Research Article

In vitro Mitochondrial β-oxidation is Enhanced by Phenolic Phytochemicals

Prabhanjana Kattepura Venkatesh[*1], **Deepti Mahishi Vasuki**[2], **Shirin Tarbiat**[3], **Cletus JM D'Souza**[4]
[1]Department of Biochemistry, Government Science College, Hassan, Karnataka, India.
[2]Department of Molecular reproduction, Development and Genetics, IISc, Bangalore, India.
[3, 4] Department of Studies in Biochemistry, University of Mysore, Mysore, Karnataka, India.
***Corresponding author's E-mail:** prabhanjana.kv04@gmail.com

Accepted on: 10-10-2014; **Finalized on:** 30-11-2014.

ABSTRACT

Obesity is a leading cause of complications such as type-2 diabetes, hypertension, osteoarthritis, asthma and cardiovascular diseases. The available anti-obesity drugs are associated with side effects. Hence there is a need for natural therapies with potential anti-obesity action. Molecules that enhance β-oxidation would have anti-obesity action. To determine the effect of phytochemicals on enhancing β-oxidation, a direct spectrophotometric measurement by intact mitochondria was used. The intactness of mitochondria was tested using succinate (0.2 M) oxidation coupled to DCPIP reduction. β-oxidation of butyrate was monitored by ferricyanide reduction and was used to test the effect of raspberry ketone, capsaicin and ethanolic extracts of *Artocarpus lakoocha* Roxb, (Moraceae) and methanolic extract of *Tamarindus indica* L., (Fabaceae), on β-oxidation. Butyrate reduced 6.5± 1.3 nmol ferricyanide /mmol butyrate. Raspberry ketone reduced 48.5± 2.1 nmol ferricyanide/mmol raspberry ketone. Enhancement of β-oxidation by capsaicin showed hormetic effect. At 10 µM concentration capsaicin reduced 37.2 nmol of ferricyanide. *Artocarpus lakoocha* extract reduced 13.8 nmol ferricyanide /mg extract. *Tamarindus indica* extract reduced 2 µmol ferricyanide /mg extract. *Tamarindus indica* extract in HPLC analysis showed one major component accounting for 73.6% by composition and corresponded to gallic acid whereas *Artocarpus lakoocha* extract had only 15% gallic acid. Our results suggest that phenolics and plant extracts containing phenolics enhanced β-oxidation of fatty acids.

Keywords: *Artocarpus lakoocha* extract, Butyrate, Capsaicin, Ferricyanide reduction, Raspberry ketone, *Tamarindus indica* extract, β-oxidation.

INTRODUCTION

The proximate cause of obesity is the imbalance in energy input and energy expenditure. The energy expenditure involves three components. They are basal metabolic rate (BMR), thermic effects of foods and activity related thermogenesis.[1] Activity related thermogenesis is a highly variable component of energy expenditure.[2] Highly active people spend up to three times more energy per day than inactive people and the energy difference is estimated to be about 2000 kcal/day.[3]

Although exercise is the most important factor that would reduce obesity, most obese people are unable to spare or are not willing to spare some time for it. Hence there is a need for drugs that can reduce energy intake or increase energy expenditure to help obese individuals. Anti-obesity drugs that target energy intake are associated with severe neurological side effects. Hence attention is now focused on drugs that can increase energy expenditure. In this direction plant extracts and phytochemicals have attracted a lot of attention because of their diverse anti-obesity effects.

Anti-obesity plants are classified based on their mechanism of action. They are pancreatic lipase inhibitors,[4] pre adipocyte differentiation inhibitor,[5] appetite suppressors,[6-9] enhancers of lipid metabolism[10-12] and enhancers of thermogenesis.[13] One of the most popular anti-obesity plant products is hydroxycitric acid of *Garcinia cambogia* which was shown to decrease appetite. Several plant extracts like *Pinellia ternate* and *Undaria pinnatifida* were shown to induce the expression of uncoupler protein 1 (UCP1) and upregulate peroxisome proliferator-activated receptor alpha (PPARα) expression.[14-17] Several other plants have been shown to reduce body weight in experimental animals but the mechanism of reduction is not known.[18,19]

Phytochemicals like caffeine[20,21] and capsaicin have been shown to enhance energy expenditure in experimental animals as well as in clinical studies.[22,23] The mechanism for anti-obesity is one of thermogenesis produced by up regulating UCP-1 rather than by increasing β-oxidation.

Tamarindus indica (Tamarind) and *Artocarpus lakoocha* Roxb (artocarpus) are both used as souring agents in south Indian cooking. Both tamarind and *Artocarpus lakoocha* have been found to have diverse biological activities and health promoting effects.

In this study we have investigated the ability of phytochemicals like raspberry ketone, capsaicin and plant extracts from the fruits of tamarind and *Artocarpus lakoocha* for their ability to increase mitochondrial β-oxidation.

MATERIALS AND METHODS

Cytochrome c, ADP, HEPES, delipidated BSA and capsaicin were from Sigma-Aldrich chemicals, India. Tris, EDTA, Sodium azide and DCPIP were from SRL, India. Mannitol

© Copyright protected. Unauthorised republication, reproduction, distribution, dissemination and copying of this document in whole or in part is strictly prohibited.

was from Denis Chem Lab Limited. Potassium ferricyanide was from Glaxo laboratories, India. Butyric acid was from Estaman Chemical Co. Germany and Raspberry ketone from NHU pharmaceutical co., China, were used for study.

Isolation of mitochondria from rat liver

Mitochondria were isolated from rat liver by the method described by Palloti & Lenaz.[24] Briefly, about 20 g of fresh liver was washed in Solution-1 (0.22M Mannitol, 0.07M Sucrose, 0.02M HEPES, 2mM Tris HCl, 1mM EDTA) and it was minced with scissors. The minced contents were washed thrice in solution-2 (solution-1 containing 0.4% BSA) in order to remove blood and connective tissue. The minced contents were homogenized in a Potter-Elvehjem homogenizer, in solution-2 in a ratio of 1:20(w/v). The homogenate was centrifuged at ~800g for 2 minutes. The supernatant was decanted and pellet was resuspended in solution-1. Again it was centrifuged at ~800g for 2 minutes. Supernatants were collected from both the steps and mixed.

The mixture was centrifuged at ~8500g for 4 minutes, supernatant was decanted and the pellet was washed in solution-1, and recentrifuged at ~8500g for 6 minutes. The pellet was resuspended in solution-3 (0.22M Mannitol, 0.07MSucrose, 0.01M Tris HCl, 1mM EDTA, pH 7.2) and centrifuged at ~8500g for 8 minutes. The pellet was finally resuspended in solution-3 at a ratio of 1 ml/g of starting material and it was stored at $4^{0}C$ until used. For each experiment freshly prepared mitochondria were used.

Intactness of mitochondria

The intactness of mitochondria was tested using the reduction of an artificial electron acceptor after blocking the flow of electrons through the mitochondrial electron transport system using sodium azide. This poison prevents the transfer of electrons from cytochrome a_3 to the final electron acceptor, oxygen. Hence the electrons from SDH-$FADH_2$ are passed on to an artificial electron acceptor 2, 6 dichloro phenolindophenol (DCPIP). The reduction of DCPIP can be followed spectro photometrically. The oxidized form of the dye is blue and the reduced form is colourless. The succinate de hydrogenase (SDH) (EC 1.3.99.1) assay was carried out with slight modification as described by Jty and King.[25] The reaction was carried out in a total volume of 5ml containing assay medium (0.3M Mannitol, 0.006M KH_2PO_4, 0.014M K_2HPO_4, 0.01M KCl, 0.005M $MgCl_2$, pH 7.2), DCPIP (0.5mM), succinate (0.2mM), and sodium azide (0.04M) treated mitochondria (about 50 mg protein). The reactants were mixed and immediately taken in cuvette. The reduction of DCPIP was monitored at 600 nm, every 5 minutes for 35 minutes.

β-oxidation assay using butyric acid

In this assay, β-oxidation dependent reduction of ferricyanide was carried out by the method described by Osmundsen and Bremer.[26] In 1938, Quastel and Wheatley used ferricyanide to measure the oxidation of substrates by various tissue preparations in the absence of oxygen.[27]

Ferricyanide is reduced to ferrocyanide by accepting one electron and generates one proton as follows:

$$2\,Fe(CN)_6^{3-} + NADH \longrightarrow 2Fe(CN)_6^{4-} + H^+ + NAD^+$$

The inner mitochondrial membrane is impermeable to ferricyanide.[28,29] The major site of ferricyanide reduction by intact mitochondria is cytochrome c (cyt c). This is facilitated by the localization of cytochrome c at the outer face of the inner mitochondrial membrane.[30]

Butyrate can be oxidized to acetate in the mitochondria through the β-oxidation pathway and the electrons are transferred to cyt c through cyt b, coQ and cyt c_1, located on the inner mitochondrial membrane. The flow of electrons from cyt c to oxygen is blocked by the use of azide which inhibits electron transport from cyt a_3 to oxygen. The reduced cyt c can then be oxidized by ferricyanide.

The reaction was carried out in a total volume of 3 ml containing HEPES buffer (10 mM pH 7.2), KCl solution (130mM), EDTA solution (0.1mM), sodium azide (1mM), K_2HPO_4(1mM), delipidated BSA (4.5 mg), $K_3Fe(CN)_6$(0.5mM), cytochrome c (0.3mg). Butyric acid (0, 10mM and 20mM). The reaction was started by adding mitochondrial suspension (~50 mg protein). The optical density was measured at 420 nm, every 5 minute for up to 35 minute. To avoid turbidity, the reaction mixture was briefly centrifuged for 30 sec at 3000g and the clear supernatant was used to measure optical density. The amount of ferricyanide reduced was calculated using the molar Extinction coefficient of 1043$M^{-1}cm^{-1}$.

Protein estimation

Mitochondria were subjected to alkaline lysis. The amount of protein present in the mitochondria was estimated using Lowry's method, taking bovine serum albumin as standard.[31]

Preparation of Tamarind extract

26.3 g of shade dried tamarind pulp was boiled with 250 ml methanol for 1 hour and filtered. Methanol was removed by distillation. The extract was concentrated under reduced pressure using a rotary flash evaporator and the final concentration was 52mg/ml.

Preparation of *A. lakoocha* extract

50 g dried *A. lakoocha* fruit was refluxed with 500 ml ethanol for 1 hour and then it was filtered. The filtrate was distilled to remove ethanol. The volume was reduced to 50 ml. Further evaporation was carried out under reduced pressure in a rotary flash evaporator and reduced to 20 ml. It was stored in an amber bottle. Final concentration was 200mg/ml.

© Copyright protected. Unauthorised republication, reproduction, distribution, dissemination and copying of this document in whole or in part is strictly prohibited.

Phytochemical analysis

Tamarind extract and *Artocarpus lakoocha* extract were subjected to qualitative phytochemical analysis.[32]

Preparation of stock solutions of phytochemicals

Capsaicin was dissolved in methanol such that the concentration was 1 mg/ml. Raspberry ketone was dissolved in acetonitrile such that the concentration was 10 mg/ml.

HPLC sample preparation for plant extracts

2ml of sample (Tamarind and *Artocarpus lakoocha* extracts) was suspended in 25ml acidified methanol (0.1 % HCl), on a magnetic stirrer for 15 minutes. Then it was filtered using whatman filter paper 1. The filtrate was flash evaporated and re-dissolved in 25ml of 20% ethanol. The ethanol fraction was taken in a separating funnel and was extracted with 20ml ethyl acetate thrice. The ethyl acetate fractions were pooled and filtered through sodium sulphate (anhydrous), to remove water.

The dried ethyl acetate fraction was flash evaporated and redissolved in 2ml of 50% methanol. This was filtered using syringe membrane filter (0.45µm) and stored at -20 °C for further use. 20µl of this was loaded to the HPLC C-18 column (Supelco, Bellafonte, PA, USA). Using a UV-visible detector (operating at 280 nm) the extracts were analyzed on a HPLC system (LC10AVP, Shimadzu, Kyoto, Japan). The mobile phase consisted of water:methanol:acetic acid (80:18:2 by volume). Optimal separations were achieved using an isocratic condition at the flow rate of 1ml/min. The eluted compounds were detected by their absorbance at 280 nm. The eluted compounds were detected by their absorbance at 280 nm.

Effect of phytochemicals and plant extracts on β-oxidation

β-oxidation of butyrate using ferricyanide reduction was carried out as described above in the presence of varying amounts of raspberry ketone (0.1,0.2 and 1mM),capsaicin (5,10 and 20 µM), tamarind extract (5,10 and 20 µg) and artocarpus lakoocha extract (0.5 mg, 1mg and 2mg). Since capsaicin, tamarind extract and artocarpus extract were made in organic solvent, the solvent was removed by evaporation prior to assay.

RESULTS

Intactness of mitochondria

The intactness of mitochondria was tested using the DCPIP reduction by the mitochondria and the assay standardization is shown in figure 1.

In the absence of azide or succinate, there was a little reduction of DCPIP. When the flow of electrons to O_2 was inhibited by azide, the oxidation of succinate resulted in the reduction of cyt c, which in turn could reduce DCPIP to a colourless form.

β-oxidation of Butyrate

Butyrate can enter the mitochondrial matrix without the help of carnitine and can undergo one cycle of β-oxidation. The NADH and $FADH_2$ generated in the reaction result in the reduction of cyt c which can then reduce ferricyanide. The reduction of ferricyanide in the presence of butyrate is shown in figure 2.

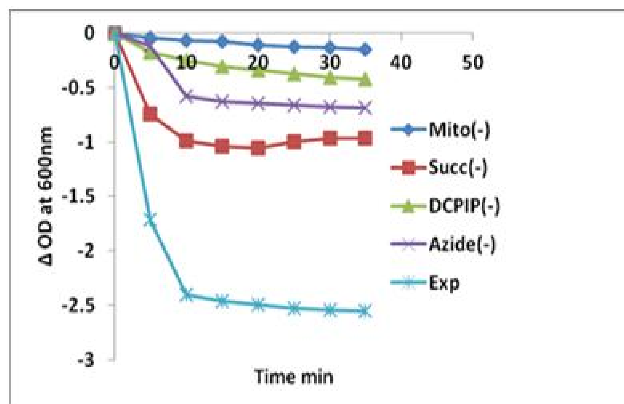

Figure 1: Standardization of DCPIP reduction assay. DCPIP reduction was measured in the absence and presence of substrate (succinate), inhibitor (azide) and artificial electron acceptor (DCPIP) as described in methods.

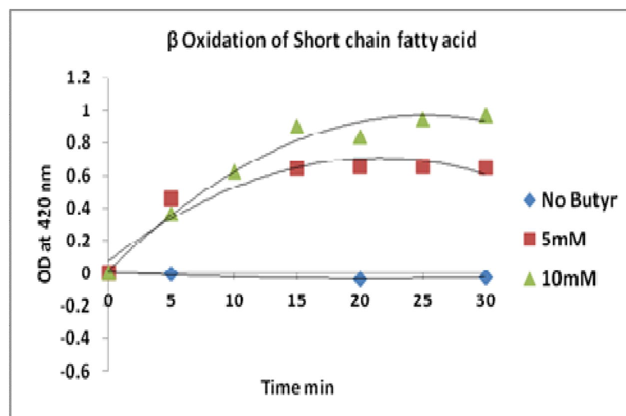

Figure 2: Standardization of β-oxidation of Butyrate. Ferricyanide reduction as a function of butyrate concentration was measured in the presence of inhibitor (azide) and artificial electron acceptor (ferricyanide) as described in the methods.

With increasing concentration of butyrate there was an increased reduction of ferricyanide. In the absence of butyrate there was no detectable ferricyanide reduction.

Raspberry ketone by itself did not induce ferricyanide reduction (data not shown). However in the presence of butyrate and the raspberry ketone, there was a dose dependent ferricyanide reduction and is shown in figure-3. Capsaicin showed a decrease in the ferricyanide reduction with increase in concentration. In the presence of 10µM capsaicin, there was an increase in the ferricyanide reduction. Ferricyanide reduction in the presence of *Tamarindus indica* extract and *Artocarpus lakoocha* extract are also shown in figure 3. While the tamarind extract showed a dose dependent increase in

© Copyright protected. Unauthorised republication, reproduction, distribution, dissemination and copying of this document in whole or in part is strictly prohibited.

ferricyanide reduction, artocarpus extract showed a dose dependent decrease in the ferricyanide reduction.

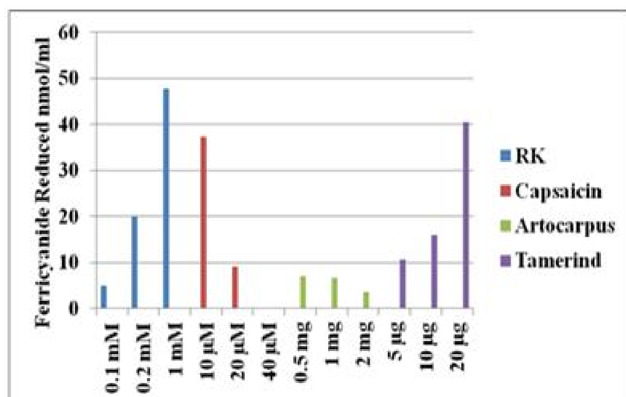

Figure 3: Effect of raspberry ketone (RK), capsaicin, *Tamarindus indica* (Tamarind) extract and *Artocarpus lakoocha* (Artocarpus) extract on β-oxidation of butyrate. Ferricyanide reduction was measured in the presence of 10mM butyrate and increasing concentrations of raspberry ketone, capsaicin, *Tamarindus indica* extract and *Artocarpus lakoocha* extract as described in the methods.

The results of phytochemical analysis of *Tamarindus indica* extract and *Artocarpus lakoocha* extract are shown in Table 1. Both the extracts contained phenolics and flavonoids among other phytochemicals.

Table 1: Phytochemical analysis of plant extracts

Extract	Tan	Phe	Alk	Gly	Sap	Ste	Ter	Fla
Artocarpus	+	+	–	+	–	+	+	+
Tamarind	+	+	–	+	+	–	+	+

Tan= Tannins, Phe= Phenolics, Alk= Alkaloids, Gly= Glycosides, Sap= saponins, Ste= Steroids, ter=Terpenes, Fla= flavonoids.

The qualitative phytochemical analysis of plant extracts was performed as described in the methods. '+' indicates presence and '–`indicates absence of the component.

The HPLC profile of *Tamarindus indica* extract and *Artocarpus lakoocha* extract are shown in figure 4 and figure 5 respectively.

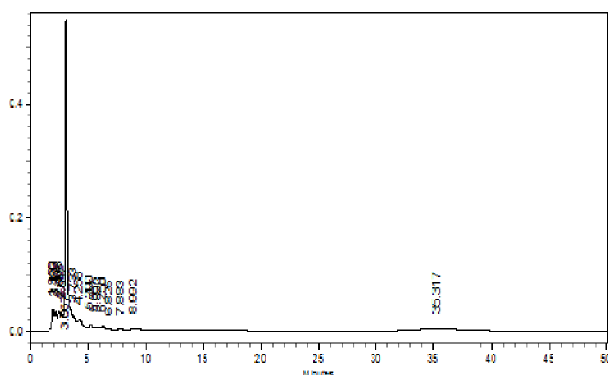

Figure 4: HPLC profile of *Tamarindus indica* extract

Tamarindus indica extract was subjected to HPLC analysis on a reverse phase column as described in methods.

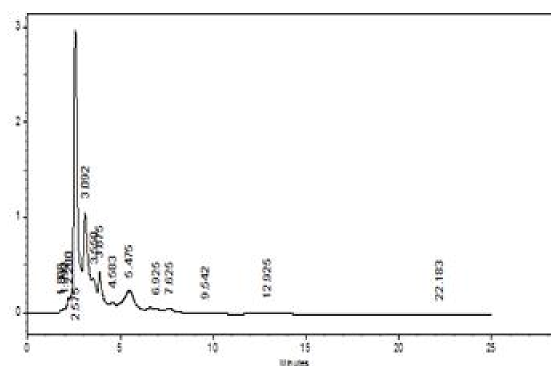

Figure 5: HPLC profile of *Artocarpus lakoocha* extract

Artocarpus lakoocha extract was subjected to HPLC analysis on a reverse phase column as described in methods.

Tamarindus indica and artocarpus extract had one major compound each, accounting for more than 73.6% and 62.1% of the total components respectively. The retention time of these major compounds are compared with the retention times of reference phytochemicals are shown in Table 2.

Table 2: Identification of HPLC fractions of plant extracts

Compound	Retention Time	
	Extracts	Reference Compounds
Myrecetin	2.575	2.633
Gallic acid	3.067	3.092

HPLC analysis of reference standards and the extracts was performed as described in the methods. The retention time of the reference compound nearest to the component in the extract are given.

DISCUSSION

Ferricyanide assay is a convenient assay to measure the β-oxidation of fatty acids. We used butyrate which does not require carnitine to transport it across the mitochondrial membranes. Also it undergoes a single cycle of β-oxidation to form acetate. The acetate that is generated can enter TCA cycle and get oxidized producing 3moles of NADH and one mole of $FADH_2$ per mole of acetyl coA in each cycle of the passage through the TCA cycle. The electrons from the reducing equivalents ultimately go to molecular oxygen via the electron transport chain. While the cytochromes b, c and CoQ are located on the inner surface of the inner mitochondrial membrane, cyt c is located on the outer surface of the inner mitochondrial membrane and is accessible to DCPIP and ferricyanide, both of which cannot pass through the inner mitochondrial membrane. Hence when azide is used to block the flow of the electrons from cyt a3 to oxygen the electron carriers would remain in the reduced form and the electrons can then be transferred from cyt c to artificial electron acceptors. This is the basis of β oxidation assay. The reducing equivalents generated by butyryl CoA dehydrogenase (EC1.3.99.3) and 3-hydorxy

© Copyright protected. Unauthorised republication, reproduction, distribution, dissemination and copying of this document in whole or in part is strictly prohibited.

butyryl CoA would both reach cytc1 from which it would go to cytochrome c. Ferricyanide can be reduced by reduced cyt c. The electron transport through the respiratory chain of mitochondria requires that the inner mitochondrial membrane be intact. In our study, we have found that freshly prepared mitochondria were able to reduce DCPIP in the presence of substrate (succinate or butyrate) and a respiratory inhibitor (azide). Ferricyanide reduction also requires an intact mitochondrial membrane. In our study we have shown that addition of butyrate enhanced ferricyanide reduction suggesting that the butyrate is getting oxidized. In the absence of butyrate, there was no reduction of ferricyanide.

Fatty acids are catabolised by the β-oxidation pathway taking place mainly in the mitochondria and also in the peroxisomes. This is the major pathway for the utilization of fatty acids since they are not excreted in any intact form or even partially degraded form like cholesterol. Hence the only mechanism available for reducing obesity is either reducing the intake of excess calories or burning the excess energy through exercise mediated β oxidation.

Phytochemicals and plant extracts that can enhance β-oxidation of fatty acids would act as anti-obesity molecules since they would promote β-oxidation of fatty acid without exercise. Capsaicin has been shown to have anti obesity property. The anti-obesity effect was attributed to increase in UCP1 resulting in thermogenesis. In this study we wanted to see whether capsaicin can also increase β-oxidation. In our study we found that capsaicin increased β-oxidation. These results are consistent with those published by Hsu and Yen.[33] However, Capsaicin showed hormetic effect where increasing concentration showed a decreased physiological effect.[34] Raspberry ketone enhanced β-oxidation of butyrate which confirms its use as anti-obesity phytochemical.[35,36] The mechanism by which Raspberry ketone may act as anti-obesity phytochemical may be through enhancing β-oxidation of fatty acids. Mechanism by which phytochemicals enhance β-oxidation is shown via several pathways in the liver where AMP activated protein kinase and PPARγ are implicated in increasing β-oxidation.[37] However, these mechanisms would not be relevant in our study since this is an *in vitro* study using only the mitochondria. The mechanisms by which phytochemicals enhance β-oxidation are not known. However, phenolics and flavonoids are the major constituents of plant secondary metabolites.[38] Flavonoids have been shown to possess anti obesity properties[39] but their role in enhancing β-oxidation *in vitro* is not known.

In our study, the phytochemical analysis showed the presence of both phenolics and flavonoids in the extracts of *Tamarindus indica* and *Artocarpus lakoocha*. *Tamarindus indica* extract showed ferricyanide reduction in a dose dependent manner whereas *Artocarpus lakoocha* showed hormetic effect. The major phenolic compound of *Tamarindus indica* extract was gallic acid whereas the major component of *Artocarpus lakoocha* extract was myrecitin, which is a flavonoid. *Artocarpus lakoocha* extract had about 15% gallic acid. The higher rate of reduction of ferricyanide by tamarind extract and lower rate of reduction of ferricyanide by *Artocarpus lakoocha* extract correlate with the relative amounts of phenolics present in these extracts. Hence it is possible that gallic acid may enhance the β-oxidation. These results are consistent with our observation that raspberry ketone and capsaicin could enhance β-oxidation; both raspberry ketone and capsaicin are phenolic compounds.

CONCLUSION

Our studies show that phenolic compounds enhance β-oxidation. Whether the enhancement in β-oxidation is a receptor mediated effect is not known at present. Our experimental results showed that phenolic compounds like raspberry ketone and capsaicin effectively reduced ferricyanide by oxidizing butyrate. Capsaicin also showed hormesis. *Tamarindus indica* extracts showed enhanced β-oxidation. *Artocarpus lakoocha* extracts showed a hormetic effect.

Acknowledgement: The authors thank Institution of Excellence IOE, University of Mysore for a research grant. The use of equipment obtained under VGST grant is gratefully acknowledged. The authors also thank Mr. Austin Richard S, Mr. Vikram Joshi and Mr Sumanth M.S. for their help in the preparation of the manuscript.

REFERENCES

1. Donahoo WT, Leveine JA, Melanson EL, Variability in energy expenditure and its components, Curr Opin Clin Nutr Metab Care, 6, 2004, 599-605.
2. Black AE, Coward WA, Cole TJ, Prentice AM, Human energy expenditure in affluent societies: An analysis of 574 doubly labelled water measurements, Eur J Clin Nutr, 50(2), 1996, 72-92.
3. Levine JA, Non exercise activity thermogenesis uberating the life force, J Interns Med, 262(3), 2007, 273-87.
4. Kim HY, Kang MH, Screening of Korean medicinal plants for lipase inhibitory activity, Phytother Res, 19, 2005, 359–361.
5. Kim MS, Kim JK, Kwon DY, Park R, Anti-adipogenic effects of Garcinia extract on the lipid droplet accumulation and the expression of transcription factor, Biofactors, 22, 2004, 193–196.
6. Kim JH, Hahm DH, Yang D, Kim JH, Lee HJ, Shiml, Effect of crudesaponin of Korean red ginseng on high-fat diet-induced obesity in the rat, J Pharmacol. Sci, 97, 2005, 124–131.
7. Saito M, Ueno M, Ogino S, Kubo K, Nagata J, Takeuchi M, High dose of Garcinia cambogia is effective in suppressing fat accumulation in developing male zucker obese rats, but highly toxic to the testis, Food Chem Toxicol, 43, 2005, 411–419.
8. Heymsfield SB, Allison DB, Vasselli JR, Pietrobelli A, Greenfield D, Nunez C, Garcinia cambogia (hydroxycitric acid) as a potential antiobesity agent: a randomized controlled trial, JAMA, 280, 1998, 1596–1600.
9. Ohia S, Opere CA, Leday AM, Bagchi M, Bagchi D, Stohs SJ, Safety and mechanism of appetite suppression by a novel hydroxycitric acid extract (HCASX), Mol Cell Biochem, 238, 2002, 89–103.

© Copyright protected. Unauthorised republication, reproduction, distribution, dissemination and copying of this document in whole or in part is strictly prohibited.

10. Rong X, Kim MS, Su N, Wen S, Matsuo Y, Yamahara J, Murray M, Li Y, An aqueous extract of Salacia oblonga root, a herb-derived peroxisome proliferator-activated receptor-alpha activator, by oral gavage over 28 days induces gender-dependent hepatic hypertrophy in rats, Food Chem Toxicol, 46, 2008, 2165–2172.

11. Huang C, Zhang Y, Gong Z, Sheng X, Li Z, Zhang W, Qin Y, Berberine inhibits 3T3- L1 adipocyte differentiation through the PPARgamma pathway, Biochem Biophys ResCommun, 348, 2006, 571–578.

12. Kim SO, Yun SJ, Jung B, Lee EH, Hahm DH, Shim I, Lee HJ, Hypolipidemic effects of crude extract of adlay seed (Coix lachrymajobi var.Mayuen) in obesity rat fed high fat diet: relations of TNF-alpha and leptin mRNA expressions and serum lipid levels, Life Sci, 75, 2004, 1391–1404.

13. Ishihara K, Oyaizu S, Fukuchi Y, Mizunoya W, Segawa K, Takahashi M, Mita Y, Fukuya Y, Fushiki T, Yasumoto KA, soyabean peptide isolate diet promotes postprandial carbohydrate oxidation and energy expenditure in type II diabetic mice, The Journal of nutrition, 133(3), 2003, 752-757.

14. Kim YJ, Shin YO, Ha YW, Lee S, Oh JK, Kim YS, Anti-obesity effect of Pinellia ternata extract in zucker rats, Biol Pharm Bull, 29, 2006, 1278–1281.

15. Maeda H, Hosokawa M, Sashima T, Funayama K, Miyashita K, Fucoxanthin from edible seaweed, Undaria pinnatifida, shows anti-obesity effect through UCP1 expression in white adipose tissues, Biochem Biophys Res Commun, 332, 2005, 392–397.

16. Maeda H, Hosokawa M, Sashima T, Funayama K, Miyashita K, Effect of medium-chain triacylglycerols on anti-obesity effect of fucoxanthin, J Oleo Sci, 56, 2007, 615–621.

17. Maeda H, Hosokawa M, Sashima T, Miyashita K, Dietary combination of fucoxanthin and fish oil attenuates the weight gain of white adipose tissue and decreases blood glucose in obese/diabetic KK-Ay mice, J Agric Food Chem, 55, 2007, 7701–7706.

18. Oben J, Kuate D, Agbor G, Momo C, Talla X, The use of a Cissus quadrangularis formulation in the management of weight loss and metabolic syndrome, Lipids Health Dis, 5, 2006, 24.

19. Oben JE, Enyeque DM, Fomekonq GI, Soukontoua YB, Aqbor GA, The effect of Cissus quadrangularis (CQR-300) and a Cissus formulation (CORE) on obesity and obesity-induced oxidative stress, Lipids Health Dis, 6, 2007, 4.

20. Dulloo AG, Ephedrine, xanthines and prostaglandin-inhibitors: actions and interactions in the stimulation of thermogenesis, Int J Obes Relat Metab Disord, 17, 1993, S35–S40.

21. Racotta IS, Leblanc J, Richard D, The effect of caffeine on food intake in rats: involvement of corticotropin-releasing factor and the sympatho-adrenal system, Pharmacol Biochem Behav, 48, 1994, 887–892.

22. Reinbach HC, Smeets A, Martinussen T, Moller P, Westerterp-Plantenga MS, Effects of capsaicin, green tea and CH-19 sweet pepper on appetite and energy intake in humans in negative and positive energy balance, Clin Nutr, 28, 2009, 260–265.

23. Shin KO, Moritani T, Alterations of autonomic nervous activity and energy metabolism by capsaicin ingestion during aerobicexercise in healthy men, J Nutr Sci Vitaminol (Tokyo), 53, 2007, 124–132.

24. Pallotti F, Lenaz G, Isolation and sub fraction of mitochondria from animal cells and tissue culture lines, methods cell biol, 80, 2001, 3-44.

25. Jty WU, King TE, Cleavage of succinate dehydrogenase from respiratory chain by cyanide, Fed proc, 26, 1967, 732.

26. Osmundsen H, Bremer J, A Spectrophotometric Procedure for Rapid and Sensitive measurements of β oxidation, Biochem J, 164, 1977, 621-633

27. Quastel JH, Wheatley AHM, Anaerobic oxidations on ferricyanide as a reagent for the manometric investigation of dehydrogenase systems, Biochem J, 32, 1938, 936-943.

28. Mitchell P, Moyle J, Estimation of membrane potential and pH difference across the cristae membrane of rat liver mitochondria, Eur J Biochem, 9, 1969, 149-155.

29. Klingenberg, M, in Kolloquium der Gesellschaft fur Biologische Chemie 19th, Mosbach, Springer-Verlag, Berlin, Heidelberg and New York, 1968, 131-133.

30. Klingenberg M, Localization of glycerol phosphate dehydrogenase in the outer phase of the mitochondrial inner membrane, Eur J Biochem, 13, 1970, 247-252.

31. Lowry OH, Rosebrough NJ, Farr AL, Randall RJ, Protein measurement with the Folin phenol reagent, J Biol Chem, 193, 1951, 265.

32. Tiwari P, Kumar B, Kaur M, Kaur G, Kaur H, Phytochemical screening and extraction: A review, Intr Phrma Sci, 1, 2011, 98-106.

33. Hsu CL, Yen GC, Effects of capsaicin on induction of apoptosis and inhibition of adipogenesis in 3T3-L1 cells, J Agric Food Chem, 55, 2007, 1730–1736.

34. Calabrese EJ, Baldwin LA, Hormesis as a biological hypothesis, Environ Health Perspect, 106 (Suppl 1), 1998, 357-362.

35. Chie M, Satoh Y, Hara M, Inoue S, Tsujita T, Okuda H, Anti-obese action of raspberry ketone, Life Sci, 77, 2005, 194-204.

36. Park KS, Raspberry Ketone Increases Both Lipolysis and Fatty Acid Oxidation in 3T3-L1 Adipocytes, Planta Med, 76, 2010, 1654–1658.

37. Kondo T, Kishi M, Fushimi T, Kaga T, Acetic acid up regulates the expression of genes for fatty acid oxidation enzymes in liver to suppress body fat accumulation, J Agric Food Chem, 57, 2009, 5982–5986.

38. Crozier A, Jaganath IB, Clifford MN, Phenols, Polyphenols and Tannins: An Overview, in plant secondary metabolites occurrence, structure and role in the human diet. (Crozier A, Clifford MN, Ashihara H UK,eds) blackwell publishing Ltd., 2006, 1-24.

39. Harmon AW, Harp JB, Differential effects of flavonoids on 3T3-L1 adipogenesis and lipolysis, Am J Physiol Cell Physiol, 280, 2001, 807-13.

Source of Support: Nil, **Conflict of Interest:** None.

© Copyright protected. Unauthorised republication, reproduction, distribution, dissemination and copying of this document in whole or in part is strictly prohibited.