Original Article

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Preventive Potential of *Citrullus Vulgaris* (Thunb.)Seeds Extracts on Cafeteria, Atherogenic Diet induced Obesity and Formaldehyde, Freund's Adjuvant Arthritis Models in Wistar Rats

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Abstract

This present study was aimed to explore the anti obesity and anti arthritic activities of seed extracts of *Citrullus vulgaris* (Thunb) in cafeteria diet (CD), atherogenic diet (AD) induced obesity and Formaldehyde and Freund's adjuvant (FA) induced arthritis in experimental animals i.e. rats. The ethanolic and aqueous extracts were prepared by using soxhlet apparatus and maceration process respectively. Anti obesity activity of seed extracts of this plant was evaluated in CD and AD induced obesity and anti arthritic activity was evaluated in formaldehyde and FA induced arthritis in rats. Phytochemical studies with both ethanolic extract (ELSCV) and aqueous extract (AQSCV) revealed the presence of carbohydrates, proteins, flavonoids, saponins, fixed oils, glycosides and steroids. No mortality or behavioral abnormality recorded in mice at the highest dose level of 2000 mg/kg tested for LD50 studies. Both the extracts with medium and high doses exhibited a significant anti obesity activity by reducing the body weight, food intake, organ and fat pads weight and serum glucose, cholesterol, triglyceride, low density lipoproteins and very low density lipoprotein cholesterol levels with an increased high density levels in CD and AD induced obesity models in rats. Both extracts with medium and high doses exhibited a significant anti arthritic activity and reduced serum biochemical parameters in FA induced arthritis model in rats. The anti obesity and anti arthritic activities with extracts conformed the above mentioned activities because presence of flavonoids, saponins, fixed oils, steroids, alkaloids and glycosides.

Keywords: *Citrullus vulgaris* seeds, obesity, sibutramine, arthritis.

1. Introduction

Obesity is a medical condition in which excess of body fat has accumulated to the extent that produce adverse effect on health, leading to reduced life expectancy and increased health problems (1). Body Mass Index (BMI), a measurement which compares weight and height, defines a person as overweight (preo-bese) when their BMI is between 25-30 kg/m^2, and obese when it is greater than 30 kg/m^2 (2). Obesity is one of the 21st century's leading health problems and the prevalence of obesity also increasing and the World Health Organization (WHO) estimates that more than 1 billion adults are overweight worldwide and at least 350 millions of them are clinically obese. Over all 2.5 millions of death are attributed to overweight/obesity worldwide (3). Obesity research shows that the condition of obese increase with age and around 28% of men and 27% of women aged 16-24 are obese in the world. Further about 76% of men and 68% of women between 55 and 64 of ages are obese (4). Obesity is nowadays a common and not only challenging health problem but

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also a predisposing factor for many other adverse health outcomes like non-insulin dependent diabetes mellitus (NIDDM), insulin resistance, atherosclerosis, dyslipidemias, cardiovascular diseases and all-cause mortality. The World Health Organization (WHO) has described it as an 'escalating epidemic and one of the greatest neglected public health problems of recent times with an impact on health as great as smoking (5). The modern sedentary lifestyle, with an abundant palatable nutrient supply and reduced physical activity, has resulted in dramatic increase in Obesity-associated diseases and metabolic syndromes (6).

Rheumatoid arthritis (RA) is a chronic, systemic inflammatory disorder that may affect many tissues and organs, but principally attacks synovial joints. The pathology of the disease process often leads to the destruction of articular cartilage and ankylosis of the joints. RA can also produce diffuse inflammation in the lungs, pericardium, pleura, sclera and also nodular lesions, most common in subcutaneous tissue (7). About 10% of the world's population is afflicted by RA, women 3 times more often than men. Onset is most frequent between the ages of 40 and 50, but people of any age can be affected. It can be a disabling and painful condition, which can lead to substantial loss of functioning and mobility if not adequately treated (8). *Citrullus vulgaris* which is commonly known as watermelon, belongs to a family cucurbitaceae (Figure 1). It is a native to Africa, Asia and America and also popular in Russia. The fleshy juicy fruit is eaten fresh and in Asia the seeds are eaten (9). The fruit is greenish yellow, the seeds are small light brown to black, about 0.4-1.1 cm long and 0.2-0.3 cm wide (10). Leaf of *Citrullus vulgaris* was tested for its mosquito repellent activity (11). The effect of seed extract was investigated for its spermatogenesis activity in rat (12). The seeds of Citrullus vulgaris also reported to be used in the treatment of bed wetting, dropsy, urinary tract infection, renal stone, alcohol poisoning, diarrhoea and hypertension (13-19). However, the seed extract of Citrullus vulgaris have not been tested for arthritis and obesity. The scientific investigation is essential to prove the potency of seeds and to extent their scope for future use. In this present work, it was planned to verify the therapeutic usefulness of locally available plants. Literature survey revealed that the plant *Citrullus vulgaris* apart from other medicinal uses was used as an ethnic folklore medicine for obesity and arthritis. Hence, the alcoholic and aqueous extracts prepared with seeds of *Citrullus vulgaris* have been selected to prove the therapeutic efficacy of the seeds as an anti-arthritic agent against, formaldehyde-induced arthritis, Freund's complete adjuvant-induced arthritis in rats. And also to investigate the cafeteria induced as well as Atherogenic diet induced obesity in rats.

2. Materials and methods

The *Citrullus vulgaris* fruits were collected near Raichur district, Karnataka, India in the month of April 2018.The 15-20 cm long fruits were cut into pieces, brownish to black seeds were collected and dried. The plant sample, fruit and seeds samples were authenticated by the University of Agriculturural Science, Bangalore, with an authentication reference number of UAS/SA/076/2018.

2.1. Chemicals and drugs

Sibutramine was procured from Symed Laboratories, Hyderabad, India, Cholesterol and lard oil Cholic acid was obtained from Sigma Aldrich, Bangalore, India. Formalin was collected from Nice-Cochin, India. Triglycerides kit, Serum glucose kit, HDL – cholesterol kit, Total – cholesterol kit, SGOT kit, SGPT kit, Serum Alkaline Phosphatase kit, Serum Albumin kit, Serum Total Protein kit, Serum Calcium kit, Serum BUN (Blood Urea Nitrogen) kit was purchased from Erba Diagnostics Mannheim,GmbH, Germany. Formaldehyde was procured from Karnataka fine chemicals Bengaluru. Freund's adjuvant was collected from GeNeiTM Mumbai.

2.2. Experimental animals

Albino rats (Wistar strain) of either sex weighing between 150-200 g and Albino mice 16-25 g were procured from National Centre for Laboratory Animal Sciences, C/0 Sri. Venkateswara Enterprises, Bangalore for experimental purpose. Then the animals were acclimatized for 7 days under standard husbandry condition.i.e.room temperature- 26±20 °C, relative humidity-45-55%, Light/

dark cycle-12:12 h. The animals were fed with a synthetic standard diet from Amrut laboratories & Pranav Agro Industries Ltd. Sangli, Maharashtra. Water was allowed ad libitum under strict hygienic conditions. All animal studies were performed in accordance to Guidelines No. 425 of CPCSEA and Institutional Animal Ethical Committee (IAEC) of V.L. College of Pharmacy, Raichur (Karnataka). CPCSEA registration number was 557/02/c/CPCSEA and all the procedures were followed as per rules and regulations.

2.3. Preparation of Ethanolic Extract

The seed powder was packed in soxhlet apparatus and extracted with 95% ethanol for 18 h. Appearance of colorless solvent in the siphon tube was taken as the termination of extraction. The extract was then transferred into a previously weighed empty beaker and evaporated to a thick paste on the water bath, maintained at 50oC to get ethanolic Seeds Extract of *Citrullus vulgaris* (ELSCV) extract. The extract was finally air dried thoroughly to remove all traces of the solvent and the percentage yield was obtained 90.

2.4. Preparation of Aqueous Extract

About 100 g of seed powder was taken in a round bottom flask (2000 ml) and macerated with 500 ml of distilled water with 10 ml of chloroform (preservative) for 7 days with occasional shaking for every hour in a closed vessel. Then the marc was removed by filtering the extract and then it was concentrated on a water bath maintained at < 50 °C to get aqueous seeds extract of Citrullus vulgaris (AQSCV). Both the extracts were stored in airtight containers in a refrigerator below 10 °C. The two extracts were examined for their colour and consistency and percentage yield was calculated with reference to air-dried powder sample used for the extraction.

2.5. Phytochemical investigation

The extract was subjected to several chemical tests to detect the chemical constituents present in them.0.5 gm of extract was dissolved in 5 ml of distilled water and filtered. The filtrate was used to determine the presence of various phytoconstituents as describe in the Table1.

2.6. Determination of Acute toxicity study (LD50 study)

The acute oral toxicity study (20) of fruit extracts of Citrullus vulgaris (Cucurbitacae) was determined in female albino mice (16-25 g) maintained under standard husbandry conditions. The animals were fasted 4 h before the experiment and up and down procedure (OECD Guidelines No. 425) method of CPCSEA were adopted for acute toxicity studies. Animals were administered with single doses of each extract and observed for their mortality during 48 h study period (short-term toxicity). Based on the short-term profile of extracts, the doses for the next animals were determined. All the animals were observed for long-term toxicity (7 days). The LD_{50} studies of the test extracts were conducted up to the maximum dose level of 2000 mg/kg body wt. 1/20, 1/10 and $1/5^{th}$ doses of the LD_{50} dose of the individual extracts were selected for the study as low, medium, and high doses.

2.7. Determination of anti obesity activity

2.7.1 Cafeteria diet induced obesity model in rats

In this method (21) the cafeteria diet (CD) consisted of three diets (48 g of condensed milk, 48 g of bread),(18 g of chocolate,36 g of dried coconut) and (48 g of cheese,60 g of potatoes). The three diets will be given to groups of 6 rats on days 1,2 and 3 respectively and then repeated for 40 days in same succession in addition to normal pellet chow diet. Group-1 Normal control which receives normal pellet chow and water ad libitum (40 days), Group-2 Cafeteria diet control, which receives CD + normal pellet Chow diet 40 days,Group-3 CD+ Sibutramine (5 mg/kg, p.o for 40 days) and six drug treated groups of (ethanolic and aqueous extracts) low, medium, and high. The parameters like change in body weight, weights of fat pads, observation of food consumption was observed. On day 41, and the animals were anesthetized with ether. Blood was collected from the retro orbital puncture later sacrificed by overdose of ether. Serum was separated and subjected to serum analysis of biochemical parameters. The values were expressed as the mean with standard deviation (SD) from 6 animals.

Table 1. Anti obesity effect of ELSCV and AQSCV on in Cafeteria Diet (CD) induced obesity in rats.

Groups	Dose	Serum biochemical parameters (mg/dL)						Organ and Fat pad weights (g/100 g)							
										Kidney					
		GLU	CHO	TRG	HDL	LDL	VLDL	Liver	Heart	Left	Right	Spleen	Perire-nal FP	Uterus FP	Mesent -ric FP
Normal Pellet diet	-	103.3 ±4.4	74.10 ±8. 0	94.2 ±6.9	20.19 ±1.3	35.05 ±8.8	18.85 ±1.3	3.39 ±0.090	0.43 ±0.01	0.55 ±0.01	0.63 ±0.01	0.72 ±0.014	0.25 ±0.015	3.34 ±0.10	1.71 ±0.03
CD Control	-	311.13 ±23.5**a	184.55 ±20.28**a	217.35 ±23.8**a	14.0 ±0.5**a	127.01 ±21.7**a	43.47 ±4.7**a	4.75 ±0.52*a	0.85 ±0.09**a	0.77 ±0.01**a	0.86 ±0.01**a	1.23 ±0.07 **a	0.59 ±0.014 **a	5.26 ±0.02**a	3.25 ±0.06**a
Standard	SBT5 mg/kg	110.7 ±4.7 **b	102.29 ±7.79 **b	122.78 ±5.1**b	36.7 ±3.4**b	40.99 ±9.0 **b	24.55 ±1.0**b	3.71 ±0.6**b	0.51 ±0.03**b	0.60 ±0.01**b	0.58 ±0.01**b	0.85 ±0.01 **b	0.43 ±0.09 **b	3.97 ±0.12**b	2.09 ±0.08**b
ELSCV	100 mg/kg	165.83 ±30.8 **b	153.30 ±14.63 ns	159.33 ±8.2 *b	16.1 ±0.45 ns	105.26 ±14.6 ns	31.86 ±1.6 *b	4.55 ±0.09ns	0.75 ±0.2**b	0.78 ±0.01**b	0.78 ±0.01**b	1.15 ±0.09 ns	0.52 ±0.017 *b	4.85 ±0.05**b	2.96 ±0.08**b
ELSCV	200 mg/kg	111.12 ±4.8 **b	132.73 ±12.0 ns	156.02 ±10.0 *b	21.94 ±1.1 **b	79.58 ±11.56 ns	31.20 ±2.0 *b	4.22 ±0.08**b	0.65 ±0.01** b	0.70 ±0.01**b	0.70 ±0.04**b	0.98 ±0.02 **b	0.47 ±0.005 **b	4.60 ±0.03**b	2.63 ±0.06**b
ELSCV	400 mg/kg	104.75 ±4.4 **b	120.44 ±23.55*b	155.88 ±11.8 *b	31.01 ±1.4**b	58.24 ±19.0 *b	31.17 ±2.3 *b	3.96 ±0.17**b	0.55 ±0.01** b	0.63 ±0.07**b	0.64 ±0.01**b	0.86 ±0.07 **b	0.44 ±0.09 **b	4.27 ±0.05**b	2.34 ±0.05**b
AQSCV	100 mg/kg	176.98 ±18.9**b	158.83 ±21.7 ns	164.85 ±18.7 *b	15.85 ±0.46 ns	110.01 ±22.3 ns	32.97 ±3.7 *b	4.51 ±0.10ns	0.75 ±0.01**b	0.72 ±0.07**b	0.84 ±0.01**b	1.19 ±0.11 ns	0.54 ±0.019 ns	4.94 ±0.06**b	2.96 ±0.06**b
AQSCV	200 mg/kg	116.86 ±14.4**b	132.6 ±14.5 ns	160.0 ±9.7 *b	21.72 ±1.2**b	78.86 ±16.72 ns	32.01 ±1.9*b	4.17 ±0.10*b	0.65 ±0.01 **b	0.70 ±0.07**b	0.72 ±0.01**b	0.90 ±0.05 **b	0.46 ±0.007 **b	4.46 ±0.07**b	2.61 ±0.07**b
AQSCV	400 mg/kg	114.4 ±14.6**b	118.3 ±3.2 *b	142.42 ±9.7 **b	27.03 ±1.0**b	62.78 ±3.5 *b	28.48 ±1.9 **b	4.02 ±0.13**b	0.54 ±0.01 **b	0.65 ±0.01**b	0.65 ±0.01**b	0.84 ±0.01 **b	0.44 ±0.011 **b	4.30 ±0.05**b	2.20 ±0.11**b

n = 6, Significant at P < 0.05*, 0.01** and 0.001***, ns = not significant. a – compare to normal control, b – compare to CD control ELSCV – Ethanolic extract of seeds of C. vulgaris, AQSCV- Aqueous extract of seeds of C. vulgaris . SBT- Sibutramine. FP- Fat p

C.vulgaris plant Seeds of C. *vulgaris*

Figure 1. Image of Citrullus Vulgaris fruit and seeds.

2.7.2. Atherogenic diet induced obesity model in rats

This method (22) involves the atherogenic diet consisted of 1% cholesterol (Loba chemie), 0.5%cholic acid (Loba chemie) and 5% Lard oil (sigma) in addition to normal pellet Chow diet all the groups of animals were supplied with Atherogenic diet. Group-1 received normal pellet chow and water ad libitum for 40 days, Group-2 Atherogenic diet control, which receives AD + normal pellet Chow diet for 40 days, Group-3 receives AD+ Sibutramine (5 mg/kg, p.o for 40 days) and six drug treated groups given with (ethanolic and aqueous extracts) low, medium, and high doses for 40 days. The parameters like change in body weight, weights of fat pads, observation of food consumption was observed. On day 41, the animals were anesthetized with ether. Blood was collected from the retro orbital puncture later sacrificed by overdose of ether separated serum was subjected to serum analysis of biochemical parameters and weights of fat pads. The values were expressed as the mean with standard deviation (SD) from 6 animals.

2.7.3. Data analysis

The obtained values in all three models were expressed as mean with SD from 6 animals, subjected to statistical analysis using one-way analysis of variance followed by Dunnett's- t-test to verify significant difference if any among the groups. P<0.05*, 0.01**, and 0.001*** was considered statistically significant.

2.8. Determination of Anti-arthritic activity

2.8.1. Formaldehyde induced arthritis

Male albino rats weighing between (150 and 200 g) were divided into nine groups of 6 rats in each, i.e., normal control (1% CMC), toxicant control, standard (Indomethacin 10 mg/kg), and six drug treated groups of (ethanolic and aqueous extracts) low, medium, and high. All the groups administered with 2% v/v formaldehyde except normal control (23). Afterward daily, the paw volume was measured for 10 days. The values were expressed as the mean with standard deviation (SD) from 6 animals.

2.8.2. Freund's adjuvant induced arthritis

2.8.2.1. Induction of arthritis

The arthritis was induced in rats by injected with single dose of 0.1 ml of Freund's adjuvant in the left hind paw. The adjuvant contained heat killed Mycobacterium tuberculosis (H37Rv strain, Tuberculosis Research Centre, ICMR, Chennai) in sterile paraffin oil (10 mg/ml). The paw volumes were measured plethysmographically at 2, 4, 6, 8, 10, 12 and 14 days after the injection of Freund's complete adjuvant.

2.8.2.2.Experimental setup

Male albino rats weighing between (150 and 200 g) were divided into nine groups of six rats in each, i.e., negative control (1% CMC, 1 ml/1 kg body weight), toxicant control (23). The standard control was treated with Indomethacin 10mg/kg as a positive control, and six drug treated groups of (ethanolic and aqueous extracts) low, medium, and high. All the groups administered with 2% v/v

formaldehyde except negative control were injected with single dose of 0.1 ml of Freund's adjuvant and were treated with extract for 14 consecutive days. Paw volumes of both paws were measured plethismographically, and body weights are recorded on the 1st and 14th day of injection.

On the days 2, 4, 6, 8, 10 and 14 the volume of injected paw is measured again plethismographically to note the primary lesion and to study the influence of standard and extracts on this phase. The severity of adjuvant induced disease is followed by measurement of non-injected paw (secondary lesions) with a plethysmometer. On the day 15, the animals were sacrificed by overdose of ether, blood was collected in plain and EDTA containing tubes, respectively for plasma/serum separation. The plasma/serum and homogenized samples were subjected to biochemical examination like total protein and albumin, globulin, acute phase proteins- Fibrinogen, Ceruloplasmin. separated serum was subjected to serum analysis of biochemical parameters. For the study of histopathology, the proximal inter phalangeal joints were removed, cleaned with saline and stored in solution of 10% formalin. The obtained inter phalangeal joint sections were stained with eosin-haematoxylin stain and viewed under 100 X magnifications. The X-ray of the arthritis induced hind legs of the experimental rats were taken, and examined for the soft tissue swelling, bony erosions and narrowing of the spaces between joints. The volume of edema was measured at prefixed time interval, i.e., 3, 5, 9, 13, and 14 days. The difference between paw volumes of the treated animals was measured, and the mean edema volume was calculated. Percentage reduction in edema volume was calculated using the formula,

Percentage reduction= (Vc − Vt/Vc) × 100

Vc = Mean volume of paw edema in control Group A.

Vt = Mean volume of paw edema in drug treated group of animals.

2.8.2.3. Arthritis Index

Arthritis index (22) were calculated for formaldehyde and Freund's adjuvant models where rats were observed daily for clinical signs of arthritis, and each paw was scored on a scale of 0-4 (arthritis index) as follows: 0=unaffected, 1=1 type of joint affected, 2=2 types of joints affected, 3=3 types of joints affected, 4=3 types of joints affected and maximal erythema and swelling. The total score for each mice was calculated as an arthritis index.

2.8.2.4. Data analysis

The obtained values in all three models were expressed as mean with SD from 6 animals, subjected to statistical analysis using One-way analysis of variance followed by Dunnett's-t-test to verify significant difference if any among the groups. P<0.05*, 0.01**, and 0.001*** was considered statistically significant.

3. Result

3.1. Preliminary Phytochemical screening

The obtained ELSCV and AQSCV are subjected for preliminary phytochemical screening using standard identification test methods. Both extracts ELSCV and AQSCV found to contain proteins flavonoids fixed oils, glycosides and tannins. Remaining other phytochemical tests were found negative.

3.2. Acute toxicity study

In the present study the ELSCV and AQSCV are subjected to toxicity studies for the LD50 dose determination. Both extracts are administered up to a maximum dose level of 2000 mg/kg body weight and did not produce any mortality. Hence 1/20th (low), 1/10th (medium), 1/5th (high) doses of the maximum dose tested for LD50 are selected for the present study.

3.3. Anti obesity activity (Cafeteria diet induced obesity model in rats)

3.3.1. Effect of ELSCV and AQSCV on body weight

In this model in normal control animals the initial and final body weight is noted as 172.21 g on 1st day and 194.25 g 40th day experimental study model. A significant increase in final body weight with 16.62 % 226.54 g is noted in CD induced obese rats. Sibutramine (5 mg/kg) significantly reduced the final body weight with 11.53 % (200.42 g) in CD induced obese rats. All the three doses of ELSCV reduced the final body weight

low dose by 3.22 % medium dose by 6.21 % and high dose by 7.63 % respectively. All the three doses of AQSCV reduced the final body weight low dose by 4.49 % , medium dose by 6.28 % and high dose by 8.97 % respectively.

3.3.2. Effect of ELSCV and AQSCV on food intake

In normal control animals, the daily food intake is noted as 20.16 g/day. A significant increase in daily food intake is noted in CD induced obese rats as 30.28 g/day. Sibutramine (5 mg/kg) reduced the daily food intake in CD induced obese rats i.e. 17.22 g/day. ELSCV and AQSCV with medium and high doses significantly reduced the daily food intake as 25.24, 22.01 g/day and 23.97, 21.90 g/day respectively. ELSCV and AQSCV with low doses also reduce the daily food intake i.e. 28.88 g/day.

3.3.3. Effect of ELSCV and AQSCV on Biochemical parameters

Both the extracts ELSCV and AQSCV, reduces the serum glucose level in a dose dependent manner i.e. 165.83, 111.12, 104.75 mg/dL and 176.98, 116.86, 114.4 mg/dL respectively. In serum cholesterol level the effect of ELSCV and AQSCV reduce the serum cholesterol with high doses only (120.44 and 118.3 mg/dL). Low and medium doses of the both extracts slightly reduced the serum cholesterol with the value of 153.30 and 132.73 mg/dL and 158.83, 132.6 mg/dL respectively. In serum LDL cholesterol level the effect of ELSCV and AQSCV reduce the serum LDL cholesterol level at high doses only noted as 58.24 and 62.78 mg/dL respectively. Low and medium doses of both extracts reduced serum LDL cholesterol level to non significant extent as 105.26, 79.58 mg/dL and 110.01, 78.86 mg/dL respectively. The effect of both extracts in other biochemical parameters such as, cholesterol, triglyceride HDL and VLDL, has been cited in the Table 2. The above mentioned all parameters of lipid profile were shown in Figure 2.

3.3.4. Effect of ELSCV and AQSCV on Organ Weights (g/100 g)

The effect of both Extracts (ELSCV and AQSCV) on liver was significantly reduced the liver weight with medium and high doses recorded as 4.22, 3.96 g and 4.17 g, 4.02 g. Effect of both the extracts on heart significantly reduced the heart weight in a dose dependent manner i.e. 0.75, 0.65, 0.55 g and 0.75, 0.65, 0.54 g respectively. ELSCV and AQSCV significantly reduces the weight of other studied organs such as kidney, spleen, uterus fat pads. The details of the weight values has been included in the Table 1.

3.4. Anti obesity activity (Atherogenic diet induced obesity model in rats)

3.4.1.Effect of ELSCV and AQSCV on body weights

In normal control animals the initial and final body weight is noted as 172.21 g on 1 st day and 194.25 g on 40th days model. An increase in final body weight with 20.84 % (234.75 g) is noted in AD induced obese rats. In Sibutramine (5 mg/kg) significantly reduced the final body weight by 14.62 % (200.42 g) in AD induced obese rats. All three doses of ELSCV reduced the final body weight low dose by 9.03 % (213.54 g), medium

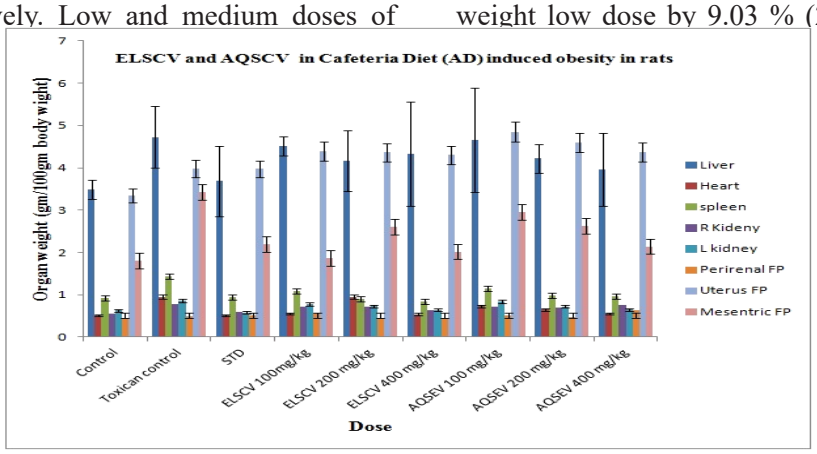

Figure 2. Effect of ELSCV and AQSCV on the lipid profile in Cafeteria diet induced obesity.

Table 2. Anti obesity effect of ELSCV and AQSCV on in Atherogenic Diet (AD) induced obesity in rats.

Groups	Dose	Serum biochemical parameters (mg/dL)						Organ and Fat pad weights (g/100 g)							
										Kidney					
		GLU	CHO	TRG	HDL	LDL	VLDL	Liver	Heart	Left	Right	Spleen	Perirenal FP	Uterus FP	Mesent -ric FP
Normal Pellet diet	-	103.35 ±4.4	74.12 ±8. 0	94.25 ±6.9	20.19 ±1.3	18.85 ±1.3	35.05 ±8.8	3.29 ±0.09	0.42 ±0.01	0.63 ±0.010	0.52 ±0.013	0.72 ±0.014	0.35 ±0.015	3.34 ±0.10	2.71 ±0.03
CD Control	-	299.35 ±3.41**a	232.44 ±9.76 **a	212.50 ±5.2 **a	12.6 ±2.4 **a	42.46 ±1.02 **a	224.29 ±22.38**a	4.82 ±0.052**a	0.85 ±0.09**a	0.86 ±0.01**a	0.67 ±0.01**a	1.23 ±0.07**a	0.59 ±0.014**a	5.38 ±0.02**a	3.03 ±0.06**a
Standard	SBT5 mg/kg	125.90 ±1.41 **b	175.80 ±2.12 **b	139.70 ±1.04 **b	38.68 ±0.49**b	27.94 ±0.20*b	109.46 ±1.89 **b	3.69 ±0.06**b	0.51 ±0.03**b	0.58 ± 0.02**b	0.80 ±0.01**b	0.75 ± 0.01**b	0.45 ±0.009**b	3.97 ±0.12**b	2.19 ±0.08**b
ELSCV	100 mg/kg	258.03 ±6.41 **b	213.18 ±2.83 *b	205.87 ±3.74 ns	17.86 ±0.36**b	41.17 ±0.30 ns	154.01 ±6.54 **b	4.51 ±0.10ns	0.75 ±0.015**b	0.78 ±0.0**b	0.72 ±0.015ns	1.29 ±0.11 ns	0.54 ±0.019*b	4.94 ±0.06**b	2.96 ±0.06**b
ELSCV	200 mg/kg	186.87 ±1.56 **b	207.63 ±2.63**b	177.27 ±1.21 **b	23.2 ±1.17**b	35.45 ±0.24**b	148.98 ±3.28 **b	4.87 ±0.10**b	0.65 ±0.010**b	0.70 ±0.04**b	0.70 ±0.01**b	0.90 ±0.01**b	0.46 ±0.007**b	4.46 ±0.07**b	2.31 ±0.07**b
ELSCV	400 mg/kg	139.73 ±1.34**b	185.77 ±1.17 **b	140.02 ±5.97 **b	28.43 ±1.71**b	27.99 ±1.19 **b	128.51 ±2.44 **b	4.45 ±0.13**b	0.47 ±0.011**b	0.64 ±0.01**b	0.63 ±0.07**b	0.64 ±0.01**b	0.44 ±0.011 **b	4.30 ±0.05**b	2.40 ±0.11**b
AQSCV	100 mg/kg	259.47 ±4.06 **b	219.07 ±1.967 ns	209.75 ±2.23 *b	15.13 ±0.64 ns	41.91 ±0.45 ns	158.98 ±3.70 **b	4.46 ±0.09ns	0.75 ±0.02**b	0.84 ±0.09 ns	0.72 ±0.007ns	1.05 ±0.09 ns	0.53 ±0.017**b	4.85 ±0.05**b	2.91 ±0.08**b
AQSCV	200 mg/kg	217.58 ±4.89**b	205.47 ±3.34**b	198.13 ±1.16 **b	24.80 ±0.77**b	39.62 ±0.21 *b	141.04 ±2.97 **b	4.22 ±0.08**b	0.65 ±0.01 **b	0.77 ±0.01**b	0.70 ±0.05**b	0.98 ±0.05**b	0.47 ±0.005 **b	4.60 ±0.03**b	2.60 ±0.06**b
AQSCV	400 mg/kg	142.53 ±6.70**b	181.50 ±3.46 **b	166.57 ±1.71**b	28.5 ±1.09**b	33.20 ±0.34**b	104.26 ±1.33 **b	3.36 ±0.17**b	0.70 ±0.01**b	0.65 ±01**b	0.65 ±0.01**b	0.86 ±0.07**b	0.44 ±0.009**b	4.27 ±0.05**b	2.64 ±0.05**b

n = 6, Significant at P < 0.05*, 0.01** and 0.001***, ns = not significant. a – compare to normal control, b – compare to AD control , ELSCV – ethanolic extract of seeds of C. Vulgaris, AQSCV- Aqueous extract of seeds of C. Vulgaris . SBT- Sibutramine p

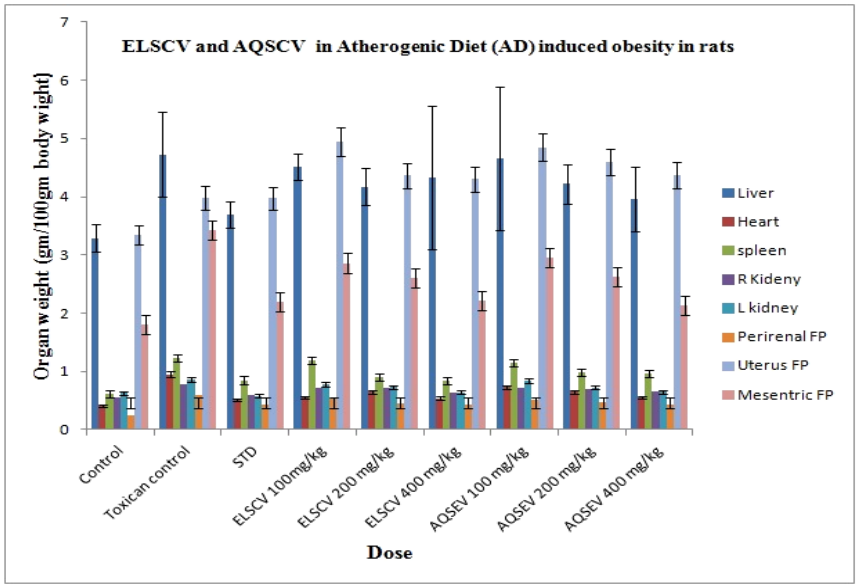

Figure 3. Effect of ELSCV and AQSCV on the lipid profile in Atherogenic diet induced obesity.

by 11.19 % (208.46 g) (P<0.01) and high dose by 12.04 % (185.3 g) respectively. Similarly all three doses of AQSCV reduced the final body weight has shown in Table 2.

3.4.2. Effect of ELSCV and AQSCV on food intake

In normal control animals the daily food intake is noted as 20.16 g/day. A significant increase in daily food intake is noted in CD induced obese rats as 30.37 g/day. Sibutramine (5 mg/kg) significantly reduced the daily food intake in CD induced obese rats i.e. 17.22 g/day. ELSCV with medium and high doses significantly reduced the daily food intake as 25.24, 22.03 g/day. AQSCV also significantly reduce the daily food intake in with medium and high doses i.e. 24.97, 21.94 and 24.09, 22.64 g/day respectively.

3.4.3.Effect of ELSCV and AQSCV on Biochemical parameters

In serum glucose level the effect of both extracts (ELSCV and AQSCV) reduced the serum glucose level in dose a dependent manner i.e. 258.03, 186.87, 139.73 mg/dL and 259.47, 217.58, 142.53 mg/dL respectively. The serum cholesterol level also reduced in dose dependent manner i.e. 213.18, 207.63, 185.77 mg/dL and 219.07, 205.47, 181.50 mg/dL respectively. The effect of both extracts in other biochemical parameters such as, cholesterol, triglyceride, HDL and VLDL, has

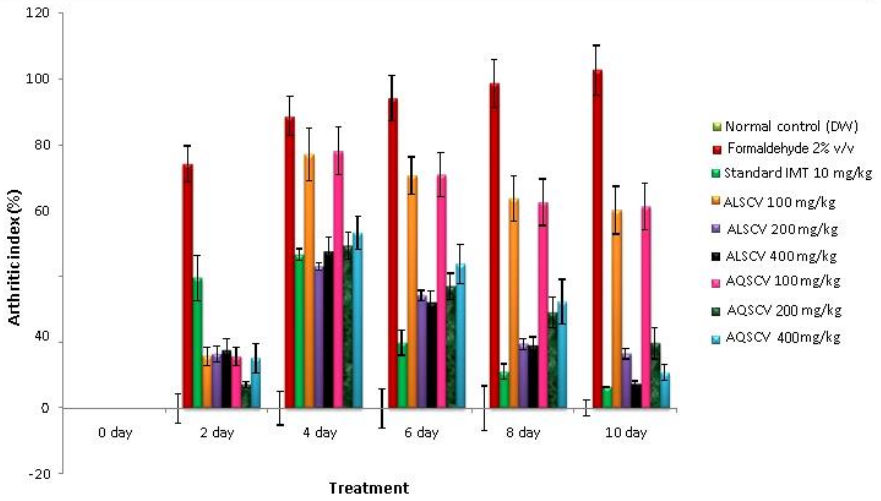

Figure 4. Effect of ELSCV and AQSCV on Formaldehyde induced Arthritis.

Table 3. Anti arthritic effect of ELSCV and AQSCV in formaldehyde induced arthritis in rats

Groups	Dose	(%) Arthritic index (mean±SEM)					
		0 day	2nd day	4th day	6th day	8th day	10th day
ormal	Distilled water	00	00	00	00	00	00
Toxicant	Formaldehyde 2% v/v	00	74.23 ±5.5	88.87 ±5.9	94.35 ±6.9	98.74 ±7.2	102.91 ±7.5
Standard	Indomethacin 10 mg/kg	00	39.65 ±6.9 **	46.77 ±1.8 **	19.99 ±3.9 **	11.14 ±2.4 **	6.54 ±0.17 **
ELSCV	100 mg/kg	00	15.73 ±2.8 **	77.24 ±8.0 ns	70.80 ±5.6 *	63.73 ±6.8 **	60.22 ±7.3 **
ELSCV	200 mg/kg	00	16.51 ±2.5**	43.12 ±0.98**	34.29 ±1.6 **	19.51 ±1.6 **	16.62 ± 1.6 **
ELSCV	400 mg/kg	00	17.62 ±3.6 **	47.63 ±4.5**	32.17 ±3.4 **	19.01 ±2.7 **	7.45 ±0.8 **
AQSCV	100 mg/kg	00	15.73 ±2.8 **	78.27 ±7.3 ns	70.99 ±6.7 *	62.60 ±7.1 **	61.41 ±7.1 **
AQSCV	200 mg/kg	00	7.18 ±1.0 **	49.43 ± 4.0**	37.10 ±3.9**	29.11 ±4.7**	19.82 ±4.8 **
AQSCV	400 mg/kg	00	15.20 ±4.5 **	55.373 ±5.1**	43.88 ±5.9 **	32.40 ±6.8**	10.8 ±2.4 **

n=6, Significant at P<0.05*, 0.01** and 0.001***, ns=not significant

ELSCV – Ethanolic extract of seeds of C. vulgaris, AQSCV- Aqueous extract of seeds of C. vulgaris.

been cited in the Table 2. The above mentioned all parameters of lipid profile were shown in Figure 3.

3.4.4. Effect of ELSCV and AQSCV on Organ Weights (g/100 g)

Both the extracts has shown its effect on liver weight and has been reduced to 4.17, 4.02 g and 4.22, 3.96 g. Heart weight (g/100 g) is also reduced in dose dependent manner i.e. 0.75, 0.65, 0.54 g and 0.75, 0.65, 0.55 g respectively. ELSCV and AQSCV significantly reduces the weight of other studied organs such as kidney, spleen, uterus fat pads. The details of the weight values has been included in the Table 2.

3.5. Anti arthritic activity (Formaldehyde induced arthritis)

3.5.1. Effect of ELSCV and AQSCV on Arthritic index (%)

In normal control (negative control) rats the arthritic index of this method was noted on day 2, 4, 6, 8 and 10 days as 74.23, 88.87, 94.35, 98.74 and 102.91% respectively. Indomethacin (10mg/kg) treatment has reduced the Arthritic index noted as 39.65, 46.77, 19.99, 11.14 & 6.54 % respectively with the above mentioned days in Formaldehyde induced arthritis rats. All three doses of ELSCV reduced the Arthritic index with low dose as 15.33 , 77.24, 70.80, 63.73, 60.22 %, medium dose as 16.51, 43.12, 34.29, 19.51, 19.51 and 16.62% and high dose as 17.62, 47.63, 32.17, 19.01 and 7.45% respectively with the above mentioned days. All three doses of AQSCV reduced the Arthritic index with low dose as 15.73, 78.27, 70.99 , 62.60 , 61.41% , medium dose as 7.18, 49.43, 37.10, 29.11 and 19.82% and high dose as 15.20, 55.37, 43.88, 32.40 and 10.8% respectively with the above mentioned days. The details of the results were shown in Table 3 and Figure 4.

3.6. Anti arthritic activity (Freund's Adjuvant induced arthritis)

3.6.1. Effect of ELSCV and AQSCV on Arthritic index (%)

Arthritic index of this method was noted on day 2, 4, 6, 8, 10, 12 and 14 day as 75.19, 98.68,

Table 4. Anti arthritic effect of ALSCV and AQSCV in Freund's adjuvant (FA) induced arthritis in rats.

Groups	Dose	Serum biochemical parameters (mean ±SEM)							(%) Arthritic index (mean ±SEM)						
		BUN (mg/dL)	CAL (mg/dL)	PRO (g/dL)	ALB (g/dL)	ALP (U/dL)	SGPT (U/dL)	SGOT (U/dL)	2nd day	4th day	6th day	8th day	10th day	12th day	14th day
Normal	Distilled-water	10.13 ±0.25	11.09 ± 0.52	10.16 ±0.29	9.66 ±0.49	610.13 ±28.02	11.84 ±0.49	16.93 ±1.44	00	00	00	00	00	00	00
CD Control	-	299.35 ±3.41**a	232.44 ±9.76 **a	212.50 ±5.2 **a	12.6 ±2.4 **a	42.46 ±1.02 **a		224.29 ±22.38**a	4.82 ±0.052**a	0.85 ±0.09**a	0.86 ±0.01**a	0.67 ±0.01**a	1.23 ±0.07**a	0.59 ±0.014**a	5.38 ±0.02**a
Toxicant	FA 0.5 w/v	25.89 ± 0.90**a	30.20 ±0.78**a	23.71 ±0.8**a	3.36 ± 0.22**a	765.74 ±6.73 **a	39.97 ±1.6**a	47.07 ±1.11 **a	75.19 ±5.41	98.68 ±17.7	104.9 ±8.07	107.25 ±7.30	107.25 ±7.30	107.25 ±7.30	107.25 ±7.30
Standard	IMT10 mg/kg	13.17 ± 0.35**b	16.6 ±0.38**b	14.56 ±0.2**b	11.18 ± 0.60**b	651.62 ±26.79 **b	15.87 ±1.1**b	21.12 ±0.85 **b	16.92 ±4.9**	76.5 ±17.1ns	28.52 ±6.43 **	23.51 ±14.0**	14.49 ±3.36 **	9.13 ±1.15 **	5.27 ±1.6 **
ELSCV	100 mg/kg	22.84 ± 0.28**b	27.69 ±0.31 **b	22.63 ±0.63ns	7.17 ± 0.5 *b	750.46 ±26.65 ns	28.68 ±2.03**b	30.71 ±0.60 **b	22.8 ±3.9 **	95.78 ±10.0ns	96.73 ±8.16ns	89.61 ±9.05ns	87.11 ±8.29ns	81.87 ±6.32 **	78.54 ±7.45**
ELSCV	200 mg/kg	19.89 ±0.4 **b	25.76 ±0.35 **b	20.98 ± 0.4ns	9.51 ±1.26**b	712.51 ±18.60 ns	23.39 ±0.5**b	25.04 ±0.73 **b	21.02 3.2**	78.7 ±12.6ns	70.81 ±6.57 **	63.88 ±16.2**	51.97 ±5.94 **	29.83 ±4.7 **	17.95 ±3.56**
ELSCV	400 mg/kg	17.36 ±0.27**b	22.82 ±0.43**b	17.51 ±0.4 **b	10.47 ±0.70 **b	681.40 ±14.55 *b	17.17 ±1.6**b	21.85 ±0.53 **b	29.64 ±4.8**	79.17 ±0.17ns	71.19 ±20.2 **	61.33 ±7.33**	31.85 ±3.97 **	17.38 ±3.59**	7.59 ±2.16**
AQSCV	100 mg/kg	23.86 ±0.41*b	27.22 ±0.71**b	23.63 ± 0.8 ns	7.65 ±1.00**b	751.25 ± 25.12 ns	25.66 ±2.2**b	31.63 ±0.73 **b	15.7 ±7.02 **	94.7 ±4.83ns	94.6 ±4.56**	86.6 ±5.6ns	82.7 ±5.38 *	79.04 ±4.86**	75.79 ±6.9 **
AQSCV	200 mg/kg	17.74 ±0.1 **b	25.86 ±0.41 **b	22.80 ±0.72 ns	9.18 ±1.29 **b	716.37 ±16.17 ns	20.60 ±1.0**b	24.72 ±1.07 **b	12.2 ±3.97 **	69.08 ±6.13ns	60.14 ±3.97 **	51.64 ±5.72**	44.03 ±4.86 **	31.96 ±2.81**	15.32 ±3.0 **
AQSCV	400 mg/kg	15.69 ±0.33**b	22.12 ±0.56 **b	18.50 ±1.3**b	11.89 ±0.34*b	678.08 ±13.43*b	16.65 ±1.29**b	22.69 ±0.95**b	18.98 ±4.08 **	65.92 ±5.07**	58.32 ±5.37 **	48.57 ±6.31**	27.28 ±3.07 **	13.25 ±1.99**	6.55 ±0.21**

n=6, Significant at P<0.05*, 0.01** and 0.001***, ns = not significant. a – compare to normal, b – compare to Adjuvant ELSCV – Ethanolic extract of seeds of *C.vulgaris*, AQSCV- Aqueous extract of seeds of C.vulgaris.

Table 5. X ray observation of the effect of ELSCV and AQSCV on rat paw

Groups	Dose	X- Ray
Normal	Distilled water	No soft tissue swelling with normal joint spaces
Toxicant	FA 5 % w/v	Soft tissue swelling and bone destruction
Standard	Indomethacin 10 mg/kg	Prevented bone destruction and no soft tissue swelling
ELSCV	100 mg/kg	Soft tissue swelling and bone destruction
ELSCV	200 mg/kg	Less bone destruction and slightly soft tissue swelling
ELSCV	400 mg/kg	Prevented bone destruction and no soft tissue swelling
AQSCV	100 mg/kg	Soft tissue swelling and bone destruction
AQSCV	200 mg/kg	Less bone destruction and slightly soft tissue swelling
AQSCV	400 mg/kg	Prevented bone destruction

104.95, 107.25, 107.25, 107.25 and 107.25 % respectively. Indomethacin (10mg/kg) treatment is significantly reduced the Arthritic index treatment as 16.92, 76.53, 28.52, 23.51, 14.49, 9.13 and 5.27 % respectively with the above mentioned days in Freunds Adjuvant induced arthritic rats. All three doses of ELSCV reduced the Arthritic index with low dose as 15.7, 94.7, 94.6, 86.6, 79.04, 75.79 %, medium dose as 21.02, 78.7, 70.81, 63.88, 51.97, 23.83 and 17.95% and high dose as 29.64, 79.17, 71.19, 61.33, 31.85, 17.38 and 7.59 % respectively with the above mentioned days. All three doses of AQSCV reduced the Arthritic index with low dose as 22.85, 95.78, 96.73, 87.11, 81.87, 78.54 %, medium as 12.2, 69.08, 60.14, 51.64, 44.03, 31.96 and 15.3 and high dose as 18.98, 65.62, 58.32, 48.57, 27.28, 13.25 and 6.55 % respectively with the above mentioned days. The details of the results were summarized in Table 4 and Figure 5.

3.6.2. Effect of ELSCV and AQSCV on Biochemical parameters

Effect of both the extracts on serum blood urea nitrogen level was significantly reduced the serum BUN level in dose a dependent. Effect of both the extracts on serum calcium level has significantly reduced the serum calcium level in dose a dependent manner as shown in (Table.4). Effect of both the extracts on serum total protein level has significantly reduced the serum total protein level at higher doses as 17.51 and 18.50 g/dL. Low and medium doses of ELSCV and AQSCV also reduced the serum total protein level (Table.4). Effect of both the extracts on serum albumin level has significantly increased the serum albumin level in dose a dependent manner. (Table.4). Effect of both the extracts on serum alkaline phosphate level significantly reduced the serum ALP as 681.40 and 678.08 U/dL. Low and medium doses of ELSCV and AQSCV also reduced the serum

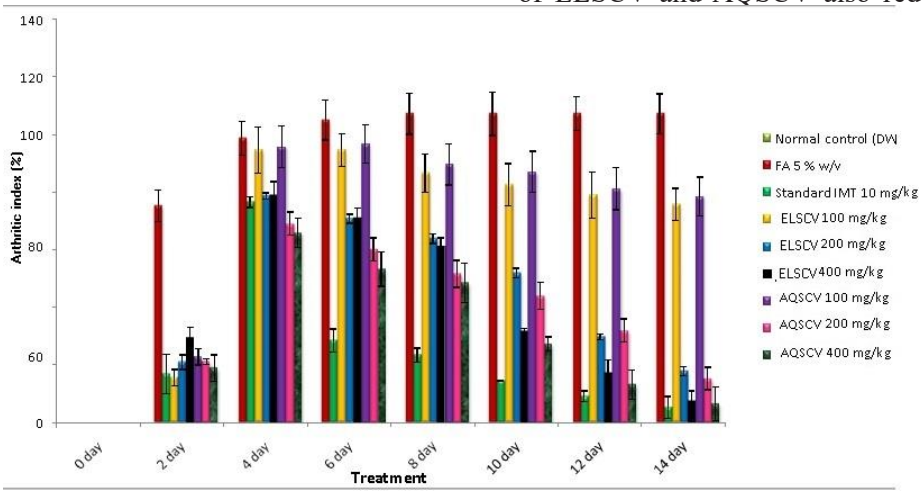

Figure 5. Effect of ELSCV and AQSCV on Freund's Adjuvant induced Arthritis.

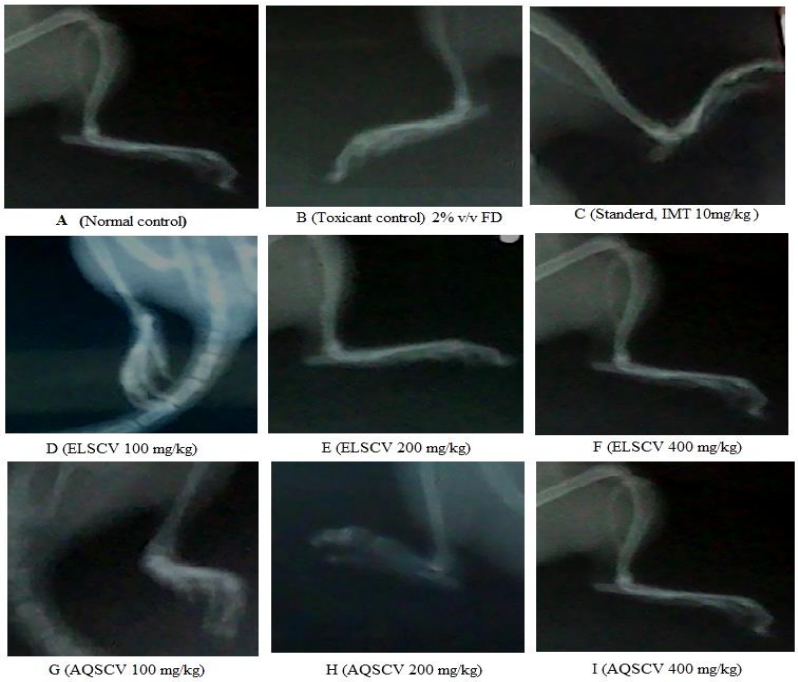

Figure 6. Effect of ELSCV and AQSCV on rat paw by radiological study.

ALP level to the non significant extent. Effect of both the extracts on serum SGPT level has significantly reduced the serum SGPT level in dose a dependent manner. Effect of both the extracts on serum SGOT level has significantly reduced the serum SGOT level in dose a dependent manner as shown in Table 4.

3.6.3. Effect of ELSCV and AQSCV on rat paw X-ray

Radiological studies on paws in FA induced arthritis model in rats

In normal control group no swelling of soft tissue and normal joint spaces is observed. Treatment with FA, swelling in soft tissue with bone destruction is noted. Standard drug Indomethacin prevented swelling in soft tissue and also bone destruction. ELSCV and AQSCV with three different doses exhibited soft tissue swelling and bone destruction seen with low doses of both the extracts. The inflammation reduced with medium dose and no swelling in soft tissue or bone destruction seen with the high doses of both extracts. The results are shown in Table 5 and Figure 6.

3.6.4.Histopathological studies on the knee joints in FA induced arthritis in rats

In control animals knee joint shows with normal articular cartilage without any inflammation. FA induced arthritic rat's shows with the mild periarticular and perivascular inflammation with edema. Indomethacin treated group (10 mg/kg) shows with normal articular cartilage without any inflammation. Both extract with high doses only shows with normal articular cartilage without any inflammation. The results are shown diagrammatically in Figure 7.

4. Discussion

TPreliminary phytochemical screening reveals the presence of proteins, flavonoids, fixed oils, glycosides and tanins in both aqueous and ethanolic extracts. The acute toxicity study results reveals the safety of Both extracts are up to a maximum dose level of 2000 mg/kg body weight and did not produce any mortality, therefore 1/20th, 1/10th and 1/5th doses were prepared and selected for the present study.

Both extracts (ELSCV and AQSCV) has significantly reduced the physical parameters like body weight, food intake, organ and fat pads weight in cafeteria induced diet and atherogenic diet induced obesity models in rats. The increased consumption of food intake with high calories is due to lack of

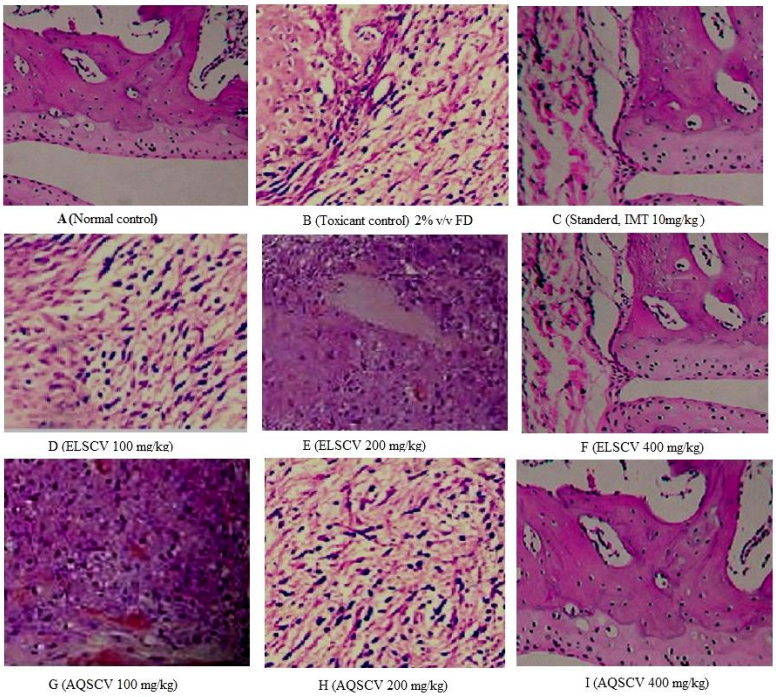

Figure 7. Effect of ELSCV and AQSCV on histopathology of knee joints.

physical activities, reduced frequency of ambulation, rearing and grooming when compared to normal rats fed with balanced diet. Treatment with ELSCV and AQSCV has significantly increased ambulation, rearing and grooming which is important for the maintenance of physical activity. In the present study, cafeteria and atherogenic diet induced obesity study in rats has showed increased blood glucose levels. Treatment with ELSCV and AQSCV has significantly reduced the levels of glucose. Animals treated with ELSCV and AQSCV in both the models has significantly showed the decreased levels of cholesterol, triglycerides, LDL and VLDL cholesterol and increased levels of HDL cholesterol. Decrease in serum cholesterol and LDL level is due to reduced intestinal absorption of cholesterol. The decreased VLDL levels in treated groups may be directly correlated to a decrease in triglyceride levels as it is well known that VLDL particles are the main transporters of triglycerides in plasma. The reduction in triglycerides level is due to an increased endothelium activity and hydrolyzes the triglyceride into fatty. In our present study, rats fed with ELSCV and AQSCV were found to significantly increase serum HDL levels suggesting its cardioprotective nature and may be due to an increased activity of lecithin cholesterol acyl transferase.

In our present study ELSCV and AQSCV exhibited a significant anti-arthritic activity in a dose dependent manner and inhibited the progression of the rheumatoid arthritis satisfactorily in treated animals. Both the extracts significantly suppressed the swelling of the paws in both acute and chronic phases and it is due to the suppression of inflammatory mediator released due to induction of Freund's adjuvant. Assessment of the levels of BUN, CAL, ALP, PRO, SGOT, SGPT and albumin provides an excellent and simple tool to measure the anti-arthritic activity of the target drug. The activities of these enzymes were significantly increased in arthritic rats. These are good indicators of liver and kidney impairment, which are also considered to be features of adjuvant arthritis. ELSCV and AQSCV had significantly reduced the (%) Arthritic index in Formaldehyde and Freund's adjuvant induced arthritis in rats. A significant improvement in biochemical parameters was observed which are the indications of antiarthritic action. Both extracts had significantly reduced the all serum biochemical parameters except the ALB level, which is increased in Freund's adjuvant induced arthritis in rats.

The histopathological study reveals that

the FA induced arthritic rats showed mild periarticular and perivascular inflammation with edema. Both extract with high doses showed the normal articular cartilage without any inflammation similar to the control group.

Radiographic changes in RA conditions are useful diagnostic measures which indicate the severity of the disease. Soft tissue swelling is the earlier radiographic sign, whereas prominent radiographic changes like bony erosions and narrowing of joint spaces can be observed only in the developed stages (final stages) of arthritis (24). The radiographic features of the rat joints in adjuvant induced arthritic model are shown in figure 6.

5. Conclusion

The anti obesity activity and anti arthritic activity of both the extracts were evaluated in CD, AD induced obesity in rats and Formaldehyde, FA induced arthritis models respectively in rats. From the experimental findings it has been concluded that both the extracts showed anti obesity and anti arthritic activities due to presence of flavonoids, saponins, fixed oils, glycosides and alkaloids which were already reported for their anti obesity and anti arthritic activities. The drug is a promising anti obesity and anti arthritic agent of plant origin in the treatment of obesity and inflammatory disorders.

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Conflict of Interest

None declared.

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