ANTI-OBESITY ACTION OF RESVERATROL AGAINST TRITON-X INDUCED OBESITY IN WISTAR ALBINO RATS

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Abstract

The some natural sources like nuts, berries, grapes and red wine are the very good source of Resveratrol. This used in various diseases all over the world.

Aim of the study: The aim of this study was to find out the antiobesity activity of Resveratrol against Triton-X induced obesity.

Materials and methods: Thirty Wistar albino rats were divided into five groups. First group received normal saline; second group received Triton-X (100 mg/kg) i.p in single dose; third group received Triton-X (100 mg/kg) i.p in single dose and the Simvastatin (10 mg/kg/day) orally for seven days; fourth group received Resveratrol (50 mg/kg/day) orally for 14 days after the single i.p. dose of Triton-X (100 mg/kg); fifth group received same single i.p. dose of Triton-X (100 mg/kg) for obesity, but Resveratrol given in (100 mg/kg/day) orally for 14 days to treat obesity.

Results: The obesity effect of Triton-X is due to hyperlipidemic action. It is well known that high level of lipid in body produces the changes in cell redox balance and leads to oxidative stress. The obesity was evidenced by high lipid peroxidation (MDA level), GSH depletion, and increased body weight as well as histological changes in liver as well as fat pads. The study indicates that Resveratrol (50 mg/kg/day as well as 100 mg/kg/day) were showed appreciably improvement in the obesity.

Conclusion: The results of this study revealed that Resveratrol have free radical scavenging potency against increased production of reactive oxygen species (ROS) as well as lipolytic action, reduced the fat gathering processes by Triton-X and act as potent antiobesity agent.

INTRODUCTION

Obesity

Obesity is the most significant emergency which is urbanized in planet. Obesity is scarcely influencing the grown-ups just as kids. Basic element of the obesity is the fat collection, which make the negative outcome on wellbeing. On the off chance that the body spending a littler measure of vitality that will be put away in the body as triglyceride in fat tissue. The BMI >30 is represent fat. Obesity is the principle causative mediator of the different medical issues like diabetes, cardiovascular, hypertension, malignant growth, and so on **(Karine Clement et al., 2003).** The most significant reasons of obesity in youngsters and grown-ups are unevenness diet, less physical development, and sitting conduct. In children orthopedic burden is additionally caused because of fat. Heftiness is certifiably not a specific issue however a various bunch of circumstance with various causes all of which is inevitably enunciated as overweight phenotype. Over the most recent ten years, obesity has more prominent than previously, 12–20% in men and 16–25% in ladies **(Nammi Srinivas et al., 2004).**

Rationale connected with the obesity:

In general, obesity is initiated by over eating and less bodily movement.

a. Calories associated with obesity: The power cost of food material is determined in units perceived like calories. The standard physically unique man wants regarding the matter of 2,500 calories every day to save a decent physical shape and weight, on the other side standard physically dynamic lady needs around 2,000 calories per day **(Nammi Srinivas et al., 2004).**

b. Be deficient in of physical activity: Absence of physical movement is the central factor to starting point of weight. People groups have calling that hold sitting at a work area for overall day, additionally practice vehicles (cars), as opposed to strolling or cycling. For rest, numerous individuals tend to watch T.V., peruse the web, dependably make the showing on PC, and rarely take normal exercise **(Nammi Srinivas et al., 2004).**

Genetics: A few people say that there's no point attempting to get more fit since "it keeps running in my family" or "it's in my qualities". There are some uncommon hereditary conditions that can cause obesity, for example, Prader-Willi disorder, there's no motivation behind why the vast majority can't get more fit.

The facts may confirm that some hereditary characteristics acquired from your parents –, for example, having a huge hunger – may make getting more fit progressively troublesome, however it doesn't make it inconceivable **(Karine Clement et al., 2003).**

c. Poor diet: Obesity doesn't happen overnight. It develops progressively over time, as a result of poor diet and lifestyle choices, such as:

- Eating large amounts of fast food – that does contain high amount of fat and sugar.
- Drinking alcohol too much – alcohol contains a lot of calories, and people who drink denselyare often overweight.
- Eating out a lot – people may be tempted to also have a starter or dessert in a restaurant andthe food can be higher in sugar and fat.

- Eating larger amount than we need – people may be encouraged to eat too much if our friendsor relatives are also eating large amount.
- Drink large amount of sugary drinks – including soft drinks and fruit juice.
- Comfort eating – if people have low self-esteem or feel depressed, they may eat to makeourself feel better.

Risk Factors:-

Obesity usually reaction from a combination of causes and contributing factors, including:

Genetics: - Our gene may influence the measure of muscle versus fat we store, and where that fat is appropriated. Hereditary qualities may likewise assume a significant job in how productively our body changes over nourishment into energy and how our body consumes calories during activity or physical exercises **(Ali AT. et al., 2009).**

Family lifestyle: - Obesity will in general keep running in their qualities. In the event that either of our parents is obese, our danger of being weighty is expanded. That is not a result of hereditary qualities. Relatives will in general offer comparative eating and action tendencies **(Beyerlein Andreas et al., 2011).**

Inactivity: - In the event that we are not exceptionally dynamic, we don't consume the same number of calories. With an inactive way of life, we can without much of a stretch take in a larger number of calories consistently than we consume exercise and routine every day exercises. Having medical issues, for example, joint pain, can prompt diminished movement, which gives to weight gain **(Beyerlein Andreas et al., 2011).**

Detrimental diet: - An eating routine that have high in calories, poorly in products of the soil, brimming with fatty food, and loaded down with unhealthy refreshments and larger than average segments adds to weight gain.

Medical problems: - In certain individuals, weight can be brought about by high fat issue, for example, Prader-Willi disorder, Cushing's disorder and different conditions. High fat issues, for example, joint inflammation, additionally can prompt diminished action, which may resultin weight gain.

Certain medications: - A few drugs can prompt weight gain whether we don't remunerate through eating routine or action. A few antidepressants, against seizure prescription, diabetes medicine, antipsychotic meds, steroid and beta blockers, these are a few medications which cause obesity.

Age: - Obesity can show up at any age, even in youthful kids. Be that as it may, at some age, hormonal changes and a less dynamic way of life increment our danger of fat. Also, the measure of muscle in our body will in general abatement with age. This lower bulk prompts a lessening in digestion. These progressions additionally lessen calories needs, and can make it harder to keep off overabundance weight. In the event that we don't intentionally control what we eat and become all the more physically dynamic as our age, we will probably put on weight.

Pregnancy: - A lady's weight essentially increments during pregnancy. A few ladies confronted troubles to get thinner after the infant is conceived. This weight increase may dedicate to the improvement of stoutness in ladies.

Quitting smoking: - Stopping smoking is regularly correspond with weight gain. For certain individuals, it can prompt enough weight gain that the individual ends up hefty. In the long haul, notwithstanding, stopping smoking is as yet a more prominent advantage to your wellbeing than proceeding to smoke.

Lack of sleep: - On the off chance that individuals not getting enough rest or getting an excessive amount of rest can cause changes in hormones that expansion your craving. We may likewise desire sustenance's high in calories and starches, which can add to weight gain. Regardless of whether we have at least one of these hazard factors, it doesn't imply that we are bound to wind up fat. We can neutralize most hazard factors through eating regimen, physical action and exercise, and conduct changes **(Beyerlein Andreas et al., 2011).**

Complications:-

If we are obese, more likely to develop a number of potentially serious health problems, including:

- Increased the level of high triglycerides and low high-density lipoprotein (HDL) cholesterol
- Type 2 diabetes
- High blood pressure
- Metabolic syndrome- A combination of high blood sugar, high blood pressure, high triglycerides and low HDL cholesterol
- Heart disease
- Stroke
- Cancer, like; cancer of uterus, cervix, endometrium, ovaries, breast, colon, rectum, esophagus,liver, gallbladder, pancreas, kidney and prostate
- Gallbladder disease
- Gynaecological problems- such as infertility and irregular periods
- Erectile dysfunction and sexual health issues
- Non-alcoholic fatty liver disease, is a condition in which fat builds up in the liver that cancause inflammation or scarring
- Osteoarthritis

Other weight-related problems that may affect your quality of life include:

- Depression
- Disability
- Sexual problems
- Shame and guilt
- Social isolation
- Lower work achievement **(D Segula et al., 2014), (D. Kinlen et al., 2018).**

Reasons of obesity:-

There are different ways by which we induced obesity like:-

- Diet induced obesity
- Over eating
- Drug induced obesity

Diet induced obesity: - Carbohydrates increment blood glucose levels, which thusly invigorate insulin discharge by the pancreas, and insulin advances the development of fat tissue and can cause weight gain. The basic starches (sugars, fructose, pastries, soda pops, brew, wine, and so on.) add to weight gain since they are more quickly consumed into the circulatory system than complex carbs like pasta, darker rice, grains, vegetables, crude natural products, and so forth and hence cause a more articulated insulin discharge after dinners than complex sugars. This higher insulin discharge, a few researchers accept, adds to weight gain **(Lakshmi .T et al., 2013).**

Over eating: - Eating prompts weight gain, for the most part if the eating regimen is high in fat. Food's high in fat or sugar (for instance, cheap food, browned nourishment, and desserts) have high energy thickness (food's that have a ton of calories in a modest quantity of sustenance). Epidemiologic investigations have demonstrated that diets high in fat add to weight gain **(Karine Clement et al., 2003).**

Drugs induced obesity: - Meds related with weight addition incorporate certain antidepressants, anticonvulsant, for example, carbamazepine and valproate, some diabetes meds, certain hormones, for example, oral contraceptives, and most corticosteroids, for example, prednisone. Some hypertension meds and antihistamines meds cause weight gain. **(Beyerlein Andreas et al., 2011).**

Triton-X: - Triton X-100 is a non-ionic surfactant which has a hydrophobic polyethylene oxide gathering and a hydrophobic lipophilic or hydrophobic gathering covalently attached to a focused benzene ring **(Supekar Aarati Ramesh et al., 2015).**

The IUPAC name of Triton-X 100 is Octoxynol-10.

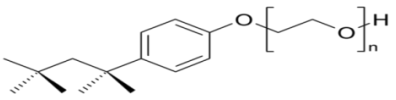

Figure: Structure of triton-X

Mechanism of Triton-X 100

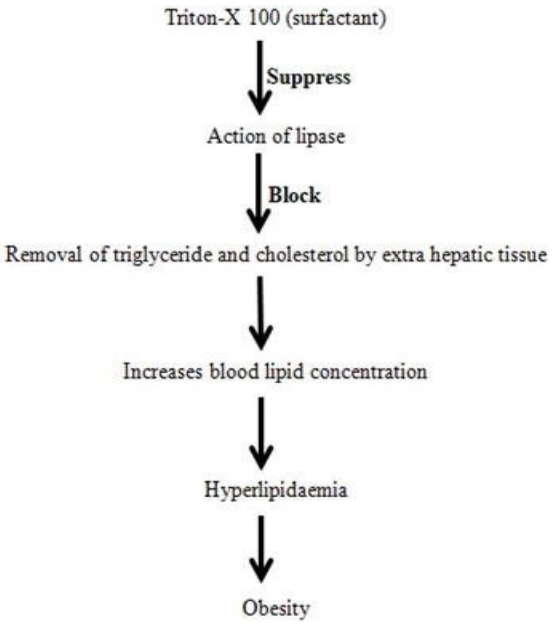

Figure: Mechanism of Action of Triton-X 100

Management

Right now overweight or a sound weight, you can find a way to counteract unfortunate weight gain and related medical issues. A few stages to anticipate weight addition are equivalent to the means to get more fit: day by day work out, a solid eating regimen, and a long haul responsibility to watch what we eat and drink.

Dietary and Lifestyle Interventions:-

Dietary and way of life intercessions determined at diminishing energy consumption and expanding energy use through a reasonable dietary and exercise program are a fundamental segment of all weight the board programs. Diets depend on the digestion and work by decreasing the admission of calories (energy) to make a negative energy balance (i.e., more energy is utilized than is expended). Diet projects may create weight reduction over the present moment however keeping up this weight reduction is regularly troublesome and frequently requires making exercise and a lower-energy diet a lasting piece of an individual's way of life. Physical exercise is a fundamental piece of a weight the executives program, particularly for weight support.

MATERIAL AND METHODS

Drugs

Simvastatin - CAS No. 79902-63-9

Resveratrol – CAS No. 501-36-0 Triton-X 100:- CAS No. 9002-93-1

Chemicals

Chloroform: - CAS No. 67-66-3 Formaldehyde: - CAS No. 50-00-0 Acetic Acid Glacial: - CAS No. 64-19-7

Potassium dihydrogen orthophosphate: - CAS No. 7778-77-0 Sodium chloride: - CAS No. 7647-14-5

Sodium carbonate (anhydrous): - CAS No. 497-19-8 Sodium bicarbonate: - CAS No. 144-55-8

Sodium tartrate AR: - CAS No. 6106-24-7 Potassium chloride: - CAS No. 7447-40-7 Sodium hydroxide: - CAS No. 1310-73-2 Sulphosalicylic acid: - CAS No. 5965-83-3 Copper (II) sulphate: - CAS No. 7758-99-8 Sodium lauryl sulphate: - CAS No. 151-21-3

Ethylene diamine tetra-acetic acid (EDTA): - CAS No. 60-00-4 Thiobarbituric acid: - CAS No. 504-17-6

Ellman’s reagent: - CAS No. 69-78-3

Bovine albumin fraction: - CAS No. 9048-46-8

Folin and ciocalteu phenol reagent: - product code- 835020

Apparatus

Electronic balance	Mettler-Toledo India Private Ltd, Mumbai
Homogenizer	Remi, Mumbai
Laboratory centrifuge	Remi, Mumbai

Micropipette	J. Mitras & Bros., New Delhi
Ultra low temperature freezer	New Brunswich Scientific, England UV
Spectrophotometer	1800, Shimadzu, USA
Water bath	Remi, Mumbai

Animals

All investigations were performed on grown-up Wistar albino rats weighing 150-250 g. The animals were acquired from the Animal House, I.T.S College of Pharmacy, Muradnagar, and Ghaziabad. Animals were housed in groups of 3 for every cage, kept up at 23±2º C, 55±5 % dampness in a characteristic light and dull cycle with free access to water and estimated access to food. The investigations were performed amid the light cycle in conscious, unreservedly moving animals that were acclimated to research facility conditions before continuing with the analyses. Procedures for the all animals were assumptive by the ethical committee at our organization (**CPCSEA registration number: 1044/PO/Re/S/07/CPCSEA, 27th Feb 2007**) and performed in conformity with institutional guidelines for the supervision and handling of experimental animals.

Experimental Design

Thirty Wistar Albino rats weighing 150-250 gm was used. The rats were isolated into five groups, Six in each groups.

Group 1 (controls): In this group rats was receive a volume of vehicle according to weight.

Group 2 (Negative Control): In this group rats was received Triton-X (100mg/kg) i.p injection of single dose.

Group 3 (Standard): In this group rats was received Simvastatin 10 mg/kg/day, p.o for 7 days after the administration of Triton-X.

Group 4 (Test): In this group rats was received Resveratrol 50 mg/ kg/day, p.o. for 14 days after the administration of Triton-X..

Group 5 (Test): In this group rats was received Resveratrol 100 mg/kg/day, p.o. for 14 days after the administration of Triton-X.

By the single intraperitoneal injection of freshly prepared solution of Triton-X- (100 mg/kg) in physiological saline after overnight fasting for 18 hours was induced hyperlipidemia in wistar albino rats.

Group	Drug	Dose	Duration	Route of administration
Group1	Control	Normal saline	7days	Oral route
Group 2	Triton-X	100mg/kg	Single dose	i.p injection
Group 3	Simvastatin	10mg/kg	7days	Oral route
Group 4	Resveratrol	50mg/kg	14 days	Oral route
Group 5	Resveratrol	100mg/kg	14 days	Oral route

Table: Experimental Design

Experimental Protocol

Collection of sample

After the complete treatment, all animals were sacrifice. Blood was gotten through the retro-orbital plexus under light anesthesia and the rats were the sacrifice. Serum for the investigation of different biochemical parameters will be isolated by centrifugation at 3000 rpm at 4oC for 20 min. The confined liver tissues were washed twice with super cold saline, blot, dried and after that weigh of each group. One half was put away at −20°C to quantify liver MDA and GSH substance; the rest was settled for 24 h in 10% buffered formaldehyde **(Ayhanci, A. *et. al.*, 2010).**

(A) Measure the physical parameters:

(i) Locomotor activity:

Generally locomotor/physical activity is reduced in obese animal, which are measured by the help of Actophotometer.

(ii) Adipose tissue weight:

The rats will sacrificed by cervical dislocation and then from various organs adipose tissue (periovarian, perirenal, and mesenteric fat pad) will isolated and weighted

(iii) Percentage change in body weight:

1. Body wt. of individual animal was taken for each group and record was maintained.

2. Body wt. was taken daily from the starting day of the study till the last dosing was do andalso before sacrificing the animal.

3. If death of any animal occurs in between the study time, its weight was also to be taken.

4. Any change in the body wt. of the animal was record.

(iv) Measurement of food intake:

Food intake was estimated on every day. To take exact food intake estimation, consideration was given to isolating the spillage food from the husk over the span of food utilization by the experimental animals **(Todd, K. S., *et al*., 1983).**

(B) Measurement of the serum triglycerides, cholesterol:

The serum triglyceride, cholesterol levels are estimated by commercial kit in different groups of rats.

(C) Measurement of MDA/TBARS level:

The affectability of estimating Thiobarbituric Acid Reactive Substances (TBARS) has made this examine the strategy for decision for screening and checking lipid peroxidation which is a major indicator of oxidative pressure. MDA frames a 1:2 adduct with thiobarbituric acid which can be estimated by fluorometry or spectrophotometry **(Kwon, T *.et al*., 1964).**

Protocol for the estimation of MDA in liver homogenate:

1. 1 gm of rat liver was taken in a tube. In that 4.5 ml of "phosphate buffer" (pH-7.4) and 3mM EDTA will take.
2. Homogenize at 2000 rpm using 10 strokes.
3. Centrifuge at 65000 G for 10 min.
4. Supernatant liquid was removed from the tube.
5. Take supernatant and add 100 µl of (8.1% SDS) + (750 µl of 20% acetic acid) + (750 µlof 0.8% thiobarbituric acid) (TBA) in a glass tube.
6. Making the volume up to 2 ml with Distilled Water (D.W.).
7. Heat it over water bath at 95ºC for 60 minutes.
8. In the above step cover the mouth of test tube with an Aluminum Foil.
9. Then test tube was taken out and cools below tap water. In this step color of the samplewas changed into pinkish.
10. Again centrifuge at "10,000" rpm for 10 min.
11. Take 300 µl supernatant in a microliter plate with blank as D.W. in a cuvette which will ready for absorbance and absorbance will be taken at 532 nm using spectrophotometer **(Ohkawa, H. et al., 1979).**

Determination of glutathione level in liver homogenate:

GSH a key antioxidant biomarker is a superoxide radical scavenger where it protects thiol group required for maintain the cell integrity against oxidation. It is an endogenous, non-enzymatic antioxidant component present at highest concentration in the liver assumes an essential part of keeping up the intracellular redox equilibrium. **(Pompella, A. *et al*., 2003).**

Protocol for glutathione:

Take 1 gm of liver in a test tube containing 5 ml of chilled phosphate buffer (PB) (pH-7.4) and 3mM EDTA(75µl of 200 mM EDTA)

1. Homogenise it at 2000 rpm at 10 strokes.
2. Centrifuge at 65000 rpm for 10 min.
1. Take 500 µl of supernatant and add 500 µl of 5% chilled sulfosalicylic acid.
2. Vortex it and keep in an ice for 30 min.
3. Again centrifuge at 10,000 rpm for 10 min.

4. Supernatant was separated from pellet and can be stored in freezer.

5. For test, take 450 µl of PB (pH 7.4) and add 50 µl ofsample.

6. For blank, take 500 µl of PB (pH 7.4) in a test tube.

7. For standard, take seven test tubes containing different concentration of standard GSHand PB (pH 7.4).

8. Vortex all the test tubes.

9. Add 3 times 1500 µl of 'Ellman'sreagent'i.e 5.5'-dithio-bis- (2-nitrobenzoic acid).

10. Vortex it.

11. Keep it for 10 min (reaction time).

12. Take absorbance at 412 nm **(Dolphin, D. *et al*., 1989).**

Total tissue protein estimation

It was done by Lowry's method.

Reagents:
Solution A = 2 gm Na2CO3 + 400 mg NaOH and volume make up the 100 ml D.WSolution B = 2% Na-Tartrate solution
Solution C = 1% CuSO4 solution.
Solution D = 96 ml solution A + 2 ml solution B + 2 ml solution C.

Standard protein solution = 1mg/ml Bovine serum albumin (BSA) (frees) solution. Folin's reagent = dilute with same volume of water at the time of use.

50 l of tissue homogenate was taken, in which 400 µl D.W was added. Then we prepared standard solution of protein with different concentration of BSA and make up the volume with D.W. up to 500 µl. For blank 500 µl D.W was taken. Then in all test tubes 2.5 ml solutionD was added. Vortexes the entire test tubes and then incubated at 370C in water bath for 10 min. Then added 250 µl Folin's reagent solution in each test tube and vortexes it, then incubated at 370C for 30 minute. Then absorbance was taken at 660 nm

Adiposity Index:

Adiposity index will be calculated by dividing total weight of omental, retroperitoneal and epididymal adipose tissue with the total bodyweight and multiplied by 100.

Adiposity Index (%) = [∑(fat pad)/(body weight×100)]

(D) Histopathological examination of Liver:

1. Under anesthesia liver was carefully removed from rats and immediately place in formalinsolution.

2. After 24 hour formal saline should be changed.

3. When used the tissue first of all wash the tissue with PBS with shaker for 1 hour.

4. Again place in PBS for 1 hour with shaking.

5. Then washing of tissue starts with gradually place in the absolute alcohol in stepwisemanner like:
Step1: Place in 70% alcohol for 2 hour with shaking. Step2: Place in 80% alcohol for 2 hour with shaking. Step3: Place in 90% alcohol for 2 hour with shaking. Step4: Place in 100% alcohol for 2 hour with shaking. Step5: Place in 100% alcohol for overnight with shaking. Step6: Place in alcohol + Xylene for 2 hour with shaking. Step7. Place in Xylene for 1 hour with shaking.
Step8: Place in Paraffin for 1hour without shaking. Step9: Place in Paraffin for 1hour without shaking.
6. Then prepare the tissue block by using mould. Place the mould on ice and maintain the temperature at -5 degree centigrade. Put the hot paraffin in between the moulds, the place tissue at one edge and put the flag at another side for indication of name of tissue.
7. Then we were prepared the slide from tissue and for this first of trim the tissue and put theblock in the microtome. Then section of size of 5 micron was started to cut.
8. It forms a ribbon of section lift the ribbon and place in water bath for proper spreading.
9. Then put the drop of Mayer's albumin (1:1 ratio of egg white and glycerin with thymolcrystal as preservative) on slide and lift the ribbon of section on that slide.
10. Then staining of tissue slide was started, first of all dip the slide in xylene for 5 minute sothat remaining wax was separated out, the dip in 100% alcohol.
11. Then dip in haematoxylene for 30 minute, then wash with distilled water, heat with absolute alcohol and put drop of Scott's reagent on this slide.
12. Then wash with distilled water and then put eosin stain which have high affinity against cytoplasm, again wash with distilled water and mount with DPx, put cover slip on it and dry in air for 2 hour.
13. Then evaluate the slide under microscope at 100x and take the picture of slide **(Derek, C.A., *et al*., 2004).**

(E) Histopathological examination of fat pad:

Isolation of fat pads

Three regions of adipose tissue will carefully dissect:

1. The periovarian fat, ovaries will take out by gentle squeezing from the peripheral fat and then by horizontal cut from all sides fat was isolated; care has been taken that too much traction was avoided on ovaries and fat.
2. The retroperitoneal, by first separating the perirenal fat and then dissecting the retroperitoneal pad in to.

3. The mesenteric, all fat found along the mesentery starting at the lesser curvature of the stomach and ending at the sigmoid colon was considered mesenteric fat; obtained by cutting the intestine below the duodenal–jejunum junction and stripping the fat by gently pulling the intestinal loops apart.

   The periovarian fat will selected for histological study. The periovarian fat of each group were excised and rinsed in 0.9% saline blotted dry of saline and excess blood. They were fixed in 12% formalin for 24 h. The tissues, after fixation, were washed in water to remove excess fixative. Washed tissues were then dehydrated through a graded series of ethyl alcohol, cleared with xylene and embedded in paraffin wax. Pieces were cut at 3μm with microtone blade and fixed on clean glass slide. The sections were routinely stained with hemotoxylin and eosin. The stained slides were observed (×200) in research microscope and photographed.

Statistical analysis:

Results were appeared as Mean ± SEM for each group. Statistical analysis was performed by utilizing Graph Pad Prism 5 statistical software. For various examinations, one-way analysis of variance (ANOVA) was utilized. In case ANOVA was indicated critical distinction, post hoc investigation finished with the Dunnet's test. $P< 0.05$ considered as statistically significant

RESULT

Results

Examination of physical parameters

Triton-X significantly increased the body weight ($p<0.050$) relative to control group as shownin Fig.6.1.1.1 The increased in body weight is significantly reduced ($p<0.050$), when Resveratrol (50 mg/kg/day and 100 mg/kg/day) were given against the Triton-X induced obesity as shown in Table 6.1.1.1 But in all above treatment groups Resveratrol (100 mg/kg/day) treated group effectively reduced the body weight as compared to other treatment groups as shown in Fig.6.1.1.1 Triton-X increased the food intake but there was no significantdifference relative to control group as shown in Fig.6.1.1.2. But treatment groups 3 and 5 significantly decreased ($p<0.050$) the food intake. Locomotion per minute significantly decreased ($p<0.050$), after inducing the obesity with Triton-X. All treatment groups Simvastatin (10 mg/kg/day), Resveratrol (50 and 100 mg/kg/day) significantly recover ($p<0.050$) the locomotion per minute shown in Fig.6.1.1.3.

Table 6.1.1.1: Physical parameters level in the rats treated with Triton-X (100 mg/kg), Simvastatin (10 mg/kg), Resveratrol (50 mg/kg, 100 mg/kg) after inducing the obesity with Triton-X.

Groups (n=6)	Body Weight (gm)	Food Intake (gm)	Locomotion /minute
Group 1	119.040 ± 2.969	9.84 ± 0.17	5 ± 0.65
Group 2	182.188 ± 3.259 **a**	10.27 ± 0.03	1.9 ± 0.12 **a**
Group 3	152.738 ± 3.723 **b**	9.64 ± 0.167 **b**	4.54 ± 0.36 **b**
Group 4	165.183 ± 1.102 **b**	9.79 ± 0.164	5.4 ± 0.42 **b**
Group 5	154.364 ± 1.525 **b**	9.36 ± 0.167 **b**	5.88 ± 0.22 **b**

All values are shown as Mean ± SEM, n= number of rats,
[a] Significantly different from Group 1 ($p<0.05$)
[b] Significantly different from Group 2 ($p<0.05$)

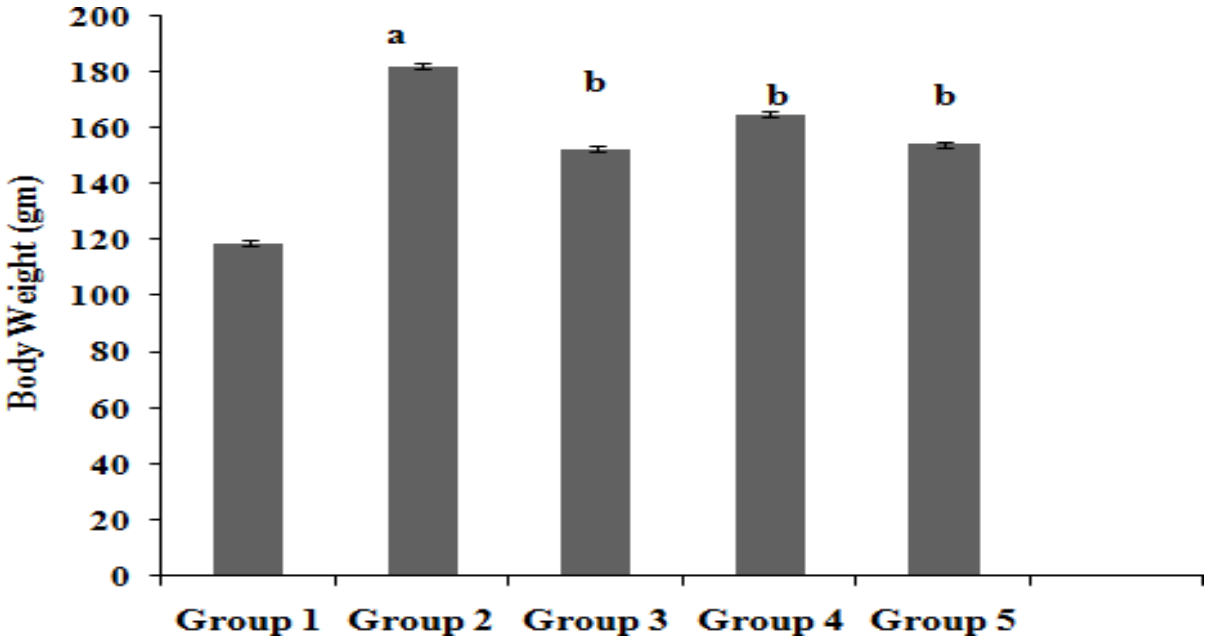

Figure 6.1.1.1: Physical parameters level in the rats. Triton-X administration significantly increased body weight as compared to the control group ($p<0.050$) which was significantly reduced ($p<0.050$) by all treatment groups (Simvastatin 10 mg/kg/day, Resveratrol 50 and 100mg/kg/day).

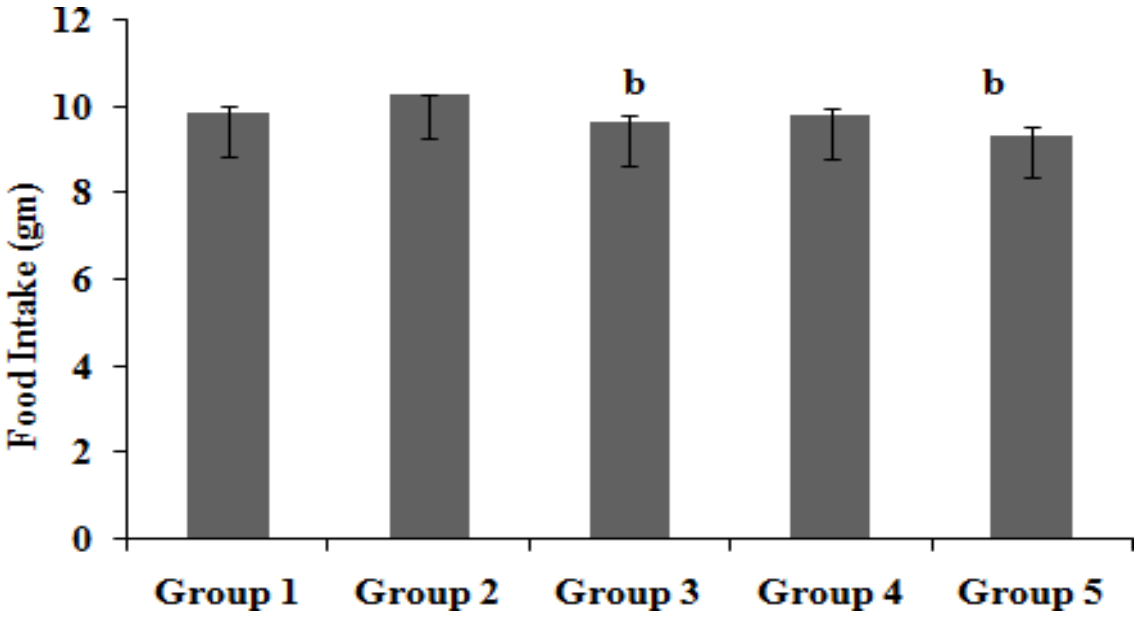

Figure 6.1.1.2: Physical parameters level in the rats. Triton-X administration increased the food intake as compared to the control group. But treatment groups 3 and 5 significantly increased ($p<0.050$), the food intake.

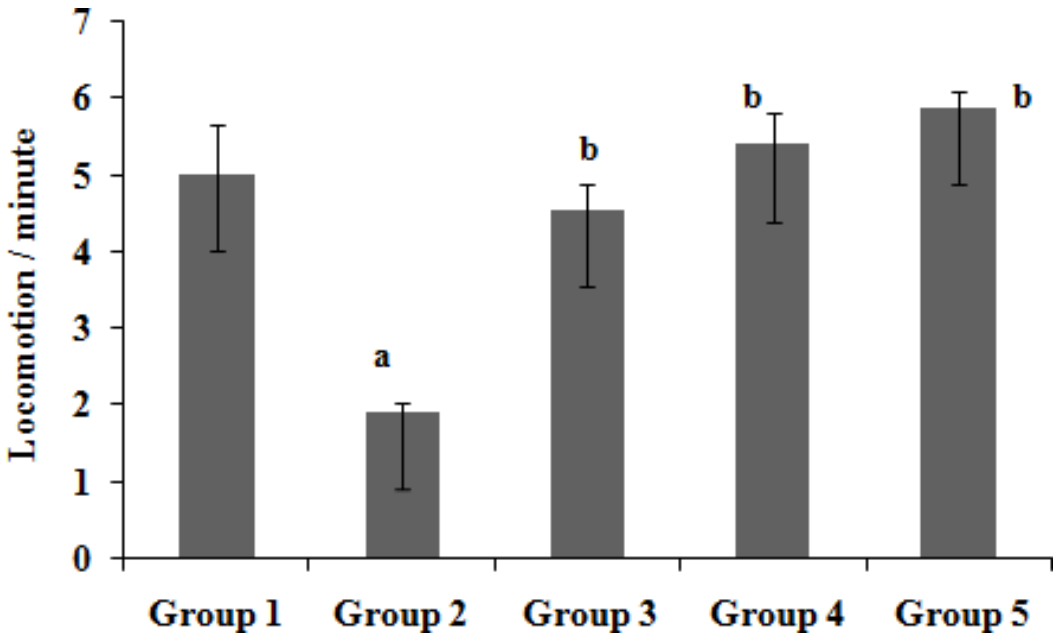

Figure 6.1.1.3: Locomotion per minute of the rats. Locomotion per minute significantly decreased ($p<0.050$), after inducing the obesity with Triton-X. All treatment groups Simvastatin (10 mg/kg/day), Resveratrol (50 and 100 mg/kg/day) significantly recover ($p<0.050$) the locomotion per minute.

Antioxidant level

The antioxidant levels are shown in Table 6.1.2.1 In the Triton-X treated group the hepatic MDA level significantly increased ($p<0.05$) relative to the control. This increased level of MDA significantly reduced ($p<0.05$) by test dose of Resveratrol (50 mg/kg and 100 mg/kg) after inducing the obesity with Triton-X. But in all above treatment groups Resveratrol (100 mg/kg) treated group highly reduced the MDA level as compared to other treatment groups as shown in Fig.6.1.2.1.

Triton-X administration also significantly decreased the GSH level as compared to the control group ($p<0.05$). Groups receiving Simvastatin (10 mg/kg/day) and Resveratrol (100 mg/kg and 50 mg/kg) after inducing the obesity with Triton-X showed a significant re-establishment in GSH level in the liver tissue ($p<0.05$) as shown in Fig.6.1.2.2.

Table 6.1.2.1: MDA and GSH level in the rats treated with Triton-X, Simvastatin (10 mg/kg/day) and Resveratrol (50 mg/kg and 100 mg/kg) after inducing the obesity with Triton-X.

Groups (n=6)	MDA (µmol/mg protein)	GSH (µmol/mg protein)
Group 1	90.56 ± 19.88	215.13 ± 50.82
Group 2	331.82 ± 95.61 [a]	54.72 ± 16.23 [a]
Group 3	201.23 ± 51.17	137.5 ± 10.85 [b]
Group 4	93.65 ± 2.10 [b]	159.93 ± 26.16 [b]
Group 5	92.03 ± 12.98 [b]	180.44 ± 6.08 [b]

All values are shown as Mean ± SEM, n= number of rats.

[a] Significantly different from Group 1 ($p<0.05$)

[b] Significantly different from Group 2 ($p<0.05$)

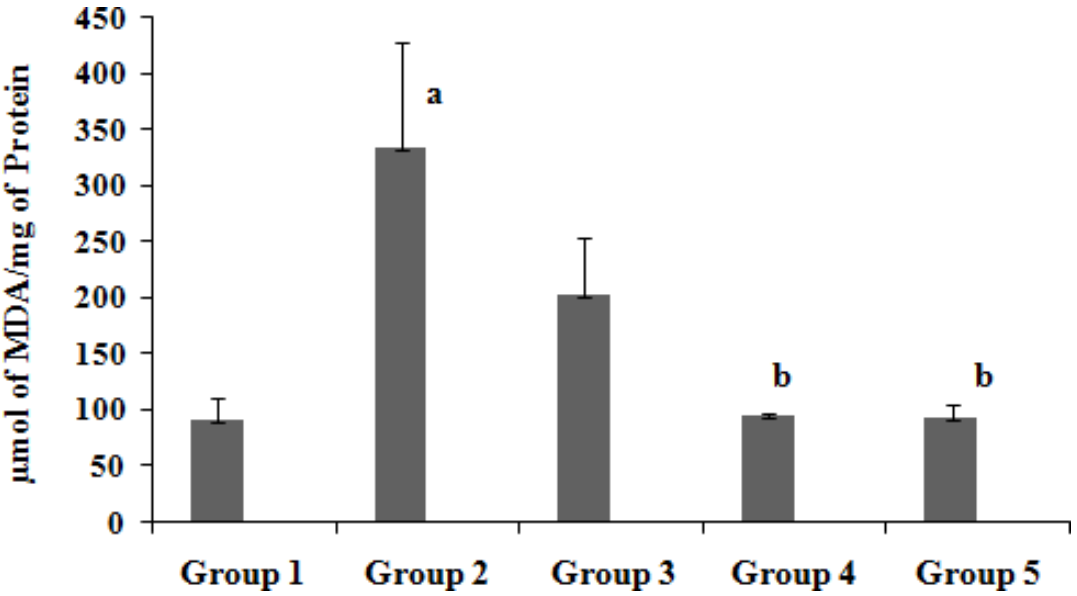

Figure 6.1.2.1: MDA level in the liver tissue of rats. This increased level of MDA significantly reduced ($p<0.05$) by test dose of Resveratrol (50 mg/kg and 100 mg/kg) after inducing the obesity with Triton-X.

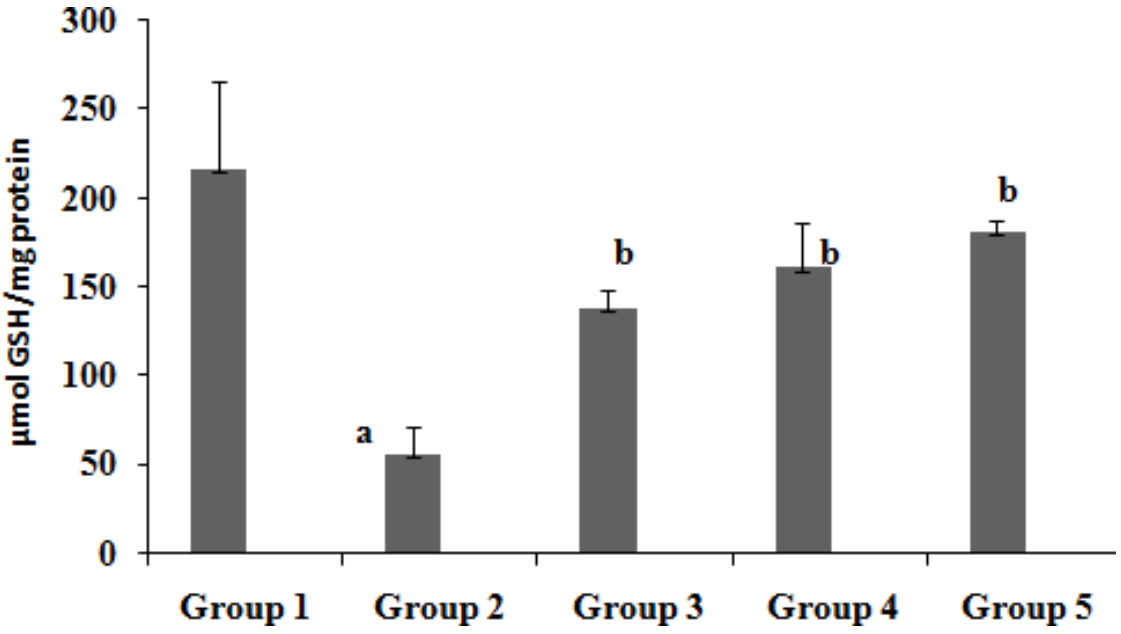

Figure 6.1.2.2: GSH level in the liver tissue of rats. Triton-X administration significantly decreased the GSH level as compared to the control group ($p<0.05$). Groups receiving Simvastatin (10 mg/kg/day) and Resveratrol (100 mg/kg and 50 mg/kg) after inducing the obesity with Triton-X showed a significant re-establishment in GSH level in the liver tissue ($p<0.05$).

6.1.3. Serum triglyceride and cholesterol level and adiposity index (%)

Triglyceride and cholesterol level in serum is shown in Table 6.1.3.1. The serum triglyceride and cholesterol level was significantly increased ($p<0.05$) by administration of Triton-X. After administration of standard drug Simvastatin (10 mg/kg/day) and test dose of Resveratrol (50 mg/kg and 100 mg/kg) after inducing the obesity with Triton-X, the serum triglyceride and cholesterol level significantly declined ($p<0.05$). The serum triglyceride and cholesterol level also shown in Fig. 6.1.3.1, Fig.6.1.3.2 respectively. Adiposity index relation with Triton-X and standard and test drug shown in the Fig.6.1.3.3.

Table 6.1.3.1: Serum triglyceride, cholesterol level and adiposity index in rats treated with Triton-X (100 mg/kg), Simvastatin (10 mg/kg), Resveratrol (50 mg/kg, 100 mg/kg) after inducing the obesity with Triton-X.

Groups (n=6)	Triglyceride (mg/dl)	Cholesterol (mg/dl)	Adiposity index (%)
Group 1	249 ± 17.81	190.4 ± 7.19	1.805 ± 0.036
Group 2	391 ± 86.35	952.2 ± 51.42 [a]	2.35 ± 0.123 [a]
Group 3	200.6 ± 21.84 [b]	401.4 ± 102.03 [b]	1.673 ± 0.09 [b]
Group 4	185 ± 18.69 [b]	465 ± 61.80 [b]	1.713 ±0.05 [b]
Group 5	138 ± 2.81 [b]	324.8 ± 54.627 [b]	1.520 ± 0.04 [b]

All values are shown as Mean ± SEM, n= number of rats.

[a] Significantly different from Group 1 ($p<0.05$)

[b] Significantly different from Group 2 ($p<0.05$)

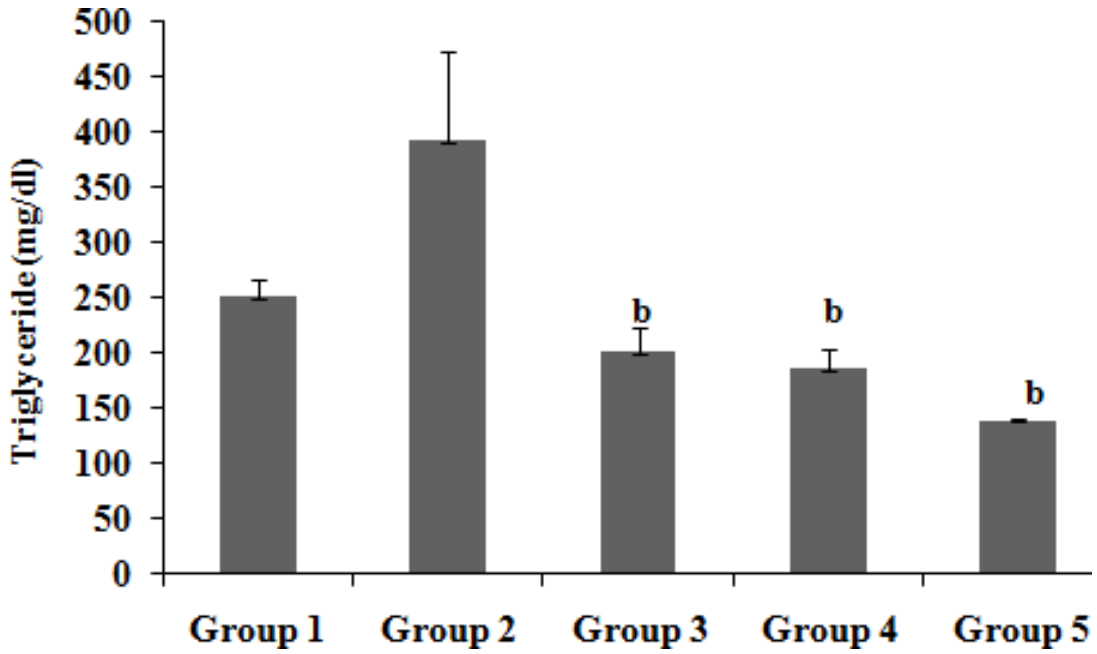

Figure 6.1.3.1: Serum Triglyceride level in the rats. Triton-X administration increased serumtriglyceride level as compared to the control, which was significantly treated by Simvastatin (10 mg/kg/day) and Resveratrol (50 mg/kg and 100 mg/kg) after inducing the obesity with Triton-X.

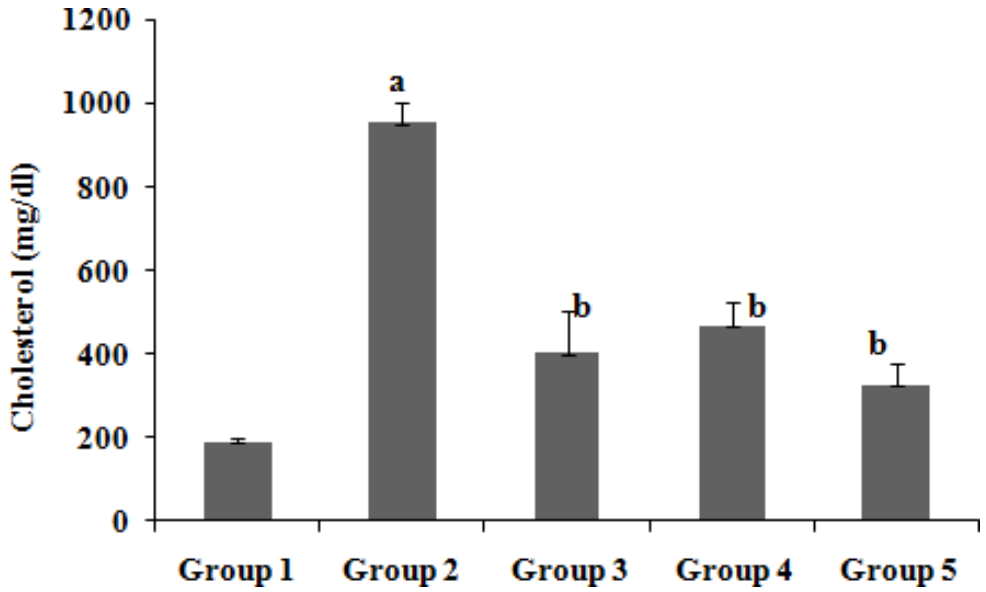

Figure 6.1.3.2: Serum cholesterol level in the rats. Triton-X administration significantly increased serum cholesterol level as compared to the control ($p<0.05$), which was significantly treated by Simvastatin (10 mg/kg/day) and Resveratrol (50 mg/kg and 100 mg/kg) after inducing the obesity with Triton-X.

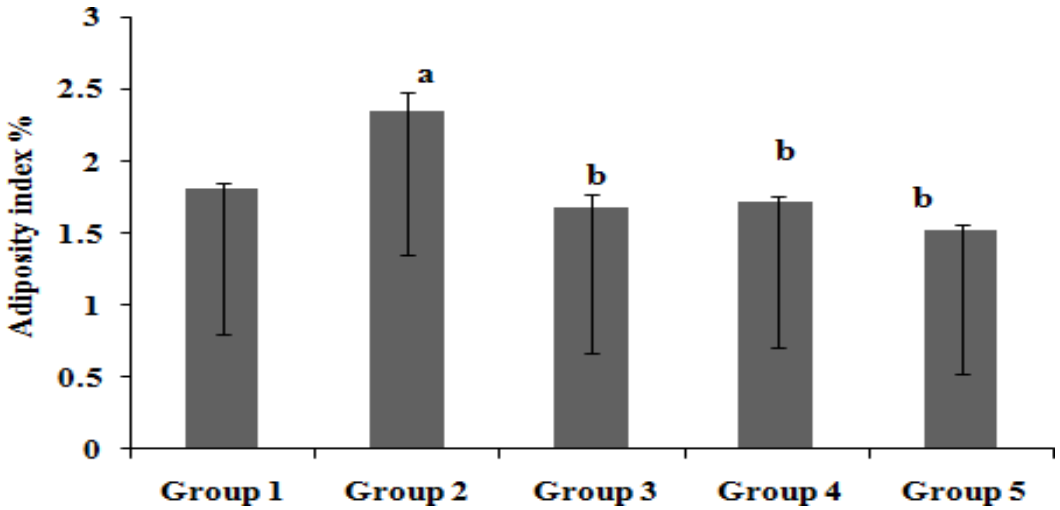

Figure 6.1.3.3: Adiposity index in the rats. Triton-X administration significantly increased adiposity index as compared to the control ($p<0.05$), which was significantly treated by treated by Simvastatin (10 mg/kg/day) and Resveratrol (50 mg/kg and 100 mg/kg) after inducing the obesity with Triton-X.

Histopathological examination

Liver Histopathological examination

The liver of a control group has shown normal histology represented by A picture of Fig.6.1.4.1 After administration of Triton- X rats showed derangement of central hepatic system and sinusoids as well as generalized haemorrhage as shown in picture B of Fig.6.1.4.1. Group 3 i.e. standard group showed the intact hepatic system and sinusoids in picture C of Fig.6.1.4.1. Group 4 means Resveratrol 50 mg/kg/day showed derangement of sinusoids and remaining cell of liver was almost normal as represented by picture D of Fig.6.1.4.1. Group 5 in which Resveratrol of 100 mg/kg/day were given showed the intact

hepatic system and normally arranged sinusoids and normal hepatocytes in picture E of Fig.6.1.4.1.

Histopathological examination of fat pads

The fat pads of a control group have shown the size 1614.161 μ^2 represented by A picture of Fig.6.1.4.2. After administration of Triton- X rats showed large size fat pads having 3546.775 μ^2 as shown in picture B of Fig.6.1.4.2. Group 3 i.e. standard group showed the 2074.014 μ^2 size of fat pad in picture C of Fig.6.1.4.2. Group 4 means Resveratrol 50 mg/kg/day showed 2632.367 μ^2 as represented by picture D of Fig.6.1.4.2. Group 5 in which Resveratrol of 100 mg/kg/day were given showed the 1640.195 μ^2 in picture E of Fig.6.1.4.2.

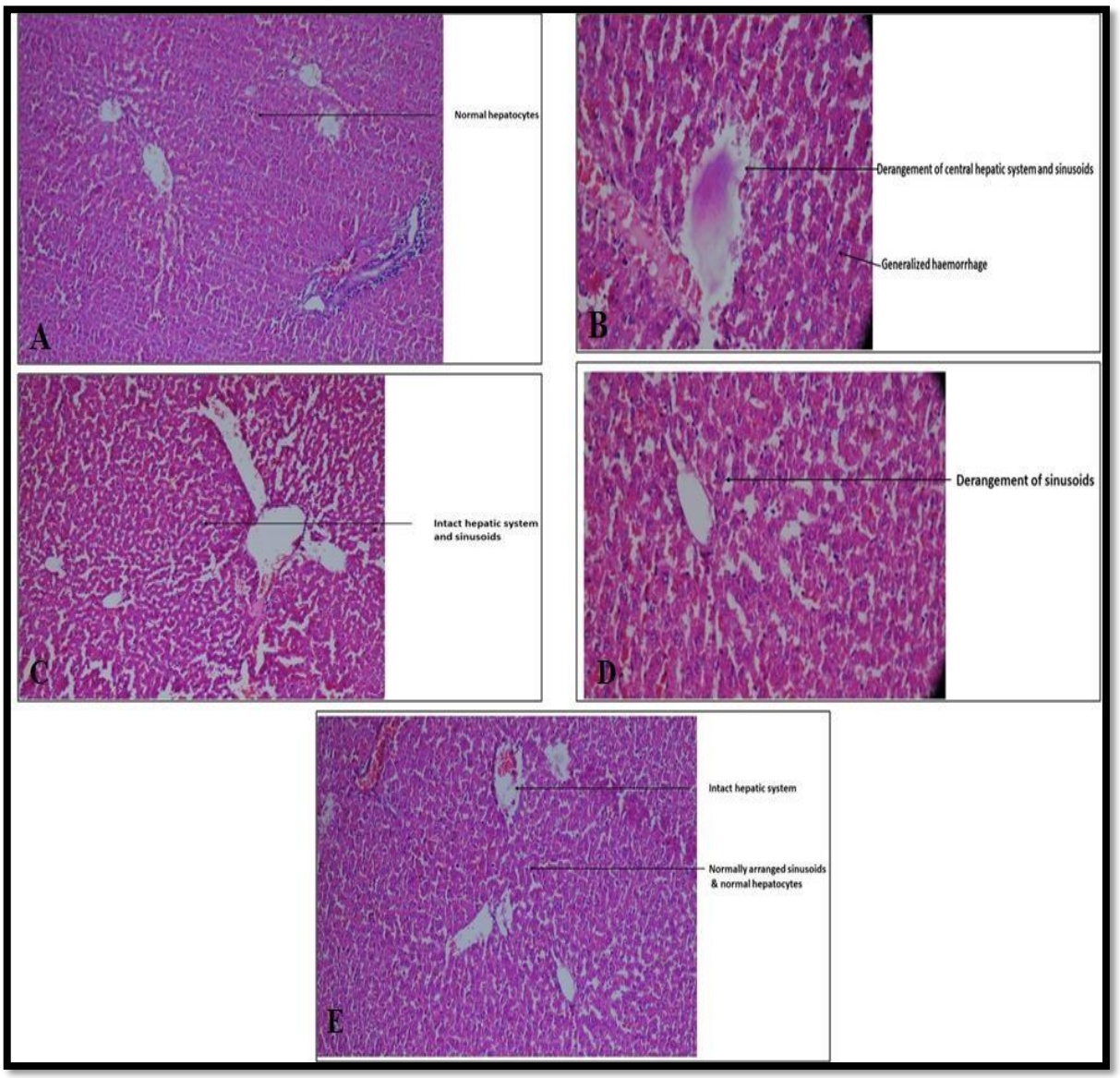

Figure 6.1.4.1: Liver Histopathological examination. A. Normal hepatocytes, B. Derangement of central hepatic system and sinusoids as well as generalized haemorrhage. C. Intact hepatic system and sinusoids. D. Derangement of sinusoids and remaining cell of liver was almost normal. E. Intact hepatic system and normally arranged sinusoids and normal hepatocytes.

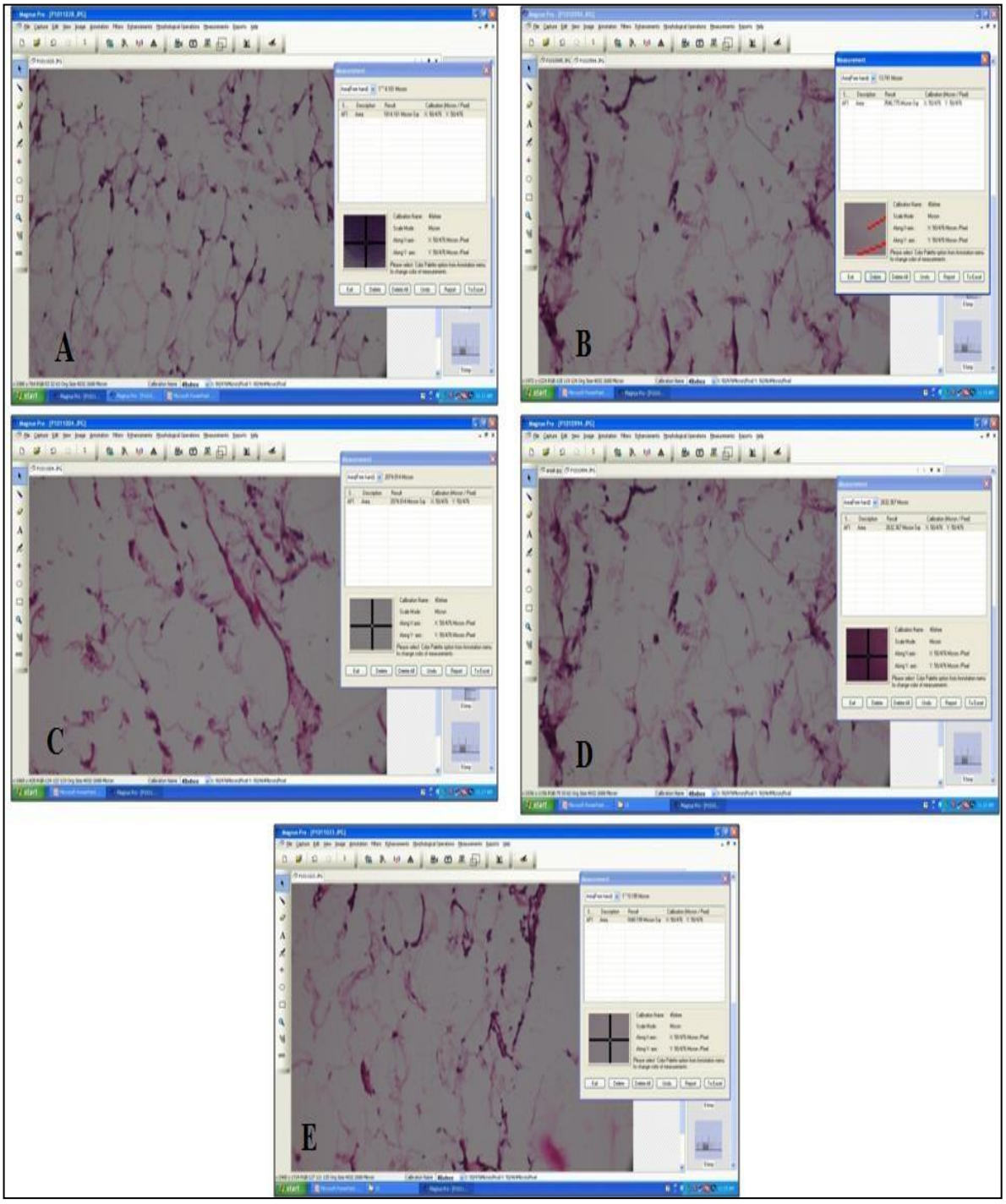

Figure 6.1.4.2: Histopathological examination of fat pads.

Discussion

In this work antiobesity action of Resveratrol against Triton-X induced obesity was observed. Hyperlipidemia was produced by freshly prepared solution of Triton-X-100 in normal saline solution by single intraperitoneal injection. Resveratrol was taken in two doses that are 50 mg/kg/day and 100 mg/kg/day as anti-obesity test drug.

Resverastrol when administered to rats with Triton-X induced obesity protected against oxidative stress, histological changes in liver and fat pads as well as produced a positive consequence on body weight, food intake, locomotion per minute, adiposity index and triglyceride and cholesterol level in serum.

In the present study, the treatment of rats with Triton-X resulted in GSH depletion, increased MDA level, and increased the triglyceride and cholesterol level in serum. Triton-X administration also leads the various histological abnormalities in liver tissue as well as increased the size of fat pads. The obesity effect of Triton-X is due to hyperlipidemic property and increased the concentration of lipid in blood. It is well known that hyperlipidemia increased the generation of ROS, produces the changes in cell redox balance and leads to oxidative stress. The obesity was evidenced by high lipid peroxidation (MDA level), GSH depletion, and increased body weight as well as histological changes in liver and fat pads.

Triton-X administered rats showed derangement of central hepatic system and sinusoids as well as generalized haemorrhage of the liver cell and large size fat pads and increased the serum triglyceride and cholesterol level. Resveratrol (50 mg/kg/day and 100 mg/kg/day) treatment after the administration of Triton-X significantly ($p<0.05$) decreased body weight, food intake, restored the GSH level ($p<0.05$) while significantly decreased serum triglyceride, cholesterol and MDA level and notably ameliorated liver and fat pads histopathological.

But these changes were highly significant in that treatment group in which Resveratrol (100 mg/kg/day) treatment were given after inducing the obesity by Triton-X. The tabular data of this study indicate that Resveratrol (50 mg/kg/day and 100 mg/kg/day at both doses) were showed appreciably improvement on the obesity.

Conclusion

Since the result of the present study, it is consummate that Triton-X generates obesity due to the hyperlipidemic action. Triton-X produces lipid peroxidation, GSH depletion, increased food intake, body weight, and increases level of triglyceride and cholesterol in serum as well as derangement of central hepatic system and sinusoids as well as generalized haemorrhage ofthe liver cell and increased the size fat pads. So increased the adiposity index.

Resveratrol may have antiobesity action against Triton-X induced obesity due to its antioxidants and lipolytic properties. Resveratrol at 100 mg/kg/ day dose was more effective as compared to 50 mg/kg/day dose and significantly ameliorate liver and fat pads histology. Resveratrol may act as a protective pharmacological agent and decreases the side effect of Triton-X. It is clearly indicate by this study that all the treatment may defend from the obesity when these treatment given with Triton-X containing preparation or medicines.

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