385-397.