Full Length Article

THE EFFECTS OF NANOFORMS OF SILIBININ AND RESVERATROL ON THE BODY WEIGHTS, FEED INTAKE, AND WATER INTAKE OF HEPATOCARCINOGENESIS INDUCED RATS

J. Venkatesh Yadav[1*], G. V. Sudhakar Rao[2], N. Pazhanivel[3], G. Sarathchandra[4] and T.M.A Senthil Kumar[5]

Department of Veterinary Pathology
Madras Veterinary College, Tamil Nadu Veterinary and Animal Sciences University
Chennai – 600 007

ABSTRACT

Hepatocellular carcinoma is one of the most common occurring malignancies in the world and has been reported in various species of animals including dogs, cats, sheep and pigs. The aim of this study was to assess the ameliorative effects of nanoforms of silibinin and resveratrol on the body weights, feed intake, and water intake of hepatocarcinogenesis induced rats. One hundred and eight male Wistar rats were randomly divided into nine groups and feed intake, water consumption, body weight gains were recorded. The study revealed a significant difference in the feed intake, water intake, body weights in carcinogen group with control and treatment groups. Nano resveratrol and nano silibinin groups effectively ameliorated compared to raw resveratrol and raw silibinin groups there by suggesting their usage in therapy for liver cancer.

Keywords: Hepatocellular carcinoma, Resveratrol, Silibinin, Diethyl nitrosamine, Rats

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INTRODUCTION

Hepatocellular carcinoma (HCC) is the most common type of primary liver carcinomas. It is one of the most common cancers all over the world that is also one of the leading causes of cancer death (Forner *et al.,* 2018). Cirrhosis, intake of aflatoxin B1-contaminated food, prolonged alcohol usage, and hepatitis B and C virus infections are all major risk factors for the development of HCC (Roy and Gadad, 2016).The threat of this fatal cancer is projected to grow in the future years, owing to alarmingly high rates of occurrence, late diagnosis, a lack of clear treatment, and

* Corresponding author Email id: venkateshhh50@gmail.com
[1] Ph.D. Scholar
[2] Professor and Head
[3] Professor
[4] Professor and Head, PLAFFS
[5] Professor and Head, Zoonosis Research Laboratory, TANUVAS, Chennai – 600 051

a terrible prognosis. Chemoprevention has been shown to improve the prognosis of liver cancer patients with a poor prognosis. Among different classes of anticancer agents, plant derivatives, particularly the dietary polyphenols, have received greater attention due to their abundance in natural foods and minimal or no adverse effects (Talari *et al.,* 2014).

Resveratrol (3, 4′, 5-trihydroxy-trans-stilbene) is a phytochemical found in grapes, berries, peanuts, and red wine, among other foods. Resveratrol can help to prevent or slow the progression of a variety of diseases, including cancer, neurodegenerative diseases, cardiovascular disease, ischemic injury, and viral infections, as well as improve stress resistance and extend the lifespan of various organisms. Resveratrol affects cellular proliferation and growth, apoptosis, inflammation, invasion, angiogenesis, and metastasis (Baur and Sinclair, 2006).

Silibinin is a major bioactive flavanone present in milk thistle seeds and has been used for approximately 2000 years to treat various liver conditions. Silibinin is clinically used in Europe and Asia as a hepatoprotective agent to treat hepatic injury due to bile duct inflammation, cirrhosis, fatty liver, mushroom poisoning, and viral hepatitis. The anticancer efficacy of silibinin is mainly through targeting proliferation, apoptosis, inflammation, angiogenesis and cancer cell metabolism (Jie *et al.,* 2018).

Despite the benefits of resveratrol and silibinin, they show poor oral bioavailability and higher metabolism, which further limits their therapeutic potential in the treatment of cancer. To overcome these problems, the use of solid lipid nanoparticles (SLN) can be an effective alternative. SLN material has already been licensed for use in pharmaceutical or cosmetic products such as triacyl glycerides, diacyl glycerides, monoacyl glycerides, waxy or fatty acids, and surfactants authorized in medical goods for use. It provides controlled drug release that is feasible for industrial scale production (Doktorovova *et al.,* 2014). Nanoparticles made from solid lipids are attracting major attention as novel colloidal drug carrier as they have been proposed as an alternative particulate carrier system. Solid lipid nanoparticles are in submicron size range between 50 – 1000 nm and composed of lipids which remain in solid state at room temperature. Main goal for the preparation of SLN are increased drug stability, controlled drug release, avoidance of organic solvent, incorporation of lipophilic and hydrophilic drugs, improved bioavailability and no biotoxicity to the carrier (Krishna Sailaja *et al.,* 2011).

To make advances in research in the prevention of HCC, several experimental models have been developed to investigate the aspects of the pathogenesis and aetiology. Due to the similarity to histological and genetic features of patients, diethyl nitrosamine (DEN) -induced HCC model, is commonly used to imitate the process of hepatocarcinogenesis (Duan *et al.,* 2014). In this study, the effects of nanoforms of silibinin and resveratrol on the body weights, feed intake and water intake in DEN induced HCC model of rats have been reported.

MATERIALS AND METHODS

Wistar albino male rats weighing 200 ± 10 g purchased from Tamil Nadu Veterinary and Animal Sciences University, Chennai, were used in this study. They were housed in polypropylene cages with 12 hrs light and dark cycle. All animal experiments were performed in accordance with the strict guidelines prescribed by the Institutional Animal Ethical Committee (IAEC) and after getting necessary approval (Approval Lr. No. 370/DFBS/IAEC/2021, dated: 16.08.2021).

Rats were divided into nine groups with twelve animals in each group. Control group (Sham control), Blank Solid Lipid Nanoparticles group (1.5 ml thrice in a week / P.O). DEN group (0.01% DEN in drinking water every day for 17 weeks). Raw Resveratrol and raw Silibinin groups (0.01% DEN in drinking water every day for 17 weeks + 50 mg/ Kg B. wt. thrice a week P. O). Nano Resveratrol and nano silibinin groups (0.01% DEN in drinking water every day for 17 weeks + 50 mg/ Kg B. wt. thrice a week P. O), Nano combination group (0.01% DEN in drinking water every day for 17 weeks +50 mg/ Kg B. wt. of nano silibinin thrice a week P. O +50 mg/ Kg B. wt. of nano resveratrol thrice a week P. O), Sorafenib group (0.01% DEN in drinking water every day for 17 weeks + 7.5 mg/ Kg B. wt. thrice a week P. O). On 30-day, 60-day, 90-day and 120-day of the trial, 3 rats from each group were sacrificed to study the tumour progression. The body weight (g) and feed intake (g) were recorded at weekly intervals for 17 weeks by weighing balance (Goldtech®, Model GTC Reg. No. 42863734) and rounded to the nearest gram. Water consumption (mL) per week was calculated. The data generated from different parameters of the experimental study was subjected to one-way analysis of variance (ANOVA), followed by the Duncan's multiple range test by using IBM SPSS software version 20 for windows.

RESULTS AND DISCUSSION

Changes in the body weight have been used to assess the cause of the disease and response to therapy of drugs. Body weight gain of the animal is directly related to feed conversion ratio and it depends on the functional capacity of liver. The mean (±SE) alterations in body weights of rats in induced and treated groups is presented in table I. The present study reports a significant ($p<0.05$) reduction in the body weights in the DEN group and Sorafenib groups from second week onwards. This decrease in body weights might be due to less feed intake following the damage of hepatic parenchyma (Kushida *et al.,* 2011). The control group and BSLNPs groups showed an increase in bodyweights steadily from week 1. Raw resveratrol and raw silibinin groups also showed a gradual increase in body weights from week 1. Nano resveratrol, nano silibinin and nano combination groups showed a decrease in body weights in week 2, but later from 3rd week there was a gradual increase. This decrease may be due to decreased water intake from the chloroform smell in solid lipid nanoparticles and which is directly related to feed intake in rats and in turn body weights. Sorafenib group rats showed a gradual decrease in body weights from week 1 to week 17 except for a slight increase on week 6, 7 and 8. These observations are in accordance

Table-I : Changes in the body weights (Mean±SE) of rats in induced and treated groups

Week/ group	w1 (n=12)	w2 (n=12)	w3 (n=12)	w4 (n=12)	w5 (n=9)	w6 (n=9)	w7 (n=9)	w8 (n=9)	w9 (n=6)
Control	283.3^{a}± 34.5	309.2^{b}± 29.2	327.5^{c}± 27.7	340.8^{c}± 26.3	335.8^{c}± 24.4	337.5^{e}± 24.1	343.3^{e}± 24.3	346.6^{e}± 23.7	283.3^{bc}± 22.1
BSLNPs	287.5^{a}± 10.0	298.3^{b}± 8.0	315.8^{bc}± 11.1	326.6^{c}± 12.2	320.8^{bc}± 10.7	325.8^{de}± 11.0	325.8^{de}± 10.5	329.2^{de}± 10.4	316.6^{c}± 25.6
DEN	288.3^{a}± 12.0	267.5^{ab}± 10.6	255.8^{a}± 13.4	248.3^{a}± 17.3	255^{a}± 10.4	185^{a}± 55.6	238.7^{ab}± 9.6	231.6^{ab}± 15.8	186.7^{a}± 26.1
Raw resveratrol	282.5^{a}± 5.5	300.8^{b}± 18.7	295.8^{abc}± 12.2	310.8^{bc}± 15.6	313.3^{bc}± 16.0	317.5^{cde}± 16.0	321.6^{de}± 16.9	324.2^{cde}± 17.3	300^{c}± 18.9
Raw silibinin	290^{a}± 6.5	280^{ab}± 11.4	300^{abc}± 11.2	322. 5^{c}± 13.9	325.8^{c}± 13.0	328.3^{e}± 13.2	224.2^{a}± 8.8	228.3^{a}± 9.3	298. 3^{c}± 8.2
Nano resveratrol	286.7^{a}± 12.6	242.5^{a}± 9.2	268.3^{a}± 10.1	268.3^{ab}± 10.6	250.8^{a}± 10.5	257.5^{b}± 10.4	274.2^{bc}± 10.9	275.8^{bc}± 10.7	197.5^{a}± 13.9
Nano silibinin	286.7^{a}± 9.5	265^{ab}± 5.1	293.3^{ab}c± 3.1	275^{ab}± 7.5	278.3^{ab}± 6.7	292.5^{bcde}± 8.1	309.1^{cde}± 8.8	310.8^{cde}± 8.7	223.3^{ab}± 24.4
Nano combination	287.5^{a}± 21.1	271.7^{ab}± 17.8	271.6^{ab}± 15.1	265^{ab}± 14.9	243.3^{a}± 18.4	263.3^{bc}± 15.4	286.7^{cd}± 18.2	288.3^{cd}± 18.5	191.6^{a}± 20.9
Sorafenib	289.2^{a}± 4.5	285.8^{ab}± 8.0	276.6^{ab}± 5.4	266.6^{ab}± 12.5	263.3^{a}± 10.9	267.5^{bcd}± 11.6	275^{bc}± 12.6	276.6^{bc}± 12.9	229.1^{ab}± 15.2

Week/ group	w10 (n=6)	w11 (n=6)	w12 (n=6)	w13 (n=3)	w14 (n=3)	w15 (n=3)	w16 (n=3)	w17 (n=3)
Control	294.1^{b}± 22.6	307.5^{bc}± 22.7	318.3^{c}± 21.2	320^{c} ±18.6	323.3^{b}± 19.2	331.7^{de}± 16.9	332.5^{c}± 15.1	336.7^{c}± 14.7
BSLNPs	319.2^{b}± 23.3	325^{c}± 20.6	332.5^{c}± 19.3	338.3^{c} ±18.8	339.1^{b}± 17.8	347.5^{e}± 16.4	315.8^{c}± 20.7	317.5^{c}± 19.8
DEN	176.6^{a}± 25.8	192.5^{a}± 7.5	205^{a}± 15.0	197.5^{a} ±17.5	172.5^{a}± 22.5	150^{a}± 10.0	135^{a}± 5.0	130^{a}± 10.0
Raw resveratrol	293.3^{b}± 17.0	297.5^{bc}± 16.1	305^{bc}± 20.9	306^{bc} ±20.8	292^{b}± 19.2	277^{cd}± 12.7	276^{c}± 12.5	278^{c}± 13.1
Raw silibinin	295^{b}± 10.0	312^{c}± 9.1	333^{c}± 9.1	334^{c} ± 10.6	324^{b}± 8.5	310de± 11.1	313^{c}± 9.4	322.5^{c}± 9.5
Nano resveratrol	181.6^{a}± 10.3	210.8^{a}± 10.5	213.3^{a}± 8.0	229.1^{a} ±11.7	201.7^{a}± 9.9	199.1^{ab}± 12.8	179.2^{ab}± 16.2	184.1^{ab}± 14.7
Nano silibinin	218.3^{a}±2 2.8	249.1^{ab}± 24.1	250.8^{ab}± 22.6	257.5^{ab} ±24.4	233.3^{a}± 22.1	237.5^{bc}± 24.3	216.7^{b}± 23.0	217.5^{b}± 23.3
Nano combination	180.8^{a}± 22.2	202.5^{a}± 21.8	219.1^{a}± 22.5	225.8^{a} ±21.9	208.3^{a}± 21.5	210.8^{ab}± 24.1	195.8^{ab}± 27.4	198.3^{b}± 28.0
Sorafenib	225^{a}± 15.1	234.1^{a}± 14.0	249.1^{ab}± 13.2	248.3^{ab} ±14.0	230^{a}± 13.8	205.8^{ab}± 17.2	195.8^{ab}± 16.1	196.6^{b}± 16.2

Means with different superscripts differ significantly ($p<0.05\%$) from each other with in the same column

Table II: Feed intake (Mean±SE) in rats during the experimental period

Week/ group	w1 (n=12)	w2 (n=12)	w3 (n=12)	w4 (n=12)	w5 (n=9)	w6 (n=9)	w7 (n=9)	w8 (n=9)	w9 (n=6)
Control	$21.0^{e}\pm$ 0.11	$19.9^{e}\pm$ 0.24	$22.1^{e}\pm$ 0.89	$22.1^{f}\pm$ 0.02	$23.4^{f}\pm$ 0.27	$23.4^{g}\pm$ 0.27	$22.8^{f}\pm$ 0.59	$22.4^{f}\pm$ 0.01	$23.1^{f}\pm$ 0.26
BSLNPs	$15.7^{c}\pm$ 0.20	$17.1^{d}\pm$ 0.16	$22.6^{e}\pm$ 0.10	$21.7^{ef}\pm$ 0.10	$21.2^{e}\pm$ 0.52	$22.5^{f}\pm$ 0.20	$22.4^{f}\pm$ 0.06	$22.4^{f}\pm$ 0.04	$21.8^{e}\pm$ 0.14
DEN	$15.6^{c}\pm$ 0.56	$13.3^{a}\pm$ 0.06	$14.5^{ab}\pm$ 0.47	$13.2^{a}\pm$ 0.01	$12.1^{a}\pm$ 0.04	$13.6^{a}\pm$ 0.14	$14.5^{a}\pm$ 0.11	$12.2^{a}\pm$ 0.01	$13.1^{a}\pm$ 0.25
Raw resveratrol	$15.7^{c}\pm$ 0.01	$14.5^{b}\pm$ 0.06	$13.4^{a}\pm$ 0.55	$14.5^{b}\pm$ 0.18	$13.2^{b}\pm$ 0.11	$15.6^{c}\pm$ 0.11	$16.5^{bc}\pm$ 0.26	$17.0^{b}\pm$ 0.79	$17.6^{b}\pm$ 0.42
Raw silibinin	$17.3^{d}\pm$ 0.38	$14.2^{b}\pm$ 0.07	$15.9^{bc}\pm$ 0.77	$16.4^{c}\pm$ 0.71	$15.6^{c}\pm$ 0.78	$14.3^{b}\pm$ 0.42	$15.5^{ab}\pm$ 1.08	$16.8^{b}\pm$ 0.52	$16.7^{b}\pm$ 0.67
Nano resveratrol	$11.9^{ab}\pm$ 0.03	$14.4^{b}\pm$ 0.13	$17.2^{cd}\pm$ 0.07	$18.0^{d}\pm$ 0.06	$17.9^{d}\pm$ 0.11	$18.0^{d}\pm$ 0.20	$18.3^{d}\pm$ 0.03	$18.7^{c}\pm$ 0.04	$18.6^{c}\pm$ 0.11
Nano silibinin	$12.^{b}\pm$ 0.03	$14.6^{b}\pm$ 0.09	$16.7^{cd}\pm$ 0.55	$18.6^{d}\pm$ 0.24	$21.7^{e}\pm$ 0.19	$22.3^{f}\pm$ 0.01	$20.6^{e}\pm$ 0.77	$19.8^{d}\pm$ 0.27	$20.4^{d}\pm$ 0.16
Nano combination	$11.5^{a}\pm$ 0.02	$14.5^{b}\pm$ 0.07	$17.8^{d}\pm$ 0.03	$20.9^{e}\pm$ 0.23	$18.1^{d}\pm$ 0.06	$17.5^{d}\pm$ 0.02	$17.5^{cd}\pm$ 0.02	$19.3^{cd}\pm$ 0.02	$19.4^{c}\pm$ 0.11
Sorafenib	$16.3^{c}\pm$ 0.48	$16.3^{c}\pm$ 0.41	$17.1^{cd}\pm$ 0.43	$17^{d}.\pm$ 0.59	$18.8^{d}\pm$ 0.47	$20.1^{e}\pm$ 0.11	$20.6^{e}\pm$ 0.07	$21.2^{e}\pm$ 0.11	$20.5^{d}\pm$ 0.21

Week/ group	w10 (n=6)	w11 (n=6)	w12 (n=6)	w13 (n=3)	w14 (n=3)	w15 (n=3)	w16 (n=3)	w17 (n=3)
Control	$23.0^{g}\pm$ 0.22	$22.7^{e}\pm$ 0.13	$22.5^{e}\pm$ 0.70	$22.0^{d}\pm$ 0.43	$22.5^{e}\pm$ 0.33	$22.5^{f}\pm$ 0.10	$21.9^{f}\pm$ 0.01	$21.6^{d}\pm$ 0.29
BSLNPs	$21.3^{f}\pm$ 0.35	$20.7^{d}\pm$ 0.22	$20.7^{d}\pm$ 0.49	$20.6^{c}\pm$ 0.49	$20.9^{d}\pm$ 0.40	$20.8^{e}\pm$ 0.38	$20.6^{e}\pm$ 0.30	$19.9^{bc}\pm$ 0.07
DEN	$12.9^{a}\pm$ 0.02	$12.9^{a}\pm$ 0.05	$12.8^{a}\pm$ 0.36	$12.8^{a}\pm$ 0.34	$12.6^{a}\pm$ 0.41	$12.1^{a}\pm$ 0.22	$11.6^{a}\pm$ 0.07	$10.5^{a}\pm$ 0.44
Raw resveratrol	$18.1^{bc}\pm$ 0.36	$18.3^{b}\pm$ 0.14	$18.6^{bc}\pm$ 0.27	$18.9^{b}\pm$ 0.27	$19.1^{b}\pm$ 0.24	$19.3^{bc}\pm$ 0.024	$19.5^{bc}\pm$ 0.03	$19.1^{b}\pm$ 0.05
Raw silibinin	$17.3^{b}\pm$ 0.58	$17.8^{b}\pm$ 0.37	$18.1^{b}\pm$ 0.23	$19.9^{c}\pm$ 0.14	$19.4^{bc}\pm$ 0.34	$19.6^{bc}\pm$ 0.34	$19.7^{bc}\pm$ 0.32	$19.2^{b}\pm$ 0.47
Nano resveratrol	$18.9^{cd}\pm$ 0.03	$19.4^{c}\pm$ 0.035	$19.5^{cd}\pm$ 0.39	$20.1^{c}\pm$ 0.17	$19.8^{bc}\pm$ 0.05	$19.9^{cd}\pm$ 0.01	$19.9^{cd}\pm$ 0.03	$19.7^{bc}\pm$ 0.09
Nano silibinin	$20.5^{ef}\pm$ 0.22	$20.1^{d}\pm$ 0.20	$20.1^{d}\pm$ 0.18	$20.3^{c}\pm$ 0.30	$19.6^{bc}\pm$ 0.27	$19.1^{b}\pm$ 0.45	$19.0^{b}\pm$ 0.42	$19.0^{b}\pm$ 0.45
Nano combination	$19.6^{de}\pm$ 0.12	$19.2^{c}\pm$ 0.10	$19.5^{cd}\pm$ 0.22	$19.9^{c}\pm$ 0.11	$19.4^{bc}\pm$ 0.22	$19.2^{bc}\pm$ 0.02	$19.3^{bc}\pm$ 0.03	$19.2^{b}\pm$ 0.03
Sorafenib	$20.5^{ef}\pm$ 0.23	$20.2^{d}\pm$ 0.36	$20.5^{d}\pm$ 0.50	$20.7^{c}\pm$ 0.42	$20.1^{cd}\pm$ 0.18	$20.5^{de}\pm$ 0.03	$20.5^{de}\pm$ 0.21	$20.5^{c}\pm$ 0.23

Means with different superscripts differ significantly ($p<0.05\%$) from each other with in the same column

Table III: Water intake (Mean±SE) in rats during the experimental period

Week/ group	w1 (n=12)	w2 (n=12)	w3 (n=12)	w4 (n=12)	w5 (n=9)	w6 (n=9)	w7 (n=9)	w8 (n=9)	w9 (n=6)	w10 (n=6)	w11 (n=6)	w12 (n=6)	w13 (n=3)	w14 (n=3)	w15 (n=3)	w16 (n=3)	w17 (n=3)
Control	38.3^{d}± 1.05	38.3^{d}± 1.05	38.3^{d}± 1.05	38.3^{f}± 1.05	38.3^{e}± 1.05	38.3^{d}± 1.05	38.3^{d}± 1.05	38.3^{d}± 1.05	38.3^{e}± 1.05	38.3^{e}± 1.05	38.3^{e}± 1.05	38.3^{e}± 1.05	38.3^{e}± 1.05	38.3^{e}± 1.05	38.3^{a}± 1.05	38.3^{e}± 1.05	38.3^{g}± 1.05
BSLNPs	35.3^{cd}± 0.21	35.3^{cd}± 0.21	35.3^{cd}± 0.21	35.3^{ef}± 0.21	35.3^{d}e± 0.21	35.3^{cd}± 0.21	35.3^{cd}± 0.21	35.3^{cd}± 0.21	35.3^{de}± 0.21	35.3^{de}± 0.21	35.3^{de}± 0.21	35.3^{de}± 0.21	35.3^{de}± 0.21	35.3^{de}± 0.21	35.3^{de}± 0.21	35.3^{de}± 0.21	35.3^{efg}± 0.21
DEN	32.5^{c}± 1.11	32.5^{c}± 1.11	28.3^{b}± 1.05	24.2^{bc}± 1.54	21.6^{b}± 1.67	18.3^{ab}± 1.70	18.3^{a}± 1.70	18.3^{a}± 1.70	18.3^{a}± 1.70	18.3^{a}± 1.70	17.5^{a}± 1.11	18.3^{a}± 1.70	18.3^{a}± 1.70	18.3^{a}± 1.70	13.3^{a}± 1.05	12.5^{a}± 1.19	12.5^{a}± 1.19
Raw resveratrol	32.5^{c}± 1.11	32.5^{c}± 1.11	32.5^{c}± 1.11	32.5^{de}± 1.11	32.5^{d}± 1.11	32.5^{c}± 1.11	32.5^{c}± 1.11	32.5^{c}± 1.11	32.5^{d}± 1.11	32.5^{d}± 1.11	32.5^{cd}± 1.11	32.5^{cd}± 1.11	32.5^{cd}± 1.11	32.5^{cd}± 1.11	32.5^{cd}± 1.11	32.5^{cd}± 1.11	32.5^{cde}± 1.11
Raw silibinin	32.5^{c}± 1.70	32.5^{c}± 1.70	32.5^{c}± 1.70	32.5^{de}± 1.70	32.5^{d}± 1.70	32.5^{c}± 1.70	32.5^{c}± 1.70	32.5^{c}± 1.70	32.5^{d}± 1.70	32.5^{d}± 1.70	32.5^{cd}± 1.70	32.5^{cd}± 1.70	32.5^{cd}± 1.70	32.5^{cd}± 1.70	32.5^{cd}± 1.70	32.5^{cd}± 1.70	32.5^{cde}± 1.70
Nano resveratrol	17.5^{a}± 1.11	17.5^{a}± 1.11	17.5^{a}± 1.11	17.5^{a}± 1.11	17.5^{a}± 1.11	16.6^{a}± 1.05	26.0^{b}± 0.44	27.5^{b}± 1.11	27.5^{c}± 1.11	27.5^{c}± 1.11	27.5^{bc}± 1.11	27.5^{bc}± 1.11	27.5^{bc}± 1.11	27.5^{b}± 1.11	27.5^{b}± 1.11	27.5^{b}± 1.11	27.5^{b}± 1.11
Nano silibinin	20^{a}± 2.23	20^{a}± 2.23	20^{a}± 2.23	20^{ab}± 2.23	20^{ab}± 2.23	22.5^{b}± 3.35	22.5^{ab}± 3.35	22.5^{a}± 3.35	25.0^{b}c± 2.23	25.0^{bc}± 2.23	27.5^{bc}± 3.35	27.5^{bc}± 3.35	27.5^{bc}± 3.35	30^{bc}± 2.23	30^{bc}± 2.23	30^{bc}± 2.23	30^{bc}± 2.23
Nano combination	16.7^{a}± 1.05	16.7^{a}± 1.05	16.7^{a}± 1.05	18.3^{a}± 2.1	21.7^{b}± 1.05	21.7^{b}± 1.05	21.7^{ab}± 1.05	21.7^{a}± 1.05	21.7^{ab}± 1.05	22.5^{b}± 1.71	23.3^{b}± 2.11	23.3^{ab}± 2.11	23.3^{ab}± 2.11	26.7^{b}± 1.05	28.3^{bc}± 2.11	28.3^{bc}± 2.11	31.7^{bcd}± 1.05
Sorafenib	28.3^{b}± 1.05	28.3^{b}± 1.05	28.3^{b}± 1.05	28.3^{cd}± 1.05	28.3^{c}± 1.05	33.3^{c}± 1.05	33.3^{c}± 1.05	34.2^{cd}± 0.83	34.2^{d}± 0.83	34.2^{de}± 0.83	36.7^{de}± 2.11	36.7^{de}± 2.11	36.7^{de}± 2.11	36.7^{de}± 2.11	36.7^{de}± 2.11	36.7^{de}± 2.11	36.7^{fg}± 2.11

Means with different superscripts differ significantly($p<0.05\%$) from each other with in the same column with earlier works (Rajasekaran *et al.,* 2011; Naina *et al.,* 2015).

The mean (±SE) feed consumption pattern in control and experimental groups is presented in table II. There is a significant difference ($p<0.05$) in feed intake between the control groups and other treatment groups. There is no significant difference among treatment groups in feed intake. DEN treated group showed a great reduction in feed intake compared to other treatment groups from 2nd week which could be due to damage to liver from carcinogen DEN. The other treatment groups including sorafenib group showed a decrease in feed intake when compared to control and blank solid lipid nanoparticle groups. These findings are similar to the findings of EI Mesallamy *et al.* (2011). Song *et al.* (2013) also observed similar effects that after eight weeks exposure to DEN, rats in the tumor control group displayed loss of appetite compared to normal control group which may be due to the alteration in the hepatic activity due to which the enzyme responsible for digestion may be altered leading to physiological and pathological changes affecting feed consumption of rats.

The mean (±SE) water intake in rats during the experimental period is represented in table III. There is a significant difference ($p<0.05$) in water intake between the control groups and other treatment groups. There is no significant difference among treatment groups in water intake. DEN treated group showed a great reduction in water intake compared to other treatment groups from 3rd week which could be due to loss of appetite from damage to liver from DEN. The nano treated groups showed less water intake than raw treated groups which might be due to taste of nano compounds as they have chloroform. Sorafenib group showed a steady increase in water intake from week 1 to week 17 when compared to other treatment groups. These findings correlate with the findings of Karabekir and Ozgorgulu (2020) and Rajasekaran *et al.* (2011).

CONCLUSION

Nano resveratrol and nano silibinin are more effective than the raw forms in ameliorating the adverse effects of DEN and thereby improving the feed intake, water consumption and body weights. Hence, solid lipid nanoparticles of resveratrol and silibinin may be used for alleviating the harmful effects caused by carcinogen DEN.

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