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Antihypertensive Activity of *Ylang Ylang* Essential Oil on Fructose Induced Hypertensive Rats

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Shreya Kanolkar[1], Sanjay Ugare[1*], V.P. Rasal[1]

[1]Department of Pharmacology, KLE College of Pharmacy, KLE Academy of Higher Education and Research, Belagavi-590010, Karnataka, India

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ABSTRACT

Objective: To evaluate the effect of antihypertensive activity of *Ylang-Ylang* essential oil in Fructose induced hypertensive rats. **Methodology:** Sprague Dawley rats were divided into 5 groups (n=6). Hypertension was induced by administration of 10% fructose in drinking water for 8 weeks. Group I (normal control) received normal feed and water. Group II (disease control) received 10% fructose in drinking water. Group III, IV and V were groups treated with 200mg/kg, 400mg/kg and 800mg/kg *Ylang-Ylang* (YYEO) essential oil respectively. Food and water intake of rats were measured daily. SBP, Heart rate, ECG and body weight of rats were measured weekly. At the end of the study period, animals were sacrificed and blood was collected. Serum was used for estimation of various biochemical parameters i.e. Complete Lipid profile, LDH, Troponin-I, uric acid. Levels of antioxidant enzymes (SOD, LPO and GSH) in heart were also estimated. Heart, Aorta and Liver dissected out and its histopathology were studied. **Result:** The acute toxicity was found to be safe at 2000mg/kg for oral administration and 200, 400 & 800mg/kg dose were selected as per LD50, for treatment. YYEO treatment significantly reduced the effects of fructose in parameters assessed such as; SBP, ECG, Heart rate and lipid variables such as TG, TC, LDL, VLDL and increasing HDL levels. It also significantly decreased the levels of SOD, GSH and MDA along with biomarkers of hypertension which showed significant dose dependent reduction in serum LDH and Uric Acid. **Conclusion:** From the above study it can be concluded *Ylang-Ylang* essential oil possesses significant antihypertensive activity.

INTRODUCTION

Cardiovascular disease is commonly known to lead reason of mortality, disability and is the worldwide biggest killer, stating 15.2 million lives a year. Hypertension is the reason for 58% of overall stroke and 25% of coronary heart illness deaths.[1] Hypertension is defined "as the persistent elevation of arterial blood force due to which there is a sustained rise in systolic or diastolic blood pressure, greater than 140/90mmHg respectively."[2-3] Hypertension is a major cause for an array of cardiovascular and other linked diseases including renal disease, peripheral arterial disease, and heart failure.[4] An untreated raised blood pressure can damage essential organs, for example, the heart, kidneys, and veins. Early monitored and controlled hypertension is crucial since the beginning of hypertension is only occasionally connected with any indications. Additionally, the prior the treatment begins the better the clinical outcomes for the patient.[5]

Some essential oils obtained from the plant used in the treatment of the Hypertension such as *Nigella Sativa Oil*[6], *fenugreek essential oil*[7], *Alpineazerumbe*[8] , Carvacrol, Eugenol, Beta-Caryophyllene, 1,8-Cineole, Thymol which are also constituents of *Cananga Odorata* essential oil have shown vasorelaxant effects in rat isolated aorta.[9-10] Recently researcher demonstrated several uses of ylang-ylang essential oil for treating various ailments. *Cananga odorata* flowers (ylang-ylang) belonging to the family Annonaceae is a wild growing tree native to tropical Asian regions and islands of India, China, Indonesia. India have introduced the production of whole *C. odorata* plants.[11] Chemical constituents of *C. odorata* extract includes 1,8-Cineole, Geraniol, Eugenol, Isoeugenol, Bisabolol, Linalool, B-Caryophyllene, etc.

Ylang Ylang essential oil has been used for treating various ailments since ancient times. Several studies have proved the efficacy and safety of essential oil. C. odorata is extracted using a water-steam distillation of the *C. odorata* flowers. *Ylang-ylang* oil has various applications in cosmetics, like massage oils, creams, perfume fragrance, and even scent candles. Recently, it contributes to the field of aromatherapy believing to have medicinal properties. Traditionally, the *Cananga Odorata* flowers were used to treat malaria, like the scent, asthma. Pneumonia and stomach ache, also to treat depression, nervousness and blood pressure lowering effect. [12-13]

As per the literature survey, many traditional uses of *ylang-ylang* essential oil are yet to be

justified scientifically for their rational and safe use. Hence, the current study is designed to evaluate the antihypertensive effect of *ylang-ylang* essential oil in fructose-induced hypertensive rats.

MATERIALS AND METHODS

Materials used in the study:

The *Ylang-ylang* essential oil was collected from Allin Exporters, B-75, sector-6, NOIDA, UP – 201301, India.

Dose selection:[14]

Ylang-Ylang essential oil at 3 dissimilar doses (200, 400 and 800 mg/kg) were administered orally for 21 days. As the drug is oil, a dose of the drug (mg) was converted into ml according to the specific gravity of *Ylang-Ylang* essential oil.

Acute Toxicity Test:[13-14]

The oil in water emulsion of *Ylang-Ylang* essential oil was prepared and the acute toxicity studies following (OECD) Guideline 423 -Acute Toxic Class Method. Rats weighing 150-200g were used for toxicity study. As per LD50 value. $1/10^{th}$, $1/20^{th}$ and $1/40^{th}$ dose was administered orally.

STUDY DESIGN:

Healthy Sprague Dawley Rats of either sex weighing from 180-200 gm were procured from *in vivo* Biosciences, Bangalore. reviewed and approved the study by the Institutional Animal Ethics Committee (IAEC), resolution number, KLECOP-CPCSEA Reg.no-221 Po/Re/ S/2000/CPCSEA, Res.25/13/2018.at KLE College of Pharmacy, Belagavi.

Either sex Sprague Dawley rats (180-210 g) will be selected and divided into following '5' groups comprising of 6 animals in each group and received the following treatment.

Sprague Dawley rats induced with hypertension by the administration of 10% fructose in drinking water for 8 weeks. Drinking water was substituted with 10% of fructose water solution. *Ylang-ylang* essential oil (200, 400 and 800 mg/ml) in emulsion form was administered orally to

rats of group III, IV, and V to evaluate the anti-hypertensive activity of the formulation.

Sr. No.	Groups(n=6)	Treatment
1	Group 1	Normal
2	Group 2	Normal Diet + fructose in drinking water for 8weeks
3	Group 3	Normal Diet + 10% fructose solution + YYEO (200mg/kg) for 21 days.
4	Group 4	Normal Diet + 10% fructose solution + YYEO (400mg/kg) for 21 days.
5	Group 5	Normal Diet + 10% fructose solution + YYEO (800mg/kg) for 21 days.

Collection of Heart tissue and Estimation of Antioxidant enzymes

Preparation of Heart Homogenate: Animals were sacrificed by overdose of anesthesia. On dissection, the hearts and liver were isolated and washed immediately using cold saline to render them free from blood clots. Heart homogenates were prepared in a cold phosphate buffer in the ratio 1:4 using homogenizer. Heart tissue sample was collected by the method of Khatib *et al.*[23] MDA, GSH, SOD, LDH, and Troponin-I were estimated by the method of Ohkawa *et al* [24], Ellaman[25], Anuradha *et al.*[24] Iwase *et al*.[25] Harsh Mohan[26] and Kumar V *et al*. respectively.

Lipid Parameters Estimations:

Blood serum prepared initially were used for estimating different lipid parameters such as TG, TC, HDL (LDL, VLDL was calculated).[27,28,29]

Histopathology:

At the termination of the treatment period, all treated animals were sacrificed, their heart, aorta, and liver were dissected out and fixed overnight in 10% formalin. Sections of the tissues fixed in paraffin were prepared, stained with eosin, hematoxylin and observed for pathological changes. Light microscope was used for observing pathological changes in the sections prepared.

***In silico* Docking Analysis**

Numerous bioactive constituents were screened and three major potent compounds were selected based on Drug likeness scores and their parameters such as ADME and toxicity were predicted. The quality of proteins selected was analyzed by Ramachandran plot. Further, the binding energies and their interactions with the selected “Target" was studied.

Statistical analysis

Results were expressed as Mean ± SEM. The differences among data Mean were determined via one-way/two-way ANOVA followed by Post –hoc tests as required (Graph pad-prism Software version 5.01). $p < 0.05$ was reflected statistically significant. All *In-silico* data are expressed in qualitative form.

RESULTS

Pharmacological investigation

***In vitro* antioxidant assay:**

DPPH assay- IC50 of ascorbic acid and YYEO was found to be 97.49µg/ml and 138.24µg/ml respectively. The results suggest that the radical scavenging activity of ascorbic acid is more as compared to *Ylang-Ylang* essential oil (Table: 1).

In vivo studies*:*

Effect of *Ylang-Ylang* essential oil on body weight (BW):

All groups started the study with comparable body weights (Group N- 185.5±12.02, GroupF-183.66±13.67, Group3-217.5±26.10, Group4-188.16±25.33, and Group5-192.5±25.14). The changes in BW of animals in different groups were compared at the end of the study. All animals were weighed once in a week. There was significant increase in BW in fructose induced hypertensive rats when compared to Group-N (287.5±57.87). Treatment of hypertensive rats with *Ylang-Ylang* essential oil showed significant dose dependent decrease in BW respectively when compared to Group- Fructose induced.

Changes in water, and food ingestion:

The changes in the water intake of modified water (10% fructose in water) in rats have been compared at the end of study. 10% fructose water was fed to rats and water intake was recorded. Initially, in week-1 there was non-significant change observed in water intake when compared to normal water. Gradual significant increase ($p<0.001$) in water intake was found by week-2 when compared to normal water fed group. *YYEO* 200mg/kg shown significant ($p<0.001$) reduction in water intake, while more significant ($p<0.001$) reduction in water intake was shown by *Ylang-Ylang* essential oil (400 and 800 mg/kg) respectively when compared with Fructose induced. YYEO 800mg/kg treated group showed significant ($p<0.01$) decreases in water intake (139.6±0.80) as compared to (200 mg/kg) YYEO treated groups (144.3±0.99) respectively. (Table: 2)

The effect of modified water (10% fructose in water) on food intake has been compared at the end of study. Significant ($p<0.001$) decrease in food intake of Group- F when compared to Group-N. *YYEO* 200 mg/kg showed significant increase in food intake, while YYEO 400 and 800 mg/kg showed significant ($p<0.01$, $p<0.001$) increase in Food intake when compared with group F. (Table: 3)

Effect on organs weight (heart and liver weight):

Fructose induced hypertensive group showed non-significant increase in heart weight with mean value of (0.974±0.02) and highly significant increase in liver weight (7.693±0.544) when compared to normal group (heart 0.894 ±0.01, liver 5.968±0.190) respectively. There was insignificant reduction in liver weight was shown in YYEO 200 mg/kg (7.305±0.331), while more significant ($p<0.05$) reduction in liver weight was shown by Ylang-Ylang essential oil (400 and 800 mg/kg) (6.282±0.229 and 6.199±0.257 respectively) and non-significant decrease in heart weight (0.855±0.03, 0.939±0.05, 0.853±0.05) was shown in (YYEO 200, 400 and 800 mg/kg) respectively when compared with group F. (Table: 4)

Effect of Ylang-ylang *on Heart Rate:*

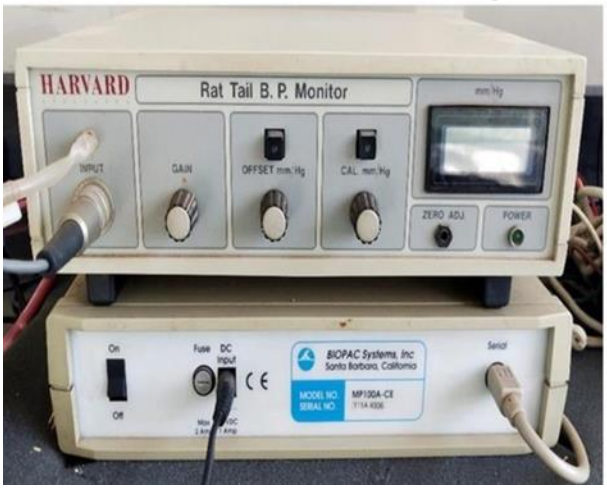
Group F showed significant ($p<0.001$) increase in heart rate (369.8±3.19) when compared to Group N (280.6±4.078). YYEO 200 mg/kg showed insignificant change in heart rate (351.6±5.395), while YYEO 400mg/kg showed significant ($p<0.01$) reduction in heart rate

(325.51±5.63) and highly significant (p<0.001) decrease shown by YYEO 800mg/kg (277.1±5.9) when compared to group F (369.8±3.19). 800mg/kg YYEO treated group showed significant (p<0.01, p<0.001) decreased in heart rate (277.1±5.9) as compared to (200 and 400 mg/kg) YYEO treated groups (351.6±5.395, 325.51±5.63) respectively. (Table: 5)

Effect of ylang-ylang *on Systolic blood pressure:*

Determination of Systolic Blood Pressure:[23]

Changes in SBP measured by Tail-Cuff method in experimental induced hypertensive rats. The experimental rats have been trained in the apparatus (animal restrainer) several times before BP measurement. SBP was measured by Tail-Cuff-method by non-invasive BP monitor (MP100 data acquisition system). Animals were pre-warmed 10 min 40 ^{0}C. Since rats had been pre-conditioned to the BP measurement procedure, they become calm during measurements. Six readings were taken and the average of six was taken as individual SBP.



BiopacSystem-MP100 Tail-Cuff

Group-F showed a significant (P<0.001) increase in mean BP (145.32±1.49) when compared with Group-N (121.66±3.38) at the end of the study. Treatment with YYEO (200 and 400 mg/kg) showed significant (p<0.01) decrease in mean BP (141.1±1.77 and 138±6.82), and highly significant (p<0.001) decrease in BP (131.6±3.77) was shown by (YYEO 800mg/kg) when compared to Group-F (145.32±1.49). 800mg/kg YYEO treated group showed significant (p<0.05, p<0.001) decreased in MBP (131.6±3.77) as compared to (200 and 400 mg/kg) YYEO

treated groups (141.1±1.77 and 138±6.82) respectively. (Table: 5)

Effect of YYEO on Heart Rate:

Group F showed significant ($p<0.001$) increase in heart rate (369.8±3.19) when compared to Group N (280.6±4.078). YYEO 200 mg/kg showed insignificant change in heart rate (351.6±5.395), while YYEO 400mg/kg showed significant ($p<0.01$) reduction in heart rate (325.51±5.63) and highly significant ($p<0.001$) decrease shown by YYEO 800mg/kg (277.1±5.9) when compared to group F (369.8±3.19). 800mg/kg YYEO treated group showed significant ($p<0.01$, $p<0.001$) decreased in heart rate (277.1±5.9) as compared to (200 and 400 mg/kg) YYEO treated groups (351.6±5.395, 325.51±5.63) respectively. (Table: 5)

Effect of YYEO on ECG parameters

Recording of ECG and evaluation of parameters:

Preparation of animal: The animals were anesthetized using Thiopentone Sodium 35mg/kg i.p.

Electrode connection:

1. Each electrode is an abraded site to reduce the amount of impedance between the skin surface and the electrode.

2. Apply the small quantity of gel at the electrode site and apply the electrode set to the right forelimb, left forelimb and left a hind limb.

3. Connection of electrode lead set to the electrode as follows–

RED	Left Leg
BLACK	Left Arm
WHITE	Right Arm

1. After connection of electrode, record the ECG for 60sec using the Biopac Software and the parameters to be analyzed are as follows-

- Heart rate(BPM)
- QRS complex(sec)

- R-R Interval(sec)

Biopack System MP 35

Group F showed significant ($p<0.001$) prolongation of QRS (0.0312±0.02), RR interval (0.551±0.02) when compared with Group N (QRS-0.020±0.0, RR-0.332±0.0) respectively. YYEO 200 mg/kg showed insignificant change in QRS and RR interval (0.0297±0.03, 0.499±0.01) respectively. while YYEO 400mg/kg showed significant ($p<0.01$) change in QRS and RR interval (0.0257±0.00, 0.4739±0.01) and more significant decrease shown by YYEO 800mg/kg (0.023±0.00, 0.403±0.001) when compared to group F. 800mg/kg YYEO treated group showed significant ($p<0.05$, $p<0.01$) decreased in QRS and RR interval (0.023±0.00, 0.403±0.001) as compared to (200 and 400 mg/kg) YYEO treated groups (QRS 0.0297±0.03, and 0.0257±0.00) (RR 0.499±0.01 and 0.4739±0.01) respectively. (Table: 6, 7)

Effect of Ylang-ylang *on Biomarkers:*

Serum LDH levels showed non-significant increase in Group F (391±17.56) compared to Group N (253.2±63.27). Treatment with YYEO 200mg/kg (364.2±19.97), showed non-significant decrease in LDH level as compared to Group- F (391±17.56) while, YYEO (400 & 800 mg/kg) (249.3±17.98, 219.1±13.36)reduced the LDH levels significantly ($p<0.05$) compared to Group-F (391±17.56). 800 mg/kg YYEO treated group showed a significant ($p<0.05$) decreased in LDH level as compared to (200 mg/kg) YYEO treated groups (364.2±19.97).

Serum UA was significantly ($p<0.01$) increased in Group-F (2.678 ± 0.19) when compared to Group-N (1.789±0.22). Treatment with YYEO 200mg/kg (2.260 ± 0.17), showed non-significant decrease in UA level as compared to Group-F (2.678 ± 0.19), whereas treatment YYEO doses (400mg/kg & 800mg/kg) (1.887 ± 0.12, 1.718 ± 0.12) showed significant ($p<0.05$, $p<0.01$)

decrease comparing Group-F (2.678 ± 0.19). (Table: 8)

Effect of *Ylang-ylang* on lipid profile (TC, TG, HDL, LDL and VLDL):

TC levels in Group F (120.2±3.20) were increased significantly ($p<0.01$) as compared to Group N (90.90± 2.44). YYEO 200 and 400mg/kg (115.6±3.57,108.1±4.24), showed non-significant decrease in TG level as compared to Group-F (120.2±3.20) while YYEO dose (800mg/kg) (98.08±3.39), showed significant ($p<0.01$) decrease in level of TC as compared to Group F (120.2±3.20). YYEO 800mg/kg (98.08±3.39), treated group showed significant ($p<0.001$) decreased in TC level as compared to YYEO treated groups (200 and 400 mg/kg) (115.6±3.57, 108.1±4.24) respectively. (Table: 9)

TG levels in Group F (202.7±4.68) were increased significantly ($p<0.01$) as compared to Group N (84.18± 2.36). YYEO 200mg/kg (186.2±3.60), showed significant decrease in TG level as compared to Group-F (30.45±3.069), while YYEO dose (400 & 800mg/kg) (162.3± 4.21, 118.7±1.549), showed highly significant ($p<0.001$) decrease in level of TG as compared to Group F (202.7±4.68) respectively. 800mg/kg YYEO (118.7±1.549) treated group showed significant ($p<0.001$) decreased in TG level as compared to YYEO treated groups (200 mg/kg) (186.2±3.60) and (400 mg/kg) (162.3± 4.21) respectively. (Table: 9)

HDL levels in Group F (24.42±1.183) were decreased significantly ($p<0.001$) as compared to Group N (51.45±2.02). YYEO 200mg/kg (25.99±1.82), showed non-significant increase in HDL level as compared to Group-F (24.42±1.183), while YYEO 400 and 800mg/kg (32.34±0.82, 44.57±1.39), showed significant increase in HDL level as compared to Group-F (24.42±1.183 as compared to Group F (24.42±1.183). YYEO 800mg/kg (44.57±1.39), treated group showed significant ($p<0.001$) increased in HDL level as compared to YYEO treated groups (200 and 400 mg/kg) (25.99±1.82, 32.34±0.82) respectively, whereas YYEO 400mg/kg (32.34±0.82), treated group showed significant ($p<0.05$) increased in HDL level as compared to YYEO treated groups (200 mg/kg) (25.99±1.82) respectively. (Table: 9)

LDL levels in Group F (55.54±3.11) were increased significantly ($p<0.001$) as compared to Group N (21.60± 1.72). YYEO 200 and 400mg/kg (52.38±3.47, 43.26±3.90), showed non-significant decrease in LDL level as compared to Group-F (55.54±3.11) while YYEO dose (800mg/kg) (29.76±2.32), showed significant ($p<0.001$) decrease in level of LDL as compared to

Group F (55.54±3.11). YYEO 800mg/kg (29.76±2.32) treated group showed significant ($p<0.05$, $p<0.001$) decreased in LDL level as compared to YYEO treated groups (200 and 400 mg/kg) (52.38±3.47, 43.26±3.90) respectively. (Table: 9)

VLDL levels in Group F (40.20±0.86) were increased significantly ($p<0.001$) as compared to Group N (16.84±0.47). YYEO 200, 400mg/kg & 800mg/kg (35.84±1.23, 33.80±1.61, 23.75±0.30) showed significant ($p<0.05$, $p<0.01$, $p<0.001$) decrease in VLDL level as compared to Group-F (40.20±0.86). YYEO 800mg/kg (23.75±0.30) treated group showed significant ($p<0.001$) decreased in VLDL level as compared to YYEO treated groups (200 and 400 mg/kg) (35.84±1.23 and 33.80±1.61,) respectively. (Table: 9)

Effect on Tissue parameters (SOD, GSH & MDA):

Antioxidant enzyme SOD levels in Group F were significantly ($p<0.01$) reduced (6.149 ± 0.448) in compare to Group N (17.39 ± 1.933) respectively, this is due to extensive utilization of SOD due to oxidative stress caused by Fructose in cardiac cells. Treatment with YYEO 200mg/kg (10.16 ± 1.099), showed non-significant increase in SOD level as compared to Group F (6.149 ± 0.448), whereas treatment YYEO doses (400mg/kg & 800mg/kg) (14.06 ± 0.63, 13.82± 1.16) showed significant ($p<0.01$) increase comparing Group F (6.149 ± 0.448). (Table: 10)

GSH levels in Group F were significantly ($p<0.001$) reduced (0.368 ± 0.02) as compared to Group N (1.040 ±0.05). Treatment with YYEO (200 and 400mg/kg) (0.641 ± 0.142, 0.536± 0.06), showed non-significant increase in GSH level as compared to Group-F (0.368 ± 0.02) while, treatment YYEO 800mg/kg (0.809±0.07) showed significant ($p<0.05$) increase comparing to 0.448 Group-F (0.368 ± 0.02) (Table:10) LPO levels in Group F(30.45 ± 3.069) were increased significantly ($p<0.01$) in compare to Group N (8.013 ± 3.069). YYEO 200mg/kg (24.83±0.89), showed non- significant decrease in LPO level as compared to Group-F (30.45±3.069), whereas YYEO dose (400 & 800mg/kg) (19.73± 0.918, 14.92±3.06), showed significant ($p<0.05$, $p<0.01$) decrease in level of LPO as compared to Group F(30.45±3.069) respectively. 800mg/kg YYEO treated group showed significant ($p<0.05$) decreased in LPO level as compared to (200 mg/kg) YYEO treated groups (24.83±0.89). (Table: 10)

Table No. 1: IC50 values of ascorbic acid and YYEO on DPPH radical scavenging activity

Sr. no.	Name of compound	$y = mx + c$	IC50 (µg/ml)
1.	Ascorbic acid	y = 0.0658x + 43.585 $R^2 = 0.9847$	97.49
2.	YYEO	y = 0.0665x + 40.807 $R^2 = 0.9806$	138.24

Table No. 2: Effect of *YYEO* on Water intake

GROUP	Week 1 (ml)	Week 2 (ml)	Week 3 (ml)	Week 4 (ml)	Week 5 (ml)	Week 6 (ml)	Week 7 (ml)	Week 8 (ml)
NORMAL	139.70±0.92	138.70±2.38	137.23±2.40	138.00±2.16	139.00±3.95	141.83±3.76	138.16±4.74	137.33±2.92
DISEASE CONTROL	149.00±1.52	149.88±1.19*	150.16±1.77*	154.66±1.59**	152.66±3.24**	157.50±4.3**	151.66±1.69*	144.66±2.35
YYEO 200mg/kg	146.31±0.95	144.43±1.89	141.83±2.91	140.33±1.37$^{\#\#}$	146.50±1.70	146.00±2.16$^{\#}$	147.83±5.24	141.16±2.47
YYEO 400mg/kg	144.51±1.93	142.66±2.19	143.00±1.52	142.66±1.97$^{\#}$	142.16±2.91	139.00±1.52$^{\#\#\#}$	139±2.03$^{\#}$	142.50±3.86
YYEO 800mg/kg	142.40±2.24	141.50±1.91	142.33±2.05	138.83±1.21$^{\#\#}$	138.26±2.32$^{\#\#}$	136.66±3.59$^{\#\#\#}$	137.16±1.86$^{\#\#}$	139.33±4.22

*p<0.05, **p<0.01 when compared with normal group.

#p<0.05, ##p<0.01, ###p<0.001 when compared with disease control group.

Results are being expressed in mean ± SEM (n=6): differences among data were determined using two-way ANOVA followed by Bonferroni posttest.

Table No. 3: Effect of YYEO on food intake

GROUP	Week 1 (g)	Week 2 (g)	Week 3 (g)	Week 4 (g)	Week 5 (g)	Week 6 (g)	Week 7 (g)	Week 8 (g)
NORMAL	59.20±3.8	61.33±1.4	62.50±2.0	58.50±2.2	60.00±1.9	59.50±3.14	62.33±2.8	58.00±3.1
DISEASE CONTROL	47.16±3.0	50.50±2.1	52.66±1.8	51.00±2.3	47.16±2.1*	59.00±4.04	49.66±1.8	43.16±3.1
YYEO 200mg/kg	49.66±1.9	55±1.49	58.50±2.6	55.83±3.1	56.50±0.9	49.16±3.57	45.50±2.8	49.00±3.4
YYEO 400mg/kg	52.33±2.6	54.16±2.8	62.16±0.9	54.00±2.4	58.16±3.4	57.00±22.5	58.00±3.9#	56.50±2.3
YYEO 800mg/kg	56.50±3.0	63±2.92	60.66±1.6	57.50±4.0	61.50±4.8#	60.33±2.21	63.16±2.7# @@@	60.83±3.2#

*p<0.05 when compared with normal group.

#p<0.05when compared with disease control group.

@@@p<0.001 when compared with YYEO (200mg/kg).

Results are being expressed in mean ± SEM (n=6): differences among data were determined using two-way ANOVA followed by Bonferroni posttest.

Table No. 4: Effect of YYEO on Bodyweight

GROUP	Week 0 (gm)	Week 1 (gm)	Week 2 (gm)	Week 3 (gm)	Week 4 (gm)	Week 5 (gm)	Week 6 (gm)	Week 7 (gm)	Week 8 (gm)
NORMAL	185.5±12.02	212±18.10	226±32.82	242.5±40.33	257.5±43.04	269.16±51.34	281.33±54.71	287.66±57.73	287.5±57.87
DISEASE CONTROL	183.66±13.67	227.83±66	239.5±52.83	258.5±61.23	269.33±76.76	293.66±89.60	298.66±53.48	298.66±53.48	305.5±54.04
YYEO 200mg/kg	196±21.18	219.66±25.03	248.33±26.07	265.33±36.87	272±40.12	279.16±42.50	283.66±44.85	289.66±45.21	293.16±45.62
YYEO 400mg/kg	188.16±25.33	210±36.23	226.33±44.90	238.5±55.70	246.5±58.11	254±61.94	258.66±58.15	252.83±57.28	276.16±39.32
YYEO 800mg/kg	192.5±25.14	220.33±39.03	238.5±37.78	259.83±48.67	255.33±43.60	272.16±49.10	275.83±59.94	275.83±55.67	267.66±543.97

Results are being expressed in mean ± SEM (n=6): differences among data were determined using two-way ANOVA followed by Bonferroni posttest.

Table No. 5: Effect of YYEO on FRC induced changes in Heart and Liver weight

GROUP	BODY WEIGHT(g)	HEART WEIGHT(g)	LIVER WEIGHT(g)
NORMAL	250.0±12.03	0.894±0.01	5.968±0.190
DISEASE CONTROL	263.9±13.66	0.974±0.02	7.693±0.544*
YYEO 200mg/kg	259.4±10.69	0.855±0.03	7.305±0.331
YYEO 400mg/kg	236.5± 8.00	0.939±0.05	6.282±0.229#
YYEO 800mg/kg	249.6±9.28	0.853±0.05	6.199±0.257#

*p<0.05 when compared with normal group.

#p<0.05 when compared with disease control group.

Results are being expressed in mean ± SEM (n=6): differences among data were determined using one-way ANOVA followed by Tukey's multiple comparison tests.

Table No. 6: Effect of *YYEO* on FRC induced changes in systolic B.P.

GROUP	Week 0	Week 1	Week 2	Week 3	Week 4	Week 5	Week 6	Week 7	Week 08
NORMAL	117.66±2.80	120.16±3.38	122.16±3.07	119.5±3.04	119.83±2.96	120.16±2.40	121.66±2.13	119.83±3.13	120.16±3.38
DISEASE CONTROL	117±2.88	122.83±1.34	126.83±2.26	131.16±2.73***	138±2.94***	141.66±1.24***	141.83±1.21***	143±1.57***	145.66±1.49***
YYEO 200mg/kg	119±4.50	124.33±3.34	127.33±2.49	133.33±2.98	139.33±2.92	139.33±2.28	140.66±1.24	141.33±1.88	141.16±1.77***
YYEO 400mg/kg	116.66±2.98	122.16±2.26	126±2.58	130.66±3.29	136.83±3.93	139.5±3.93	139.5±528	139.33±6.10	138.66±6.82###
YYEO 800mg/kg	117.5±3.09	121.33±2.56	126±3.41	131±2.88	135.5±2.43	135.83±2.43	136.83±5.17	134.66±4.34###	131.66±3.72###@@

***p<0.001 when compared with normal group.

###p<0.001 when compared with disease control group.

@@p<0.01, @@@p<0.001 when compared with YYEO (200mg/kg) group.

^^p<0.01 when compared with YYEO (400mg/kg) group.

Results are being expressed in mean ± SEM (n=6): differences among data were determined using two-way ANOVA followed by Bonferroni posttests.

Table No. 7: Effect of *YYEO* on FRC induced changes in the ECG and Heart rate

GROUP	HEART RATE (BPM)	RR INTERVAL (sec)	QRS COMPLEX (sec)
NORMAL	280.6±4.078	0.3321±0.0	0.020±0.00
DISEASE CONTROL	369.8±3.190***	0.5512±0.02***	0.03124±0.02***
YYEO 200mg/kg	351.6±5.395	0.4990±0.01	0.0297±0.03
YYEO 400mg/kg	325.51±5.63##	0.47390.01##	0.0257±0.00##
YYEO 800mg/kg	277.1±5.9###@@@^^	0.403±0.01###@@^	0.023±0.00###@@

***p<0.001 when compared with normal group. ##p<0.01, ###p<0.001 when compared with disease control group. @@p<0.01, @@@p<0.001 when compared with YYEO (200mg/kg) group. ^p<0.05, ^^p<0.01 when compared with YYEO (400mg/kg) group. Results are being expressed in mean ± SEM (n=6): differences among data were determined using one-way ANOVA followed by Tukey's multiple comparison tests.

Table No. 8: Effect of YYEO on LDH, URIC ACID and TROPONIN-I

GROUP	LDH (U/L)	SERUM URIC ACID (mg/dl)	TROPONIN -1
NORMAL	253.2±63.27	1.789±0.220	Negative
DISEASE CONTROL	391.0±17.56	2.678±0.190##	Negative
YYEO 200mg/kg	364.2±19.97	2.260±0.179	Negative
YYEO 400mg/kg	249.3±17.98*	1.887±0.126*	Negative
YYEO 800mg/kg	219.1±13.36*@	1.718±0.121**	Negative

##p<0.01 when compared with normal group. *p<0.05,

**p<0.01 when compared with disease control group.

@p<0.05 when compared with YYEO (200mg/kg) group.

Results are being expressed in mean ± SEM (n=6): differences among data were determined using one- way ANOVA followed by Tukey's multiple comparison test.

Table No. 9: Effect of YYEO on FRC induced changes in Lipid profile (TC, TG, HDL, LDL, and VLDL)

GROUP	TG (mg/dl)	TC (mg/dl)	HDL (mg/dl)	LDL (mg/dl)	VLDL (mg/dl)
NORMAL	84.18±2.368	90.90±2.446	51.45±2.027	21.60±1.723	16.84±0.475
DISEASE CONTROL	202.7±4.687$^{###}$	120.2±3.20$^{###}$	24.42±1.183$^{###}$	55.54±3.118$^{###}$	40.20±0.861$^{###}$
YYEO 200mg/kg	186.2±3.609*	115.6±3.575	25.99±1.836	52.38±3.470	35.84±1.235*
YYEO 400mg/kg	162.3±4.211***	108.1±4.247	32.34±0.8224**	43.26±3.905	33.80±1.617**
YYEO 800mg/kg	118.7±1.549$^{***@@@\wedge\wedge\wedge}$	98.08±3.399$^{**@}$	44.57±1.393$^{***@@@\wedge\wedge\wedge}$	29.76±2.320$^{***@@@\wedge}$	23.75±0.309$^{**@@@\wedge\wedge\wedge}$

###p<0.001 when compared with normal group. *p<0.05,

**p<0.01, ***p<0.001 when compared with disease control group.

@p<0.05, @@@p<0.01 when compared with YYEO (200mg/kg) group.

^p<0.05, ^^^p<0.001 when compared with YYEO (400mg/kg) group.

Results are being expressed in mean ± SEM (n=6): differences among data were determined using one-way ANOVA followed by Tukey's multiple comparison test.

Table No. 10: Effect of YYEO on Heart antioxidant enzymes (SOD, GSH and LPO)

Parameter Groups	SOD (U/mg of protein)	GSH (µM/g)	LPO (nM/g)
Normal	17.39±1.933	1.040±0.0506	8.013±3.069
Disease control	6.149±0.4482$^{###}$	0.3686±0.0224$^{###}$	30.45±3.06$^{##}$
YYEO 200mg/kg	10.16±1.099	0.641±0.1421	24.83±0.891
YYEO 400mg/kg	14.06±0.6302**	0.5367±0.0634	19.73±0.918*
YYEO 800mg/kg	13.82±1.162**	0.8099±0.0714*	14.92±3.06$^{**@}$

###p<0.001 when compared with normal group.

*p<0.05, **p<0.01 when compared with disease control group.

@p<0.05 when compared with YYEO (200mg/kg).

Results are being expressed in mean ± SEM (n=6): differences among data were determined using one-way ANOVA followed by Tukey's multiple comparison test.

***In silico* Docking studies**

Selection of compounds and identification of target

Among all the bioactive phytoconstituents from YYEO, 3 constituents namely Beta-caryophyllene, Bisabolol, Eugenol were predicted for Drug likeliness character. Drug likeliness score were shown in Table no 20.

Quality of protein molecule

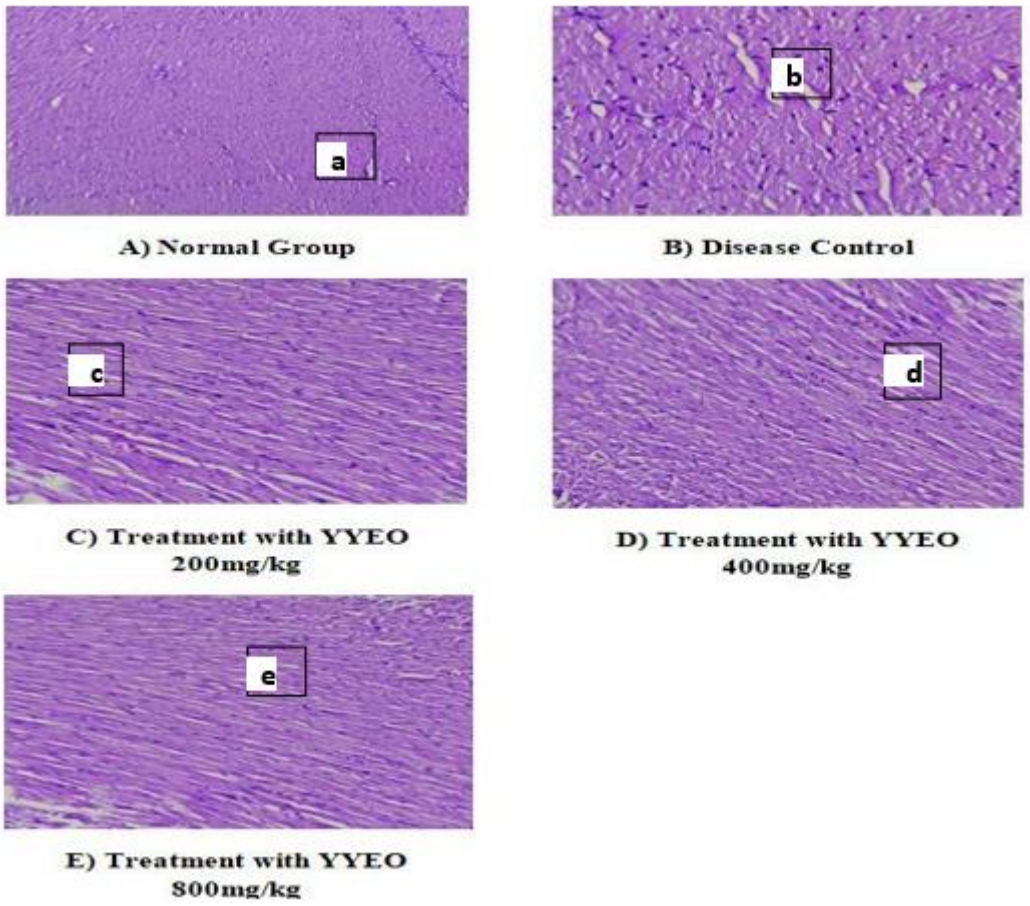
The overall quality factor checked under Errat was found to be 96.63%. The Ramachandran plot and quality of the protein is shown in Figure 35.

Docking studies of compounds with Angiotensin converting enzyme

The docking studies of three compounds from YYEO were studied for inhibition of ACE. Among all the three bioactive Phytoconstituents, Eugenol scored highest binding affinity of -4.18 kcal/mol (IC50 867.32µM), Bisabolol -4.43 kcal/mol (IC50- 563.17µM), Beta- caryophyllene -6.27 kcal/mol (IC50- 25.14 µM). The binding energy, inhibition constant was shown in Table no 21.

Histopathological data of Heart, Aorta, and Liver:

A. HEART



Normal Control- sections of heart showing normal intact cardiac muscle fibers architecture, intracellular spaces appears intact, continuity with adjacent myofibrils (a).

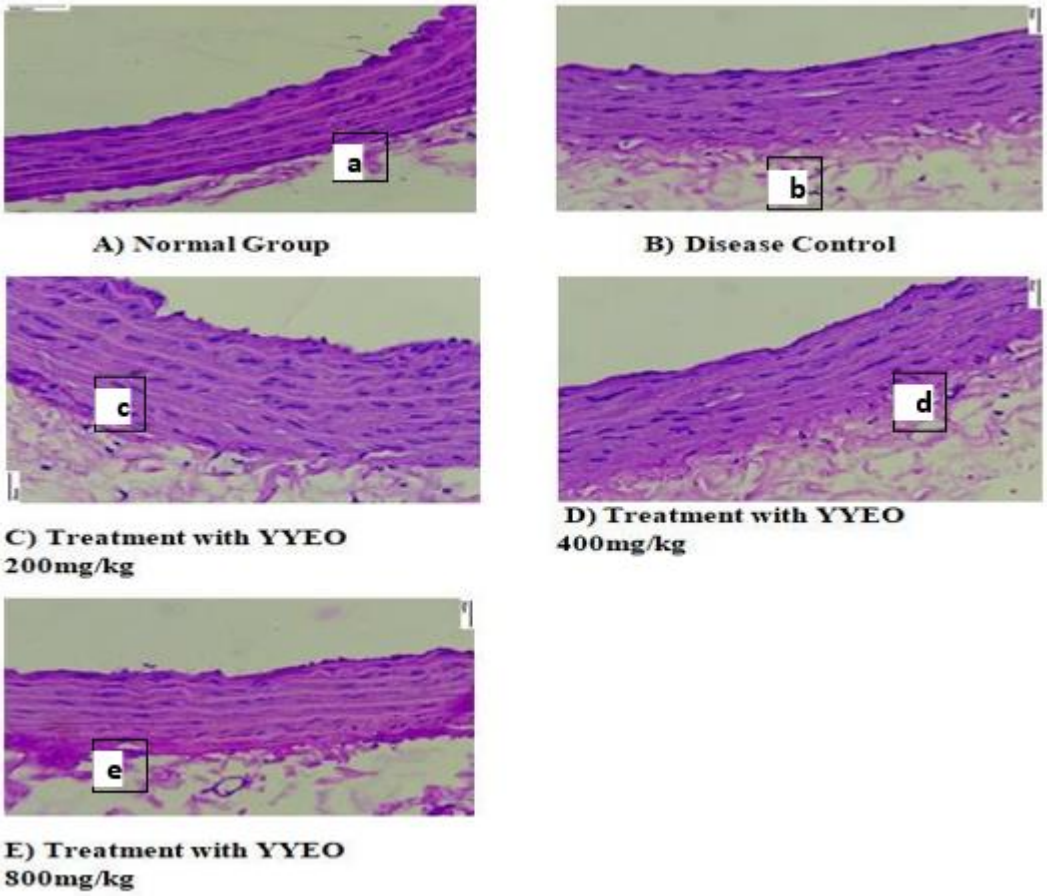
1. **Disease Control-** Degenerative Changes Bland in muscle fibre, interstitial fibrosis and congestion of hyaline/myofibrils having edema with intracyto-vacuoles. (b)

2. **YYEO 200mg/kg-** Presence of congestion and edema in cardiomyocytes but reduced muscle separation and cardio-fibrosis and moderate edema.(c)

3. **YYEO 400 mg/kg–** shows normal myofibrillar structure with moderate congestion with mild edema, reduced vacuole formations. (d)

4. **YYEO 800 mg/kg-** shows normal intact myofibrillar structure with mild congestion, mild edema, no vacuole formations. (e)

B. AORTA:



1. **Normal Control-** sections of liver showing normal intact arrangement of *T. media* and *T. intimal* (a)

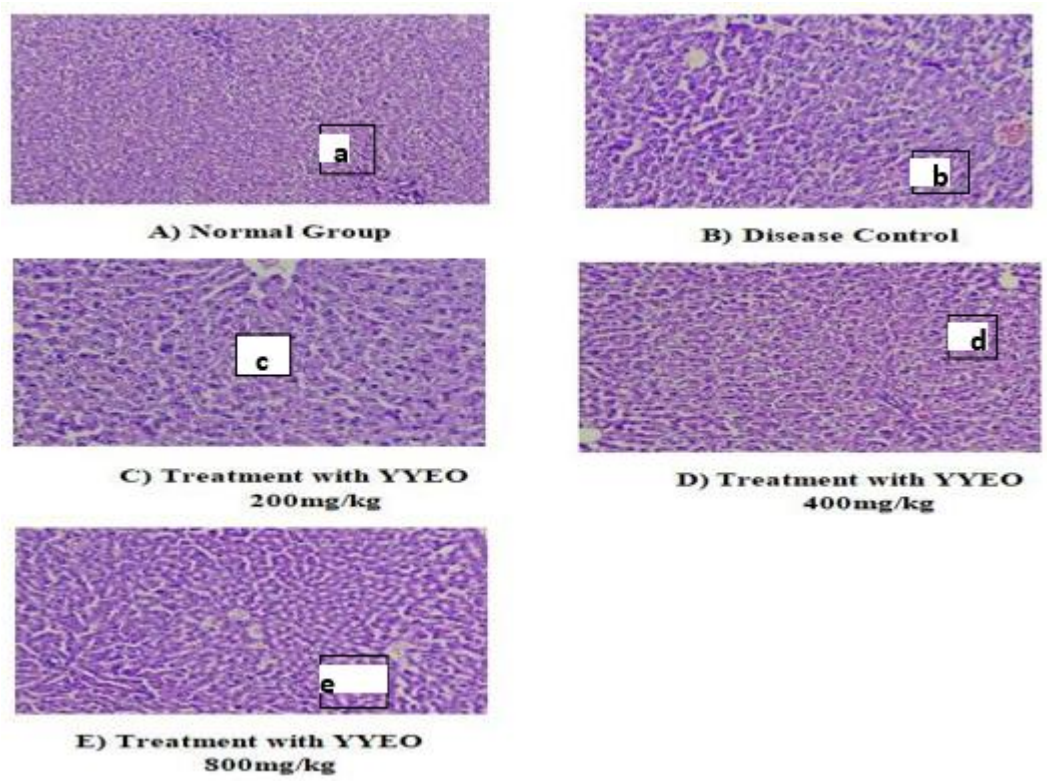
2. **Disease Control-** Thickening of *T. Media* due to the raised density of nucleus with the presence of inflammatory reactive cells, irregular arrangement of *T. Intimal* and infiltration of mononuclear cells and broken medial necrosis in elastin bands is observed.(b)

3. **YYEO 200 mg/kg -** Thickening of *T. Media* remains to improve the irregularity of intimal.(c)

4. **YYEO 400 mg/kg and YYEO 800 mg/kg** – shows slight reduction in thickening of *T. Media*, intact arranging of fibres and mild medial. (d)

YYEO 800 mg/kg – shows reduced in thickening of *T. Media*, intact arranging of fibres, regular arrangement of *T. Intimal* and mild medial. (e)

C. LIVER:



1. **Normal Control-** Sections of liver showing normal intact architectures of cell and tissues with minimal collagen fibres around hepatic-cells.(a)

2. **Disease Control-** Sections of liver showing steatosis of macrovesicles, Lipid dropout, inflammation, infiltrate, sinusoidal and venous congestion, ballooning disintegration and cellular apoptosis (b).

3. **YYEO 200 mg/kg -** Minimal existence of cellular death, the existence of congestions but minor steatosis and cellular infiltrate, reduction in lipid droplets. (c)

4. **YYEO 400 mg/kg and YYEO 800 mg/kg –** shows minor ballooned disintegrated hepatocytes, mild macrovascular steatosis, spotty necrosis, mild congestion.

IN SILICO* DOCKING STUDIES*:

Drug likeness character of active constituents of essential oil was predicted using Molsoft online server.

- Molecular weight <500g/mol
- Log p <5
- Hydrogen bond donor <5
- Hydrogen bond acceptor <10

Table No. 11: Drug likeness character of active constituents of essential oil

Name of Compound	Molecular Weight (g/mol)	HBD	HBA	Log P	Drug- likeness Score
	(<500g/mol)	(<5)	(<10)	(<5)	
Linalool	154.25	1	1	0.10	-0.99
Beta-cryophyllene oxide	204.19	0	0	5.20	-0.71
Eugenol	164.08	2	1	2.61	-0.60
Bisabolol	222.37	1	1	4.97	0.11

- **Ramachandran Plot and quality of the protein:**

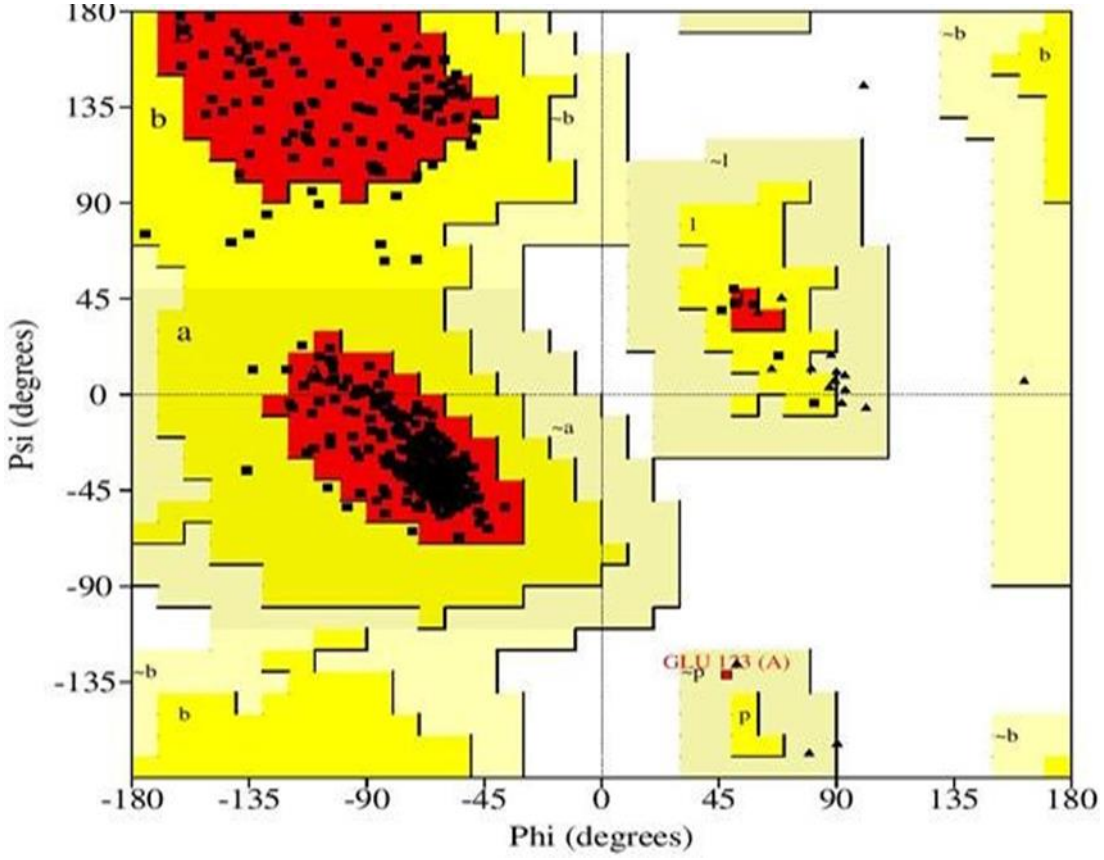


Figure No. 1: Ramachandran Plot of Homology modeled ACEs: No of residues in favored region (~98.0% expected): 567(98.3%), no. of residues in allowed region (~2.0% expected): 9(1.6%) and No. of residues in outlier region (0.2% expected): 1(0.2%).

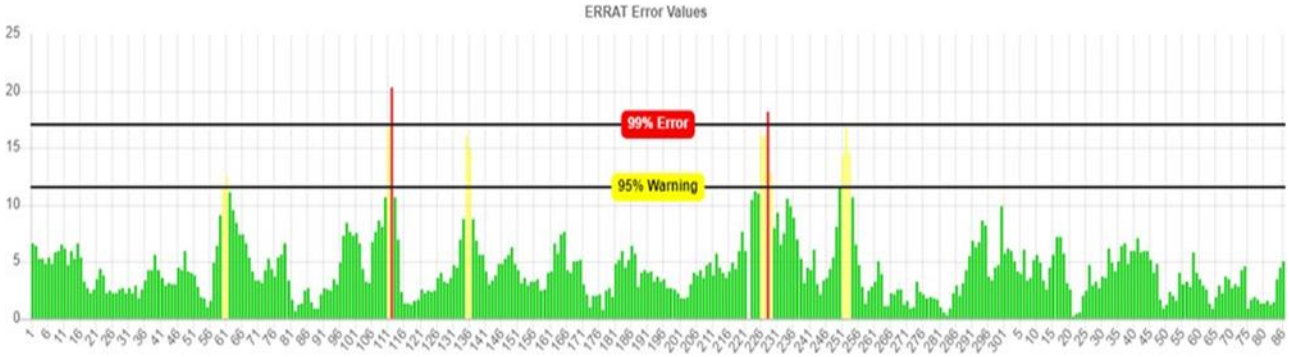


Figure No. 2: Overall quality factor of homology modeled ACE enzyme- 96.63%.

Table No. 12: In- silico ADME Prediction of YYEO major constituents Bisabolol, Eugenol, Beta-Caryophyllene oxide

Name of Protein Molecules	PDBID	Bisabolol		Eugenol		Beta-caryophyllene oxide	
		BE	IC50	BE	IC50	BE	IC50
Angiotensin Converting Enzyme	2XY9	-4.43	563.17µM	-4.18	867.32µM	-6.27	25.41 µM

Finally, the Interaction of all 3 constituents Beta-caryophyllene oxide, Bisabolol, Eugenol these molecules are targeted to protein Angiotensin-converting enzyme was predicted using Pre-ADMET online server:

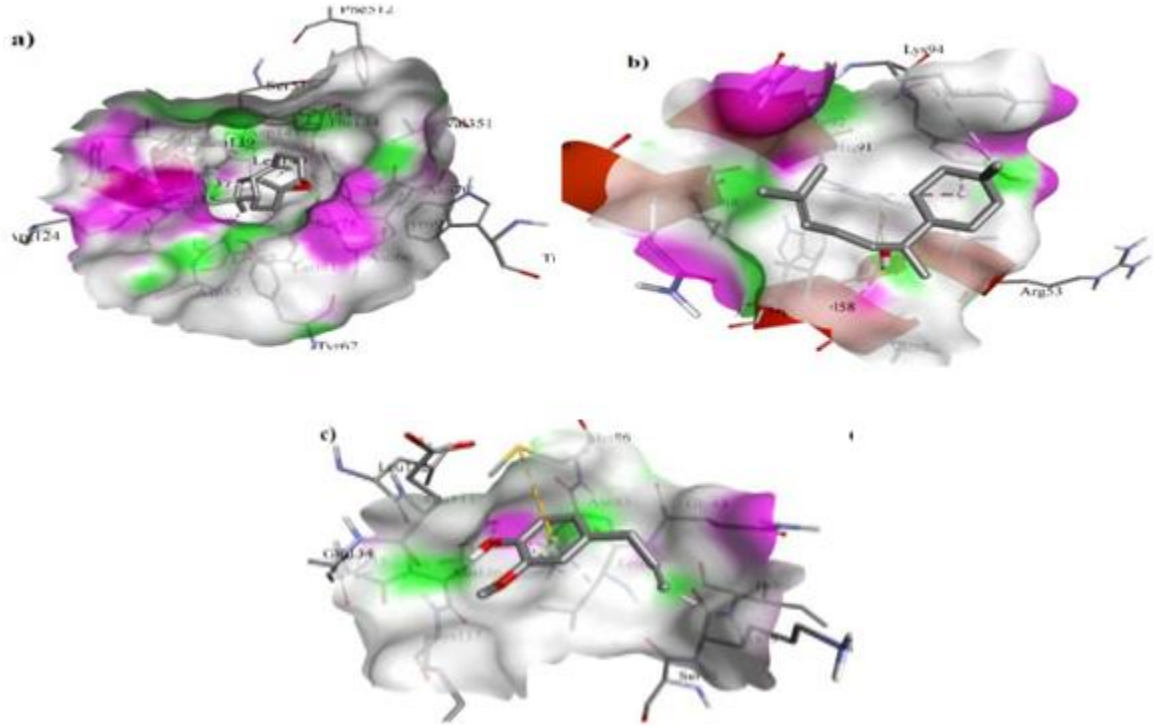


Figure No. 3: In silico *ADME Prediction of YYEO major constituents Bisabolol, Eugenol, Beta-Caryophyllene oxide.*

DISCUSSION

The current study was performed to evaluate the effect of YYEO on fructose-induced hypertension in SD rats. This study demonstrated the efficacy of YYEO to improve SBP, ECG profile, heart rate, lipid profile, serum biomarker, histopathological changes, *in-vitro* and *in-vivo* antioxidant property. Acute oral toxicity studies have shown no mortality at 2000mg/kg (LD50). Hence, based on LD50 200mg/kg, 400mg/kg and 800mg/kg was selected in the current study to assess the YYEO action on hypertensive rats. The results obtained from the current study, suggest that YYEO improves hypertension state in a dose-dependent manner further reflecting 800mg/kg as an effective dose. This oil showed antioxidant capacity through the inhibition of DPPH by 138.24%respectively.

Chronic/High fructose ingestion by a rat in study produces metabolic syndromes like hypertension, hyperlipidemia, hyperglycemia, and adiposity. This although well-known for metabolic syndrome model actuated by high fructose consumption.[24,25] The current study is designed from different investigations to utilize high concentration fructose in drinking water, YYEO had shown the curative effect on a number of its manifestations.

We noticed that intake of water was more in disease induced group compared with normal, it is probably because of fructose concentration creating sweet savor, that influenced acceptability in the rats, as previously reported in a different investigation.[26] In the present study fructose in drinking water in rats has been related to a reduction in food ingestion, agree with those reported previously.[27,28,] Nevertheless, there were no variations in body weight in any of the treatment groups, in spite of the reduction in food consumption; this is likely due to fact that the additional high drinking fructose increased total caloric admission, as recently revealed.[29,30,31]

In our examination, fructose supplementation was not related as indicated insignificant reduction in heart weight in the fructose group as compared with normal. This is similar to recently reported studies.[75]The liver weight would in general decrease in YYEO treated rats. It is understood that the lipid substance in the liver was increased in fructose-induced hypertension rats. Consequently, it is suggested that YYEO improves hypertension by suppressing lipidsynthesis.[32,33]

As per previous study, fructose in drinking for 4 weeks demonstrated raised in BP, increased HR and demonstrated change in ECG profile. In the present examination, demonstrated that high dietary fructose increases RR intervals, QRS complex and heart rate.[34,35] Administration of YYEO for 21 days improved ECG profile, RR intervals, QRS complex and heart rates compared to the disease group, which may be due to stimulation of endothelial cells resulting in vascular remodeling and relaxation as per the previous reports.[36,37]

Dyslipidemia is a trademark part of metabolic disorder, and it has been giving the idea that chronic fructose ingestion favors hepatic lipogenesis, increasing lipid aggregation in the liver; also it secretes and rises in blood content. Our information exhibited that a 21days of YYEO treatment decreased the serum triglycerides in YYEO groups and this has been accounted for by us and different authors of various oils. In the lipoprotein profile, YYEO supplements demonstrated a reduction in LDL, VLDL and raise in HDL, which agrees with the previous results.[38,39] Fructose treated rats showed a rise in serum LDH level when compared with normal rats this impact might be because of FRC induced high release of LDH from the liver and the cardiac cells, which is similar to recently reported studies. The YYEO rats have displayed a significant decrease in the LDH level compared to fructose-treated rats.[40]

In fructose treated group have appeared the low level of the SOD (antioxidant enzyme) of heart tissue sample; this is because of the broad usage of SOD because of oxidative-stress caused by FRC in cardiovascular cells. The YYEO group has indicated raised in SOD value. At the point when compared with fructose rats. This proposes the antioxidant capability of YYEO. This may be because of the defensive role of YYEO on FRC induced oxidative stress in the heart cells because of the presence of various constituents in oil which are previously reported to have antioxidant property.[41,42]

Additionally, our result demonstrated that FRC drinking water increases oxidative stress by aggregating oxidants, for example, LPO, GSH, this is also in agreement as per past reports.[82] It was recommended that oxidative stress leads to hyperinsulinemia that can prompt to hypertension. In the present study raised in Uric acid the various study demonstrated that fructose diet increase UA was associated with the pathogenesis of FRC-induced hypertension.[43,44]

Hypertension is one of the key segment of metabolic syndrome, and chronic FRC administration instigates hypertension in an obstinate way. A few components are accepted to involve in the etiology and maintenance of hypertension, for example, increase in sympathetic activity, an increase in the activity of the RAAS and Angiotensin-II, renal alteration, endothelial dysfunction, and insulin resistance. Several authors have proven that YYEO improves the histopathological characteristics of organs in several studies.

All things considered, this study additionally has a couple of drawbacks that should be considered. The chronic FRC in drinking water rat model does not absolutely reflect the progression and advancement of hypertension in human; therefore, extrapolation of the obtained information to humans is tricky because of interspecies differences. Despite these drawbacks, we were able to define the curative effect of YYEO on complications related to fructose-induced hypertension model in rat.

In addition, YYEO demonstrated additional positive effects by reducing cardiovascular oxidative stress. This examination explicates that YYEO might be considered as a promising helpful system for the prevention & treatment of hypertension. As of now, there are no reliably successful treatments for hypertension, and YYEO treatment likewise could evade the long term unwanted impacts of medications.

CONCLUSION

Ylang-Ylang essential oil demonstrated a significant dose-dependent improvement in fructose-induced hypertensive rats. Moreover, treatment of hypertensive rats with *Ylang-Ylang* essential oil exhibited an improvement in Systolic blood pressure, ECG profile, heart rate, lipid profile, serum cardiac biomarker. Furthermore, it also demonstrated antioxidant potential by significantly reducing oxidative stress induced in the animal experimental model.

Further experimentation needed to elucidate the molecular level mechanism of action of Ylang-Ylang essential oil. Hence, it could be an alternative therapy in the treatment of hypertension.

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