Chinese Herbal Medicines 11 (2019) 326–331

Contents lists available at ScienceDirect

Chinese Herbal Medicines

journal homepage: www.elsevier.com/locate/chmed

Original Article

Antiobesity and antihyperlipidemic effect of *Ixora coccinea* on Triton X-100 induced hyperlipidemia in rats: An approach to evaluate asymmetrical temperature distribution analysis using thermography

Sidhra Syed Zameer Ahmed[a], Syed Zameer Ahmed Khader[a,*], Krishnaveni Radhakrishnan[a], Vanmathi Marimuthu[a], Muniraj Chinnusamy[b], Venkatesan Thangavel[b], Karamchand Ravi[a], Manimaran Vetrivel[a]

[a] Department of Biotechnology, K.S. Rangasamy College of Technology, Tiruchengode, Tamil Nadu 637215, India
[b] Department of Electrical and Electronics Engineering, K.S. Rangasamy College of Technology, Tiruchengode, Tamil Nadu 637215, India

ARTICLE INFO

Article history:
Received 20 August 2018
Revised 18 December 2018
Accepted 11 January 2019
Available online 30 May 2019

Keywords:
anti-oxidant
hyperlipidemia
Ixora coccinea Curtis
obesity
thermography
Triton X-100

ABSTRACT

Objective: Obesity and hyperlipidemia is the major cause of many pathological diseases with an increase side effects using allopathic drugs. The present study focuses on the effect of *Ixora coccinea* on Triton X-100 induced hyperlipidemia in rats and associated complications.
Methodology: *In vitro* radical scavenging activity of *I. coccinea* was assessed using DPPH, FRAP and hydrogen peroxide. *In vivo* antiobesity and antihyperlipidimic activity of *I. coccinea* was tested in Triton X-100 induced hyperlipidemic rats and assessed for its biochemical parameters in blood and tissue samples. The relationship between physiological responses and regulation of body temperature was investigated by using animal surface temperature images captured with infrared camera.
Results: The results of mineral analysis, antioxidant, total flavonoid and phenolic content represented high amount of mineral and had the potential to scavenge free radicals tested with DPPH, FRAP and hydrogen peroxide radicals with dose dependent activity. The highest activity was observed in aqueous extract, DPPH with 71.5% inhibition, FRAP with 56.8%, H_2O_2 with 33% activity at 100 μg/mL concentration. Triton X-100 induced hyperlipidemic rats when treated with *I. coccinea* aqueous extract showed significant activity by regulating the biochemical parameters and maintaining the lipid profile by decreasing TC, LDL-C, VLDL-C, TG and improving HDL-C levels. Similarly, the elevated levels of creatinine, urea, uric acid, AST, ALT, ALP due to induction of hyperlipidemia, were brought back to near normal levels after treatment with *I. coccinea*. The levels of tissue anti-oxidants enzymes like SOD and CAT were also found to be improved in treated *I. coccinea* groups. The whole body asymmetrical temperature distribution analysis showed that significant decreases in temperature was observed in obesity induced groups but a gradual increase in temperature (2%–5%) was observed after treatment.
Conclusion: Thus, the results indicated that *I. coccinea* can be a drug of choice to decrease the risk of complications associated with hyperlipidemia and obesity.

1. Introduction

Obesity is a major health problem and one of the leading causes (preventable) of death worldwide. In 2016, globally 39% of adult aged 18 years and above were overweight and 13% were obese and 41 million children under the age of five were overweight. Obesity is a major issue for metabolic diseases, including hypertension and hyperlipidaemia potentially type 2 diabetes, cardiovascular disorder, non-alcoholic fatty liver disease, stroke and colon cancer. Obesity is mostly caused by a combination of excessive food intake, lack of physical activity and genetic susceptibility (James, 2008). Some of the cases are caused mostly due to endocrine disorders, medications, genes or mental illness. In addition to this, attenuation in adipogenesis and over expression of pancreatic lipase enzyme play a vital role in progression of obesity and overweight.

Current medication using allopathy drugs causes serious side effects; Hence, an urgent and alternative remedy is required. Scientists from different disciplines are focusing on herbal products and their secondary metabolites acting as potent therapeutic agents. Usage of these herbal products are effective with minimal or no side effects and are available at a relatively lower cost than

* Corresponding author.
E-mail address: zameerkhader@gmail.com (S.Z.A. Khader).

https://doi.org/10.1016/j.chmed.2019.05.006
1674-6384/

allopathy drugs. (Syed et al., 2017). Plants produce a several types of bioactive molecules with different activities or functions which play a major role in production of medicines. In pharmaceutical industries, plants and its natural products play a significant role in drug development process for preventing and curing many different types of diseases.

Ixora coccinea Curtis belongs to Rubiaceae family and parts such as the flowers, leaves, roots and stems have been used to treat various ailments in the Indian traditional system of medicine, the Ayurveda, and also in various folk medicines. *I. coccinea* is a small shrub which is cultivated throughout India and reported to have antimicrobial, cytotoxic, antinociceptive, hepatoprotective, anticancer and anti-inflammatory properties (Annapurna, Amarnath, Amar Kumar, Ramakrishna, & Raghavan, 2003; Latha & Panikkar, 1998). Based on the observation, an effort is made to analyse efficiency of *I. coccinea* against oxidative stress produced during obesity and combating complications associated with obesity.

2. Materials and methods

2.1. Collection and preparation of plant materials

Fresh flowers of *I. coccinea* were collected from local areas of Namakkal district, TamilNadu and authenticated. The flowers were thoroughly washed with distilled water to remove dirt and contaminations, shade dried, finely powdered using electric blender and stored in an air-tight container for further use and extraction was carried out using water as solvent (Syed et al., 2017).

2.2. Analysis of minerals, chlorophylls, carotenoids, total phenol and flavonoid content

Proximal constituent and mineral content like ash, phosphorous, potassium, magnesium, manganese, zinc, calcium, sulphur, copper, chromium, arsenic, nickel, aluminium and sodium were determined using atomic adsorption spectrophotometer as per the method suggested by the Association of Official Analytical Chemists (AOAC, 1990). Total phenols and flavonoids were determined by the Folin–Ciocalteu assay (McDonald, Prenzler, Autolovich, & Robards, 2001)

2.3. In vitro anti-oxidant assays

The radical scavenging activities of the extracts were determined with DPPH (Shimada, Fujikawa, Yahara, & Nakamura, 1992), Ferric reducing anti-oxidant power assay (Yang, Guo, & Yuan, 2008) and hydrogen peroxide assay (Ruch, Cheng, & Klaunig, 1989) according to an established protocol.

2.4. Experimental design

Adult male albino Wistar rats weighing around 100–150 g were obtained from Animal House facility, K.S.R.C.T.B.T with approval from institutional animal ethical board (KSRCT/BT/IAEC/2017/20) maintained under standard laboratory condition. Obesity was induced in overnight fasting animals by single intra peritoneal injection of freshly prepared solution of Triton X-100 (100 mg/kg b.w.) in physiological saline (Adigun, Oladiji, & Ajiboye, 2016).

Obesity induced rats were divided into three groups with six rats in each group (i) *I. coccinea* treated group (100 mg/kg b. w.) (ii) *I. coccinea* treated group (200 mg/kg b. w.) (iii) Atorvastatin (10 mg/kg b. w.) treated group (Adigun et al., 2016). All the experimental animals were maintained at ambient temperature throughout the experimental period, extracts and drug were supplemented using an intragastric tube for 25 d. Body weight and food intake was monitored every week throughout the experiment. At the end of the experimental period, animals were sacrificed and blood and tissue samples were collected for further investigation.

2.5. Biochemical profiling

Lipid profile was estimated using standard kits purchased from TransAsia Bio Medical Limited, Mumbai, India. Method of Friedwald was used to determinate very low-density lipoprotein (VLDL-C) and low density lipoprotein (LDL-C) (Friedwald, Levy, & Friedrickson, 1972). Urea, Uric acid and creatinine were estimated using standard reagent kits purchased from Coral clinical systems, Goa, India. AST, ALT and ALP were assessed using standard kits purchased from Transasia Bio Medical Limited, Mumbai, India. The activity of SOD and catalase were assayed following the previous methods (Kakkar, Das, & Vishwanathan, 1984; Abei1984).

2.6. Infrared thermal imaging of animals

Infrared camera (FLIR T420, FLIR Systems, Boston, MA, USA) with wide temperature range of −4°F to 2192 °F (−20 °C to 1700 °C), FOV 25° × 19°, and thermal sensitivity of 0.1 °C was used in this study. As a baseline anterior to posterior view of whole body, thermal images of all the rats were taken under standard conditions at constant distance of 12 cm. Thermal images were taken in normal rats and obesity induced rats during treatment and after treatment for a period of five weeks following the method of Syed et al. with slight modification (Syed et al., 2018).

2.7. Statistical analysis

Values were presented as means ± SEM. The statistical significance was evaluated by one-way using the statistical software SPSS Version 17 (Origin Lab Corporation, USA). The data were analyzed by one way analysis of variance (ANOVA) followed by Tukey's test.

3. Results and discussion

3.1. Analysis of minerals, total phenol and flavonoid content

Minerals like calcium, magnesium, sodium, potassium, and manganese are essential for body to control and prevent various diseases (Aliyu, Musa, Oshanimi, Ibrahim, & Oyewale, 2008). Table 1 depicted the presence of mineral (micro and macro elements) in flowers of *I. coccinea* with higher amount of ash content (78%) representing it was an edible substance with 88.65% and 776 µg/g of zinc and manganese present in it. Zinc plays a vital role in various metabolic activities and replication, cellular differentiation, protein synthesis, immune mechanism, proper sexual functioning (Pathak & Kapil, 2004). Previous research suggested that zinc stimulates the activity of vitamins and proper functioning of the heart and acts as a cofactor for many enzymes like DNA and RNA polymerase, superoxide dismutase, lactate dehydrogenase etc. (Ojobor Charles, Anosike Chioma, & Ani Chijiokes, 2015). Manganese is necessary for the functioning of the pituitary gland, the pineal gland and the brain.

Moreover, studies in animal model have shown that diet rich in potassium and less of sodium can reduce the risk of hypertension and stroke (Berry, 1998; FAO and WHO, 2011; Robert, Daryl, Peter, & Victor, 2003). Ca, Mg, Zn and Fe (Wenkam, 1990) are required in small amounts, to make the body function properly (Wills, 1998). Sodium plays a vital role in digestion, osmosis, and also helps to control diabetes mellitus, similarly chromium also contributes its effect in sugar metabolism and prevents the progression of disease (Khan, Batinic-Haberle, & Benov, 2009; Komorowski et al., 2012; Syed, Sidhra, Ponmurugan, & Senthil Kumar, 2016). Magnesium is an extracellular fluid helps maintain osmotic equilibrium

Table 1
Evaluation of mineral content in *Ixora coccinea* flowers.

Content	Elements													
	P/(mg·g^{-1})	K/(mg·g^{-1})	Zn/(mg·g^{-1})	Mn/(mg·g^{-1})	Cu/($\times10^{-6}$)	Cr/($\times10^{-6}$)	As/($\times10^{-6}$)	Ni/($\times10^{-6}$)	Al/($\times10^{-6}$)	Na/($\times10^{-6}$)	Mg/%	Ca/%	S/%	Ash/%
Quantity	1.47	12.31	0.088	0.776	0.11	2.53	0.06	0.19	8.32	18.51	0.19	1.6	0.45	78.01

(Thomas & Krishnakumari, 2015). Calcium is necessary for bones and teeth formation, helps to act as a co-factor during metabolism and during the process of blood clotting (Robert et al., 2003). The daily required quantity of minerals to boost the immune system and for regulation of metabolism was found in *I. coccinea.*

The total flavonoid content was found to be around 0.490−1.833 mg of RU/g at varying concentration, similarly the total phenolic content present in *I. coccinea* is 0.954−1.960 mg of RU/g (Table 2). It is well known fact that phenols and flavonoids play a major role in preventing obesity by inhibiting the fat metabolism related enzymes like pancreatic lipase, lipoprotein lipase and glycerophosphate dehydrogenase (Ajithadas, Vijayalakshmi, & Karthikeyan, 2014). Research has proved that phenolic compounds are potent chain breaking anti-oxidant due to the presence of hydroxyl groups that scavenges free radicals (Syed et al., 2018).

3.2. Free radical scavenging ability of I. coccinea

Free radicals are produced in the body and triggers oxidative damage by releasing reactive oxygen species (ROS) and reactive nitrogen species (RNS) from activated neutrophil and macrophages, leading to various diseases like diabetes, arthritis, Parkinson's disease, autism, cancer, cataracts and aging Alzheimer's dementia (Rege, Juvekar, & Juvekar, 2012) and even heart disease (Singh & Jialal, 2006). The complications can be minimized only by proving a natural substance that can scavenge the free radicals. Hence the effect of *I. coccinea* flowers extract as radical scavenger was assessed and the results were represented in Fig. 1. The scavenging activity was found to be dose dependent in both aqueous and ethyl acetate extracts of *I. coccinea* at various concentration (20 µg/mL to 100 µg/mL) and the radical scavenging activity was found in aqueous extract with H_2O_2 (29%−33%), DPPH (27%−71%) and FRAP (31%−56%). Hence the results of the present study demonstrated that *I. coccinea* has the potential to scavenge free radicals by inhibiting lipid peroxidation and reduce the complication associated with many diseases.

3.3. Effect of I. coccinea on lipid profile in experimental obesity

It is evident from the previous research that Triton X-100 induction significantly alters the lipid profile providing obesity and hyperlipidemia in experimental rats (Jaafaru Sani, Hauwa'u, Peter Maitalata, Mohammed Mustapha, & Timothy, 2016). Triton X-100 induced rats were treated with *I. coccinea* at the dosage of 100 and 200 mg/kg b. w. and the results were represented in Table 3. There were significant changes observed after Triton X-100 induction with increase in TC, TG, LDL-C and VLDL-C, whereas sudden decrease in HDL-C was observed. Treatment with *I. coccinea* extract at 200 mg/kg b. w. demonstrated significant reduction in TC, LDL-C, VLDL-C and TG with (125%, 8.9%, 56% and 15%). The HDL-C levels were found to increase with 33% when compared with initial and final day and the results were on par with the standard drug treated groups. The positive risk factor for atherosclerosis is a significant reduction in HDL-C (Poongothai, Ponmurugan, Syed, Senthil Kumar, & Sheriff, 2011; Syed et al., 2017) and from the present results it was evident that the risk associated with obesity can be minimized using *I. coccinea*.

Table 2
Estimation of total flavonoid and phenolic content in flower extract of *Ixora coccinea*.

Sample concentration/ (mg·mL^{-1})	Total flavonoid content mg of RU/g	Total phenolic content mg of GA/g
0.1	0.490 ± 0.48	0.954 ± 0.41
0.2	1.231 ± 0.55	1.410 ± 0.48
0.3	1.522 ± 0.37	1.619 ± 0.51
0.4	1.703 ± 0.25	1.845 ± 0.44
0.5	1.833 ± 0.36	1.960 ± 0.44

Note: Content of flavonoids was expressed in terms of rutin equivalents (RU) per gram of dry extract. Content of total phenols in extracts was expressed as gallic acid equivalents (GA) per gram of dry extract.

3.4. Effect of I. coccinea on kidney function and liver function test in experimental obesity rats

Table 4 demonstrated the effect of *I. coccinea* on creatinine, urea and uric acid on experimental induced obesity rats. The results revealed sudden increase in all the three parameters after Triton X-100 induction but treatment with *I. coccinea* in two different doses and standard drug brought back the raised levels by 74%−130% in creatinine, 6%−59% in urea, and 14%−34% in uric acid. Liver is the vital organ involved in detoxification of compounds and the functional status of liver can be evaluated through liver function test. During the induction of obesity and hyperlipidemia, fat accumulation in liver of obese rats causes swelling of rough endoplasmic reticulum and mitochondria in hepatocytes (Mopuri, Ganjayi, Banavathy, Naidu Parim, & Meriga, 2015) leading to improper functioning of liver. However, treatment with *I. coccinea* and standard drug on experimental rats brought back the altered levels to near normal levels, AST by 16%−60%, ALT by 36%−59%, ALP by 4%−13% respectively (Table 5). The results are on par with the drug treated groups representing that *I. coccinea* not only maintains the lipid level during obesity but also helps to ameliorate liver and kidney to function properly.

3.5. Effect of I. coccinea on tissue anti-oxidant enzymes in experimental obesity rats

Superoxide anion, hydroxyl radicals, hydrogen peroxides are implicated during hypercholesterolemic atherogenesis and these oxygen derived radical's cause's oxidative stress leads to cellular damage (Sidhra et al., 2017). Degradation of unsaturated fatty acids during abnormal condition leads to alteration in the enzyme levels (Girija, Lakshman, Udaya, Sabhya, & Divya, 2011). SOD is an important defense enzyme that catalyzes the dismutation of superoxide radicals and catalase (CAT) is the hemoprotein that catalyzes the reduction of hydrogen peroxide and protects the tissues from highly reactive hydroxyl radicals (Liu, Sun, Rao, Su, & Yang, 2013). During the present study, the levels of SOD and CAT in liver and kidney were found to be improved after treatment with *I. coccinea* and standard drug indicating its favourable action and preventing the oxidative stress (Table 6).

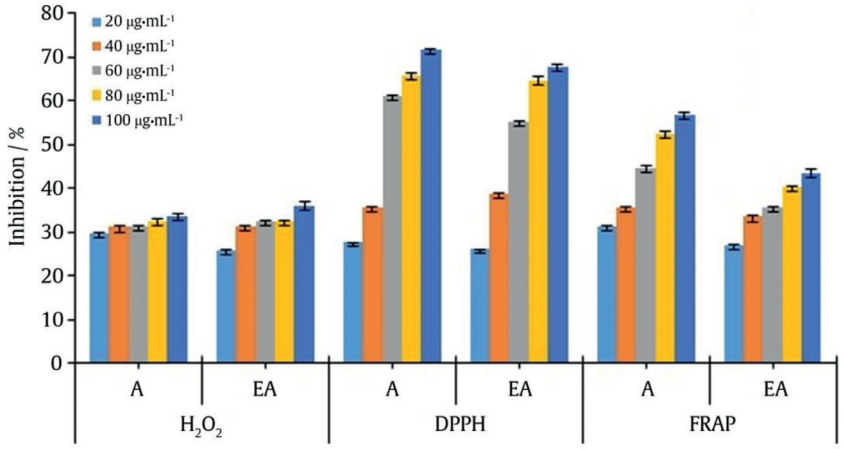

Fig. 1. Free radical scavenging ability of *Ixora coccinea*.

Table 3
Effect of *Ixora coccinea* on lipid profile in experimental rats.

Experimental animals		TC/(mg·dL^{-1})	HDL - C/(mg·dL^{-1})	LDL-C/(mg·dL^{-1})	VLDL-C/(mg·dL^{-1})	TG/(mg·dL^{-1})
Group I	Regular Intervals	204.6 ± 1.4	32.6 ± 2.11	62.8 ± 3.21	32.8 ± 3.12	194.01 ± 1.10
	Final	116.2 ± 0.2	36.89 ± 1.3	57.6 ± 3.78	26.78 ± 2.12	176.10 ± 2.20
	% of Change (R *vs* F)	−76.07	+11.62	−9.027	−22.47	−10.17
Group II	Regular intervals	201.2 ± 0.3	31.8 ± 4.22	61.9 ± 4.12	33.2 ± 2.11	174.01 ± 150
	Final	89.3 ± 2.68	47.54 ± 5.3	56.8 ± 1.45	21.15 ± 1.01	150.01 ± 2.02
	% of Change (R *vs* F)	−125.30	+33.10	−8.97	−56.97	−15.99
Group III	Regular intervals	198.7 ± 2.11	32.4 ± 2.11	63.2 ± 2.12	30.8 ± 0.12	175.02 ± 0.1
	Final	96.0 ± 3.25	52.36 ± 1.51	48.21 ± 3.12	19.39 ± 1.00	138.2 ± 2.48
	% of Change (R *vs* F)	−106.97	+38.12	−31.09	−58.84	−26.64

Note: All value represents six individual observations, and data are expressed in mean ± SEM calculated by one-way ANOVA followed by Tukey's test.
Regular intervals – Analysis carried out once a week, Final observation – end of experiment. + & – indicates percentage of change during and after treatment, same as Tables 4 and 5.

Table 4
Effect of *Ixora coccinea* on creatinine, urea and uric acid in experimental rats.

Experimental animals		Creatinine/(mg·dL^{-1})	Urea/(mg·dL^{-1})	Uric acid/(mg·dL^{-1})
Group I	Regular intervals	2.42 ± 1.14	50.6 ± 1.14	2.18 ± 0.14
	Final	1.39 ± 0.07	47.6 ± 0.09	1.90 ± 0.06
	% of Change (R *vs* F)	−74.100	−6.30	−14.74
Group II	Regular intervals	2.32 ± 0.16	51.2 ± 1.12	2.14 ± 1.14
	Final	1.18 ± 0.05	36.7 ± 0.42	1.75 ± 0.05
	% of Change (R *vs* F)	−96.61	−39.50	−22.28
Group III	Regular intervals	2.40 ± 0.02	51.80 ± 1.18	2.10 ± 0.12
	Final	1.04 ± 0.13	32.40 ± 0.54	1.56 ± 0.02
	% of Change (R *vs* F)	−130.76	−59.87	−34.61

Table 5
Effect of *Ixora coccinea* on AST, ALT and ALP levels in experimental rats.

Experimental animals		AST/(U · L^{-1})	ALT/(U · L^{-1})	ALP/(U · L^{-1})
Group I	Regular intervals	63.4 ± 2.32	69.20 ± 1.58	162.48 ± 5.09
	Final	54.36 ± 1.11	50.79 ± 0.04	154.98 ± 3.42
	% of Change (R *vs* F)	−16.62	−36.24	−4.839
Group II	Regular intervals	62.80 ± 7.09	70.40 ± 1.40	163.1 ± 1.62
	Final	39.29 ± 1.04	43.56 ± 0.02	126.00 ± 2.64
	% of Change (R *vs* F)	−59.83	−61.61	−29.44
Group III	Regular intervals	62.40 ± 1.02	69.6 ± 4.42	162.00 ± 4.62
	Final	38.92 ± 2.04	43.73 ± 6.01	142.12 ± 1.16
	% of Change (R *vs* F)	−60.32	−59.15	−13.988

Table 6
Effect of *Ixora coccinea* on antioxidant enzymes level in experimental rats.

Groups	SOD/(U · mg^{-1}·protein^{-1})		CAT/(U · min^{-1})	
	Liver	Kidney	Liver	Kidney
Group I	1.04 ± 0.09	1.23 ± 0.18	0.28 ± 0.12	0.14 ± 0.28
Group II	1.09 ± 0.36	1.13 ± 0.26	0.31 ± 0.09	0.24 ± 0.17
Group III	1.15 ± 0.42	1.34 ± 0.18	0.58 ± 0.15	0.25 ± 0.38

Note: All value represents six individual observations, and data are expressed in mean ± SEM calculated by one-way ANOVA followed by Tukey's test.

3.6. Effect of *I. coccinea* on body temperature in experimental obesity rats

Thermography based diagnostic application is a developing area for the quantification and to evaluate the progression of diseases (Syed et al., 2018; Umapathy, Vasu, & Gupta, 2017). Hence, an attempt is made to identify the temperature changes during obesity and treatment. In normal rats, the temperature was found to be normal representing proper circulation of blood flow and the healthiness of the rat. In experimental obese rat, there was a gradual decrease in mean temperature which may be due to high deposition of fat in the adipose tissue (Fig. 2A) and subsequent treatment with *I. coccinea* to rats in group I and II demonstrated gradual raise in temperature with 2%–5% asymmetric abdominal temperature changes (Fig. 2B), similar effect was observed in group III drug treated rats. Skin plays a vital role in monitoring and regulating body temperature and could be a better tool for monitoring the therapy.

Fig. 3 represented the observation recorded using FLIR camera at three different abdominal sites of experimental rats representing the temperature changes. Various studies have hypothesized and reported that infrared thermography is a fast developing diagnostic tool to identify disease severity in human and animal models (Jiang, Ng, & Yeo, 2005). Hence, the present study revealed and hypothesized that there was reduction of temperature during Triton X-100 induced obesity and brought back to near normal levels. Hence, thermography can be a valuable tool to identify the severity of the disease condition.

In conclusion, our findings demonstrated that the phytochemicals presented in *I. coccinea* had the ability to scavenge free radicals, reduce the risk of complications associated with obesity and can be a drug of choice.

Declaration of Competing Interest

The authors declare no conflict of interest.

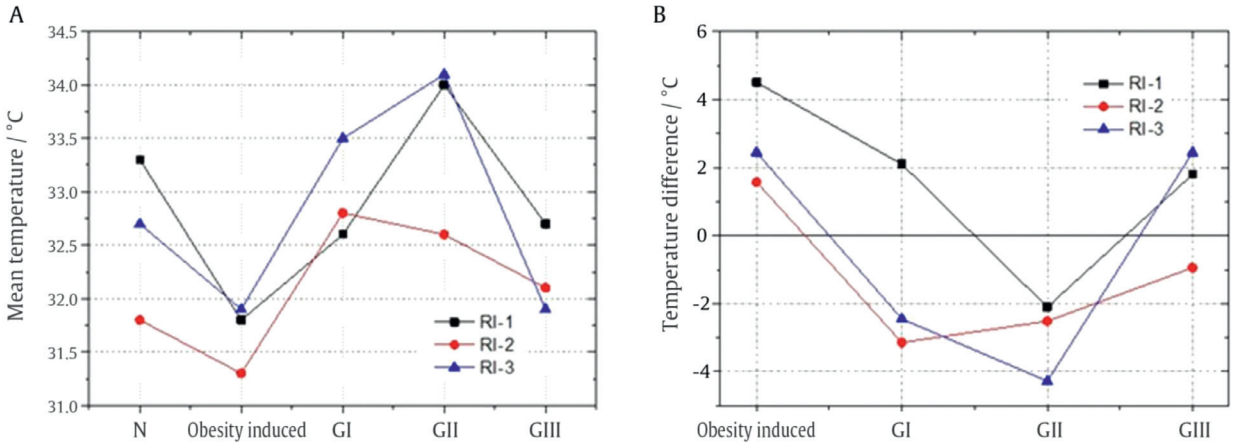

Fig. 2. Effect of *Ixora coccinea* on body temperature (A) and asymmetric temperature (B) in experimental rats.

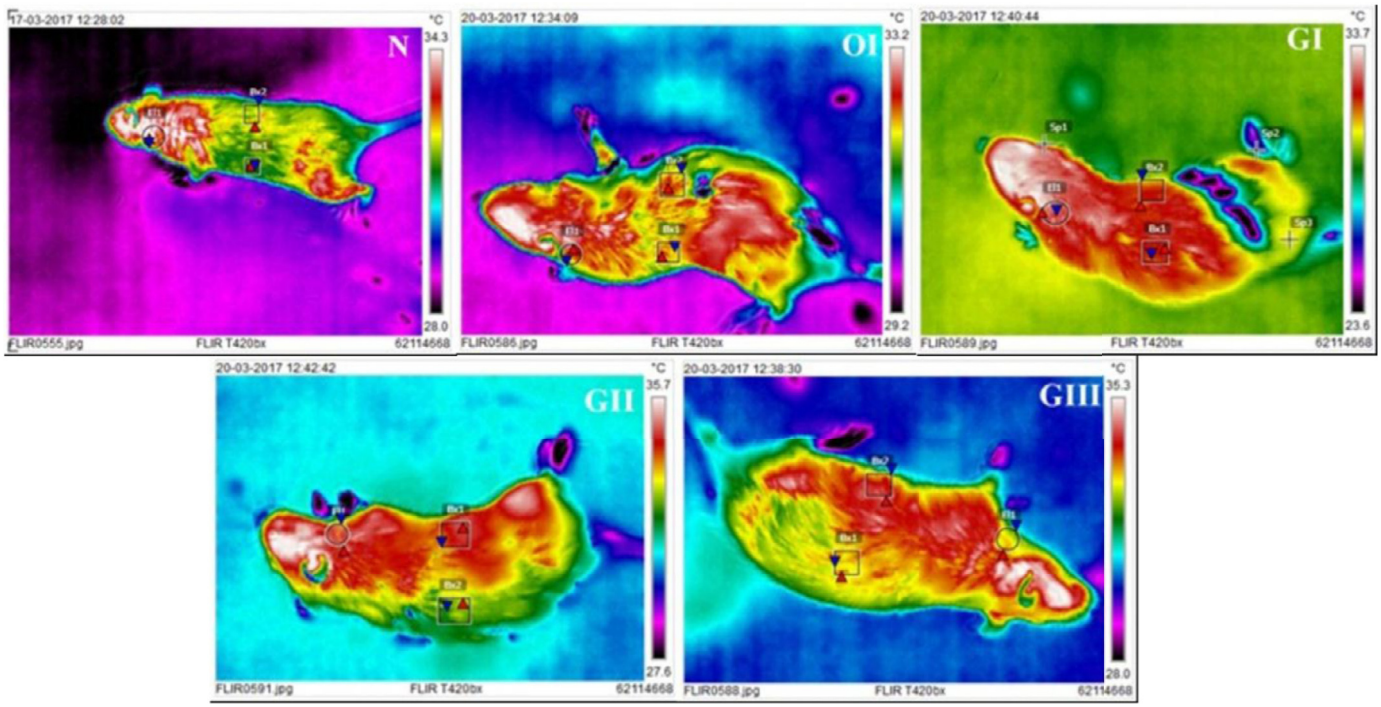

Fig. 3. Thermal imaging of diabetic rats representing thermogram of normal rats, obesity induced rats, and group I, group II and group III rats.

Acknowledgements

Authors are thankful to the Management and Principal of K.S. Rangasamy College of Technology, (DBT-FIST and DBT-STAR Scheme) Tiruchengode for providing infrastructure and facilities to carry out this research work successfully.

References

Abei, H. (1984). Catalase *in vitro*. In L. Parker (Ed.), *Methods in enzymology* (pp. 121–126). New York: Academic Press. PMID: 6727660 .

Adigun, N. S., Oladiji, A. T., & Ajiboye, T. O. (2016). Antioxidant and anti-hyperlipidemic activity of hydroethanolic seed extract of *Aframomum melegueta* K. Schum in Triton X-100 induced hyperlipidemic rats. *South African Journal of Botany, 105*, 324–332.

Ajithadas, A., Vijayalakshmi, K., & Karthikeyan, V. (2014). Pancreatic lipase inhibitory screening of *Citrullus lanatus* leaves. *The Pharma Innovation Journal, 3*(7), 44–52.

Aliyu, A. B., Musa, A. M., Oshanimi, J. A., Ibrahim, H. A., & Oyewale, A. O. (2008). Phytochemical analyses and mineral elements composition of some medicinal plants of north Nigeria. *Nigerian Journal of Pharmaceutical, 7*, 119–125.

Annapurna, J., Amarnath, P. V. S., Amar Kumar, D., Ramakrishna, S. V., & Raghavan, K. V. (2003). Antimicrobial activity of *Ixora coccinea* leaves. *Fitoterapia, 74*, 291–293.

AOAC (Association of Official Analytical Chemists). (1990). *Official methods of analysis* Washington, D.C.

Berry, T. N. (1998). The role of condensed tannins in the nutritional value. *British Journal of Nutrition, 51*, 493–504.

FAO and WHO (2011). Codex alimentarius commission food additives and contaminants: Joint FAO/Who food standards programme. In *CF/5 INF/1. Food and Agriculture Organisation of United Nations* (pp. 1–89). Geneva, Switzerland: World Health Organisation.

Friedwald, J., Levy, Y. R., & Friedrickson, S. D. (1972). Estimation of concentration of low density lipoprotein cholesterol in plasma without use of preparative ultracentrifuge. *Clinical Chemistry, 18*(6), 499–502.

Girija, K., Lakshman, K., Udaya, C., Sabhya, S. G., & Divya, T. (2011). Antidiabetic and anti cholesterolemic activity of methanol extracts of three species of *Amaranthus*. *Asian Pacific Journal of Tropical Biomedicine, 1*(2), 133–138.

Jaafaru Sani, M., Hauwa'u, Y. B., Peter Maitalata, W., Mohammed Mustapha, B., & Timothy, B. (2016). Ameliorative effect of methanolic extract of *Cassia occidentalis* (MECO) whole plant, on Triton X-100-induced hyperlipidaemia in Albino rats. *International Journal of Science and Research, 5*(2), 2141–2146.

James, W. P. (2008). The fundamental drivers of the obesity epidemic. *Obesity Reviews, 9*(1), 6–13.

Jiang, L. J., Ng, E. Y., & Yeo, A. C. (2005). A perspective on medical infrared imaging. *The Journal of Medical Engineering & Technology, 29*(6), 257–267.

Kakkar, P., Das, B., & Vishwanathan, P. N. (1984). A modified spectrophotometric assay of superoxide dismutase. *Indian Journal of Biochemistry &Amp; Biophysics, 21*, 130–132.

Khan, I., Batinic-Haberle, I., & Benov, L. T. (2009). Effect of potent redox-modulating manganese porphyrin, MnTM-2-PyP, on the Na^+/H^+ exchangers NHE-1 and NHE-3 in the diabetic rat. *Redox Report: Communications in Free Radical Research, 14*, 236–242.

Komorowski, J. R., Tuzcu, M., Sahin, N., Juturu, V., Orhan, C., & Ulas, M. (2012). Chromium picolinate modulates serotonergic properties and carbohydrate metabolism in a rat model of diabetes. *Biological Trace Element Research, 149*, 50–56.

Latha, P. G., & Panikkar, K. R. (1998). Cytotoxic and antitumor principles from *Ixora coccinea* flowers. *Cancer Letters, 130*, 197–202.

Liu, Y., Sun, J., Rao, S., Su, Y., & Yang, Y. (2013). Antihyperglycemic, antihyperlipidemic and antioxidant activities of polysaccharides from *Catathelasma ventriorrhiza* in streptozotocin induced diabetic rats. *Food Chemistry and Toxicology, 57*, 39–45.

McDonald, S., Prenzler, P. D., Autolovich, M., & Robards, K. (2001). Phenolic content and antioxidant activity of olive extracts. *Food Chemistry, 73*, 73–84.

Mopuri, R., Ganjayi, M., Banavathy, K. S., Naidu Parim, B., & Meriga, B. (2015). Evaluation of antiobesity activities of ethanolic extract of *Terminalia paniculata* bark on high fat diet-induced obese rats. *BMC Complementary and Alternative Medicine*, 15–76.

Ojobor Charles, C., Anosike Chioma, A., & Ani Chijiokes, C. (2015). Studies on the phytochemical and nutritional properties of *Tetracarpidium conophorum* (black walnut) Seeds. *Journal of Global Biosciences, 4*(2), 1366–1372.

Pathak, P., & Kapil, U. (2004). Role of trace elements zinc, copper and magnesium during pregnancy and its outcome. *Indian Journal Paediatric, 71*, 1003–1005.

Poongothai, K., Ponmurugan, P., Syed, Z. A. K., Senthil Kumar, B., & Sheriff, S. A. (2011). Antihyperglycemic and antioxidant effects of *Solanum xanthocarpum* leaves (field grown & *in vitro* raised) extracts on alloxan induced diabetic rats. *Asian Pacific Journal of Tropical Medicine, 4*(10), 778–785.

Rege, A., Juvekar, P., & Juvekar, A. (2012). *In vitro* antioxidant and anti-arthritic activities of shilajit. *International Journal of Pharmaceutical Sciences, 4*(2), 650–653.

Robert, K. M., Daryl, K. G., Peter, A. M., & Victor, W. R. (2003). Harper's illustrated biochemistry. In *Benders and Mayes vitamins and minerals* (p. 496). New York: Medical Publishing Division: Lange Medical Books/McGraw-Hill.

Ruch, R. J., Cheng, S. J., & Klaunig, J. E. (1989). Prevention of cytotoxicity and inhibition of intracellular communication by antioxidant catechins isolated from Chinese green tea. *Carcinogenesis, 10*, 1003–1008.

Shimada, K., Fujikawa, K., Yahara, K., & Nakamura, T. (1992). Antioxidant properties of xanthan on the autoxidation of soyabean oil in cyclodextrin emulsion. *Journal of Agricultural and Food Chemistry, 40*, 945–948.

Sidhra, S., Syed, Z. A. K., Krishnaveni, R., Anupriya, B., Senthil Kumar, B., & Kishore, R. (2017). Modulatory effect of *Leucas aspera* on oxidative stress and glucose metabolism against diabetic complications in experimental rats. *International Journal of Pharmaceutical Sciences, 8*(8), 27–33.

Singh, U., & Jialal, I. (2006). Oxidative stress and atherosclerosis. *Pathophysiology, 13*, 129–142.

Syed, Zameer Ahmed K., Sidhra, S., Ponmurugan, P., & Senthil Kumar, B. (2016). Ameliorative potential of *Solanum trilobatum* on oxidative stress in alloxan induced diabetic rats. *Pakistan Journal of Pharmaceutical Sciences, 29*(5), 1571–1578.

Syed, Z. A. K., Sidhra, S., Nithiya Priya, B. T., Krishnaveni, R., Muniraj, C., Venkatesan, T., Kisore, P. V., Karamchand, R., Rajadurai, S. R., & Moogambigai, S. M. (2018). *In vitro* radical scavenging activity and modulating effect of *Annona cherimola* on complications associated with diabetes in experimental diabetic rats-An approach to evaluate Asymmetrical Temperature Distribution Analysis Using Thermography. *Interventions in Obesity & Diabetes, 1*(5), 1–7.

Syed, Zameer Ahmed K., Sidhra, S., Senthil Kumar, B., Thanga, K. A., Geetha, K., Mohamed Rafi, M., Ponmurugan, P., & Kishore, R. (2017). Modulatory effect of dianthrone rich alcoholic flower extract of *Cassia auriculata* L. on experimental diabetes. *Integrative Medicine Research, 6*, 131–140.

Syed, Zameer Ahmed K., Sidhra, T. A., Sanjeeva, N., Senthil, K. B., Syed, T. S., & Ponmurugan, P. (2018). Radical scavenging potential, antiinflammatory and antiarthritic activity of isolated isomer methyl-γ-orsellinate and roccellatol from *Roccella Montagnei*. *Bulletin of Faculty of Pharmacy, Cairo University, 56*(1), 39–45.

Thomas, R. A., & Krishnakumari, S. (2015). Proximate analysis and mineral composition of *Myristica fragrans* seeds. *Journal of Pharmacognosy and Phytochemistry, 3*(6), 39–42.

Umapathy, S., Vasu, S., & Gupta, N. (2017). Computer aided diagnosis based hand thermal image analysis, a potential tool for the evaluation of rheumatoid arthritis. *Journal of Medical and Biological Engineering, 38*(4), 666–677.

Wenkam, A. (1990). In *Utilization and processing of fruits: 5* (pp. 388–506). London: Macmillan. Press.

Wills, R. S. (1998). *Post-harvest: An introduction to the physiology of handling fruits, vegetables and ornamental*: 560 (fourth Edn). Publication University of new Wales press Ltd.

Yang, J., Guo, J., & Yuan, J. (2008). *In vitro* antioxidant properties of rutin. *LWT – Food Science and Technology, 41*, 1060–1066.