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Studies on the physicochemical characteristics of heated honey, honey mixed with *ghee* and their food consumption pattern by rats

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Abstract

Honey and ghee are the two food substances used widely in our diet. In *Ayurveda*, it is quoted that heated honey and honey mixed with equal amount of ghee produce deleterious effects. Hence, it was of our interest to study the physicochemical characteristics and chemical constituents of heated honey and honey mixed with ghee, and their effect on daily food intake and organ weights of rats. The specific gravity of samples showed a significant decrease in honey and ghee samples heated to 140°C. The pH of honey heated to 140°C was elevated with a reduction in the specific gravity. There was a significant rise in hydroxymethyl furfuraldehyde (HMF) in 60° and 140°C heated honey samples. The browning and total antioxidant of honey mixed ghee samples was significantly higher when compared to ghee samples. Further, the authors have also evaluated the effects of consumption of heated honey, ghee, honey mixed with equal amount of ghee and heated honey mixed with heated ghee in rats. The feeding of heated honey and honey mixed with ghee for 6 weeks showed no significant change in the food intake, weight gain and relative organ weights. The study revealed that the heated honey mixed with ghee produces HMF which may cause deleterious effects.

Key words: Ghee, heated honey and heated ghee, honey mixed with ghee, honey, hydroxymethyl furfuraldehyde.

Introduction

In *Ayurveda*, food is considered as God *Brahma* and also one of the three basic pillars (food, sleep and celibacy, i.e., *ahara*, *nidra* and *Brahmacharya*) for a healthy life.[1] Food-related diseases include nutritional deficiency, food contamination, food intolerance and also food incompatibility. Food incompatibility may develop because of contradictory qualities, contradictory combination, opposed processing procedures and the nature of food itself. Incompatible food habits lead to a number of diseases including sterility, herpes, eye diseases, skin eruptions, ascites, fistula, leprosy, sprue, edema, fever, rhinitis, fetal distress and may even cause death.[2]

Honey is a sweet, sticky, yellowish fluid made by bees from nectar.[3] Ghee is the Indian name for clarified butter fat, and is usually prepared from cow's milk, buffalo's milk or from mixed milk.[3] Honey and ghee are the two food substances used widely in our diet. The commercial honey available in the market is heat-processed, which absolutely contradicts the accepted Ayurvedic concept. Heating of honey is much discussed under quality deterioration, wherein certain enzymes and nutrients are lost. Honey cakes, honey candies, etc., that are baked are also available for consumption. In some instances, honey and ghee are mixed and served as food. In *Ayurveda*, Acharya *Charaka* has quoted that heated honey and honey mixed with equal ghee produce deleterious effects in the body and may cause death also.[4] Hence, the authors have evaluated the physicochemical characteristics of heated honey mixed with heated ghee and its food consumption pattern by rats.

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Materials and Methods

Materials

All the biochemicals used in these investigations were of highest purity and procured from Sigma company, St. Louis street, MO, USA; Merck, Darmstadt, Germany, Sisco Research Laboratory (Mumbai, India), Across Organics (Mumbai, India), Spectrochem (Mumbai, India) and S.D. Fine Chemicals (Mumbai, India). All the organic solvents were of analytical reagent grade.

Unprocessed natural honey (raw) was procured from Madikeri forest, Karnataka. The honey samples were heated to 60 and 140°C in order to generate hydroxymethyl furfuraldehyde (HMF). Cow's ghee was procured from Salem, Tamil Nadu.

Processed honey (*Dabur*) was procured from the market. Processed ghee (*Nandini*) was procured from Mysore Dairy.

Honey and ghee mixture: Ghee samples were mixed with equal quantity of honey and similarly for the 60 and 140ºC heated samples.

Methods

Inbred male rats (*Rattue norvegicus*) of Wistar strain reared in the Defence Food Research Laboratory, Mysore, in the weight range of 100–150 g were used for the entire study. Male Wistar rats were divided into six groups of six rats in each. Group I was fed with normal pellet diet, and served as a control. Group II rats were fed with honey along with pellet diet. Group III rats were fed with heated (140ºC) honey with pellet diet. Group IV rats received with ghee with pellet diet. Group V rats were fed with equal amount of honey mixed ghee with pellet diet. Group VI rats were fed with heated honey (140ºC) mixed with heated ghee with diet. Clearance of experimental design by the Institutional Ethical committee for rats was taken.

Dose fixation

Ayurvedic classics do not contain the dose of honey and ghee that can be taken together as a part of regular diet. Hence, the dose was fixed based on clinical practice. As per Ayurvedic practice, for a 70 kg man, the normal dose of honey recommended is 48 g/day (1 *pala matra*) and recommended dose of ghee is 30 ml/day (*hraseeyasi matra*). Accordingly, the dose of honey was fixed at 102 mg of honey/day and that of ghee was calculated to be 64 µl/day for the Wistar rats of about 150 g body weight.

The animals were kept at ambient temperature and exposed to light–dark cycle of 12 hours duration each. The leftover diet was collected, dried and weighed to determine the food intake. Weekly food intake and weight gain were monitored. Blood was collected from the orbital plexus of the rats on 0 day and at the end of 6 weeks before sacrifice. The reagents used in the biochemical assays were of highest purity and procured from AGAPPE Diagnostics (Kerala, India), Crest Bio-Systems (Goa, India) and Span Diagnostics (Surat, India). After the completion of 6 weeks, the rats were sacrificed under mild anesthesia (sodium pentobarbitone, 50 mg/kg body weight i.p.) and liver, *Kidney*, heart, brain and colon tissues were quickly excised.

Total sugars and reducing sugars in honey and ghee samples were estimated as per the prescribed procedure.[5] Total ash, acid insoluble ash and specific gravity were estimated as per the method of AOAC International.[6] HMF and browning index in honey were studied spectrophotometrically. The amount of total polyphenolic compounds was measured.[7] The determination of flavonoids was performed according to a colorimetric assay.[8] The antioxidant activity was determined by the standard method prescribed.[9] Peroxide value (PV) was determined according to the AOAC method.[10] Free fatty acid (FFA) value[11] and Thiobarbituric acid value (TBA) value[12] in ghee were also estimated.

Results and Discussion

Heating of honey is contraindicated according to *Ayurveda* as it causes deleterious effects. Hence, to evaluate this concept, honey has been heated beyond the permissible temperature, i.e., 140ºC for 2 minutes. On heating ghee, there is formation of more peroxides. Hence, the study has been conducted to evaluate the effects of heating honey, honey mixed ghee and heated honey mixed heated ghee on Wistar rats.

Table 1 shows the total and reducing sugar and moisture content of honey and ghee samples. All the honey samples showed total and reducing sugars within the prescribed limits. The moisture content of honey was 17% and did not exceed 25% as prescribed by P.F.A. act A.07.03.[13] It was observed that on heating the moisture content decreased significantly. The moisture content of ghee was found to be within the prescribed limits of AGMARK standards not >0.3%.[14]

Table 2 depicts the ash value, acid insoluble ash, pH, specific gravity and organoleptic characteristics of honey and ghee samples. These results showed no significant difference in ash value of unprocessed, processed and heated honey samples. However, the ash value of ghee was significantly low. The acid insoluble ash was found to be within the normal range for all the samples. The pH of honey and ghee samples showed no significant difference. The pH of 140ºC heated honey (both processed and unprocessed) was more as compared to the unprocessed, processed and 60ºC heated unprocessed and processed honey samples, but not statistically significant. The specific gravity of honey and ghee samples showed significant decrease in specific gravity of 140ºC heated honey samples (unprocessed and processed honey) and ghee samples (cow's ghee and *Nandini* ghee). This may probably be due to the increase in the density of honey and reduced moisture content.

The organoleptic characteristics of honey and ghee samples showed the following results: unprocessed honey – "very good", processed honey – "good", 60ºC heated, unprocessed and processed honey samples – "good above fair" and 140ºC heated honey (both processed and unprocessed) – "fair", on a 9-point Hedonic scale. However, the "taste" was not included as a part of the organoleptic evaluation.

According to P.F.A act A.07.03, honey should not contain more

Table 1: Changes in sugar and moisture contents of honey and ghee on heating

Samples	Total sugars (%)	Reducing sugars (%)	Moisture content (%)
Unprocessed honey	82.0	66.1	17.1
Processed honey	81.0	65.0	17.0
60°C heated unprocessed honey	83.0	66.0	9.0
60°C heated processed honey	82.0	67.0	9.1
140°C heated unprocessed honey	83.0	67.0	1.8
140°C heated processed honey	83.0	67.0	1.8
Cow's ghee	—	—	0.1
Nandini ghee	—	—	0.2
Honey and ghee	13.02	9.76	0.1

Values are mean of three determinations

than (a) 25% of moisture, (b) 0.5% ash and (c) 5% of sucrose. The minimum reducing sugar content should be 65%. The normal pH should be 3.2–4.5 and specific gravity of honey should be 1.3–1.5. Normal FFA value of ghee is 0.5–3.0. The samples used for the assay were found to be well within these values in the raw form. However, on heating, the pH of 140°C heated honey (both processed and unprocessed) was at an elevated level, which may indicate the decrease in acid content of honey. At the same time, specific gravity of 140°C heated honey (both processed and unprocessed) sample was reduced considerably, showing the decreased quality of the honey on heating. It was reported that the boundary thermal treatment, assumed as the most severe condition able to produce a permissible quality loss, was found to be 140°C for 15 seconds in the transient stage and 30 seconds in the isothermal stage.[15]

Table 3 depicts the chemical characteristics of honey samples, viz., HMF, browning index, total phenolic compounds, flavonoids and total antioxidant activity. The table shows a significant rise in HMF in 60°C heated unprocessed and processed honey and 140°C heated unprocessed and processed honey. The European Union, in order to simplify and update the legislation in some food sectors and to follow the new standards of codex on honey, published the EU Directive 2001/110/CE (L 10/47), where in Annex II honey description and chemical composition are listed. The EU Directive follows sections 2 and 3 of ALINORM, 01/25, but with differences. Point 3.2 of ALINORM stresses the effect of overheating on chemical composition changes and quality loss.[16] The most important difference is given in the HMF level. White (1994) proposed the HMF level to be the only reliable heating/storage index in honey. The normal values of HMF must not exceed 80 mg/kg of honey coming from tropical regions with ambient temperature.[17] There was a significant increase in browning and also a rise in antioxidant activity in heated honey samples when compared to unheated honey samples. The browning observed was non-enzymatic due to the Maillard reaction which occurs when the sugars condense with free amino acids. It is believed that the Maillard Reaction Products (MRPs) act as non-nutrient antioxidants.[18] The antioxidant properties of MRPs have been reported to be strongly affected by the physicochemical properties of the system and by the processing conditions. The polyphenols, ascorbic acid and other carbonyl compounds, even if formed during oxidative reactions, can take part in the Maillard reaction itself.[19] As reported earlier, the antioxidant activity and brown pigment formation increases with temperature and time and there is a correlation between the antioxidant activity and increased brown pigment formation.[20] There is also a significant increase in total phenolic compounds and flavonoids in 140°C heated unprocessed and processed honey when compared to other samples of honey. This also suggests the increase in antioxidant activity of honey samples

Table 2: Changes in physicochemical characteristics of honey and ghee on heating

Samples	Ash value (%)	Acid insoluble ash (%)	pH	Specific gravity	Organoleptic characteristics
Unprocessed honey	0.15 ± 0.012^{a}	0.01± 0.002^{a}	4.16 ± 0.42^{a}	1.39 ± 0.14^{a}	7.5 ± 0.61^{a}
Processed honey	0.18 ± 0.013^{a}	0.01± 0.002^{a}	3.92 ± 0.43^{a}	1.41 ± 0.13^{a}	7.0 ± 0.59^{a}
60°C heated unprocessed honey	0.14 ± 0.011^{a}	0.01± 0.002^{a}	4.50 ± 0.51^{a}	1.41 ± 0.12^{a}	6.5 ± 0.62^{a}
60°C heated processed honey	0.17 ± 0.031^{a}	0.01± 0.002^{a}	4.18 ± 0.39^{a}	1.43 ± 0.13^{a}	6.5 ± 0.62^{a}
140°C heated unprocessed honey	0.14 ± 0.029^{a}	0.01± 0.002^{a}	4.73 ± 0.49^{a}	0.88 ± 0.09^{b}	5.0 ± 0.49^{ab}
140°C heated processed honey	0.18 ± 0.001^{a}	0.01± 0.002^{a}	4.64 ± 0.44^{a}	0.80 ± 0.08^{b}	5.0 ± 0.42^{ab}
Cow's ghee	0.07 ± 0.001^{b}	Nil	4.31 ± 0.41^{a}	0.90 ± 0.07^{b}	6.0 ± 0.59^{a}
Nandini ghee	0.07 ± 0.002^{b}	Nil	4.46 ± 0.42^{a}	0.89 ± 0.07^{b}	7.0 ± 0.65^{a}
Honey and ghee	0.09 ± 0.003^{b}	Nil	4.40 ± 0.41^{a}	1.24 ± 0.10^{a}	7.0 ± 0.61^{a}
F value (8,9)	447.62	—	21.11	223.63	3.47
P value	0.000	—	0.000	0.000	0.041

Mean values bearing different superscripts (a and b) are significantly different from each other (Scheffe's *post hoc* test, $P < 0.05$)

Table 3: Changes in phytoconstituents of heated honey

Samples	HMF mg/kg	Browning index units	Total phenols mg/100g	Flavonoids mg/gm	Antioxidant activity (%)
Unprocessed honey	25.00 ± 3.0^{a}	0.089 ± 0.001^{a}	1.43 ± 0.15^{a}	0.04 ± 0.003^{a}	37.80 ± 5.1^{a}
Processed honey	35.00 ± 3.8^{b}	0.134 ± 0.020^{b}	1.43 ± 0.13^{a}	0.04 ± 0.003^{a}	39.40 ± 6.2^{a}
60°C heated unprocessed honey	68.80 ± 5.9^{ac}	0.133 ± 0.020^{b}	1.48 ± 0.13^{a}	0.04 ± 0.003^{a}	50.11 ± 5.1^{b}
60°C heated processed honey	72.40 ± 7.3^{ac}	0.196 ± 0.030^{c}	1.47 ± 0.12^{a}	0.04 ± 0.003^{a}	52.07 ± 6.3^{b}
140°C heated unprocessed honey	69.70 ± 5.2^{ac}	0.210 ± 0.020^{c}	1.92 ± 0.15^{b}	0.05 ± 0.004^{b}	64.18 ± 6.5^{b}
140°C heated processed honey	73.80 ± 6.5^{ac}	0.213 ± 0.030^{c}	1.92 ± 0.17^{b}	0.05 ± 0.004^{b}	67.56 ± 7.1^{b}
F value (5,6)	87.43	0.621	193.8	14.38	530.34
P value	0.000	0.69	0.000	0.000	0.000

Mean values bearing different superscripts (a, b) are significantly different from each other (as indicated by Scheffe's *post hoc* test where $P < 0.05$)

Table 4: Changes in stability parameters and phytoconstituents of ghee

Samples	PV (meq O_2/kg)	FFA (%) oleic acid	TBA (mg MDA/kg)	Browning index (units × 10^{-3})	Total phenolics (mg/100 g)	Flavonoids (mg/g × 10^{-3})	Antioxidant activity (%)	Saturated: unsaturated fatty acid ratio
Cow's ghee	6.25 ± 0.71[a]	3.07 ± 0.32[a]	0.74 ± 0.08[a]	8.0 ± 0.2[a]	0.41 ± 0.04[a]	4.1 ± 0.20[a]	9.8 ± 0.8[a]	65:34
Nandini ghee	6.25 ± 0.70[a]	3.10 ± 0.29[a]	0.75 ± 0.07[a]	9.0 ± 0.7[a]	0.42 ± 0.03[a]	4.2 ± 0.24[a]	9.4 ± 0.90[a]	65:34
Honey and ghee	6.10 ± 0.68[a]	3.08 ± 0.29[a]	0.72 ± 0.08[a]	113.0 ± 1.2[b]	0.47 ± 0.03[a]	4.2 ± 0.14[a]	24.78 ± 1.9[b]	65:34
F value	0.757	1.113	0.865	0.621	193.8	14.38	530.34	-
P value	0.5	0.43	0.5	0.69	0.000	0.000	0.000	-

Mean values bearing different superscripts (a and b) are significantly different from each other (as indicated by Scheffe's *post hoc* test where $P < 0.05$)

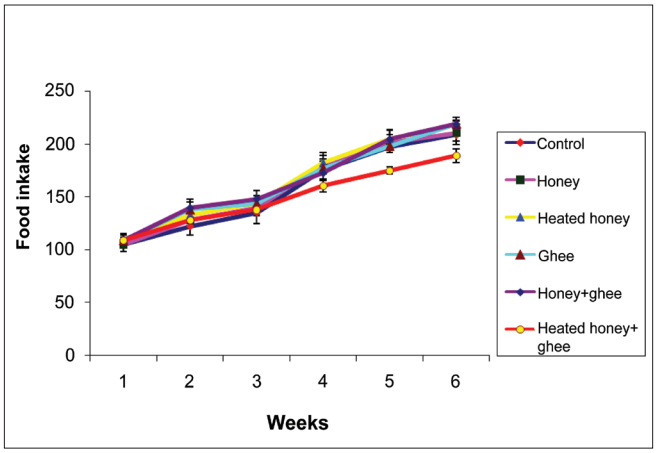

Figure 1: Food intake of rats fed with honey and ghee; values are mean ± SD of six rats

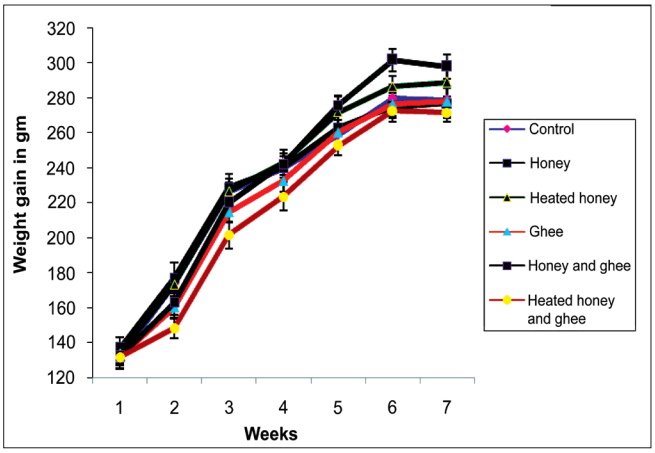

Figure 2: Weight gain of rats fed with honey and ghee; values are mean ± SD of six rats

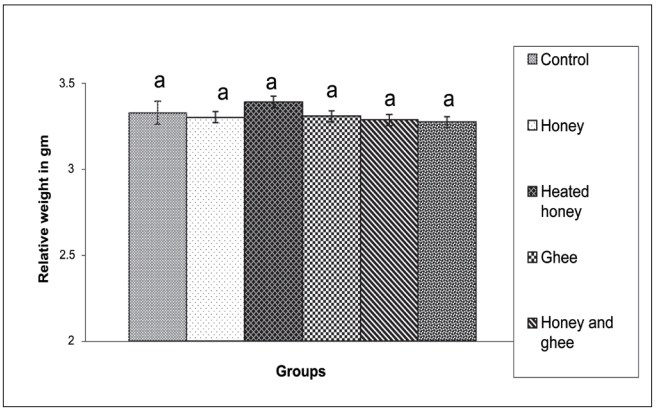

Figure 3: Effects of feeding of honey and ghee on relative weight of rat liver. Values are mean ± SD of six rats; values bearing same superscript (a) are not significantly different from each other

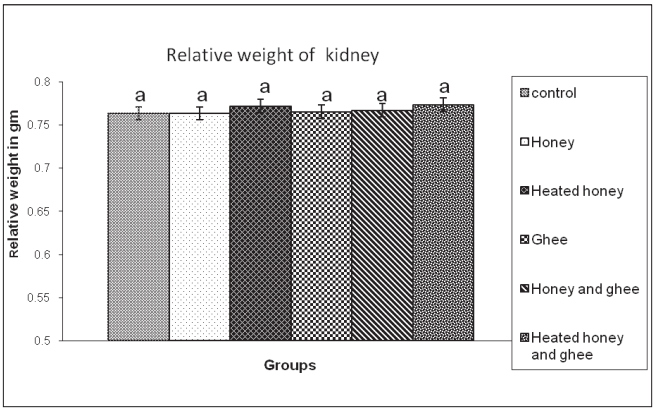

Figure 4: Effects of feeding of honey and ghee on relative weight of rat kidney. Values are mean ± SD of six rats; SD ranged from 0.075 to 0.090 and values bearing same superscript (a) are not significantly different from each other

with respect to heat.

Table 4 shows the PV, FFA, TBA value, browning index, total phenols, flavonoids and total antioxidant activity of cow's ghee, *Nandini* ghee, and honey and ghee mixture. The results show no statistical difference in PV, FFA, TBA, browning index, total phenols, flavonoids and total antioxidant activity of the ghee samples and ghee mixed honey samples. However, the browning and total antioxidant of honey mixed ghee samples was significantly high when compared to ghee samples. This may possibly be due to the mixture of honey in the ghee sample, wherein phenols present in honey impart brown color on oxidation and due to the correlation between antioxidant activity and brown pigment formation,

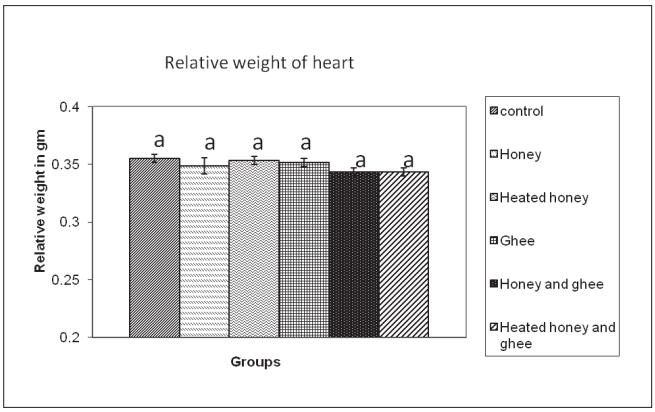

Figure 5: Effects of feeding of honey and ghee on relative weight of rat heart. Values are mean ± SD of six rats; values bearing same superscripts (a) are not significantly different from each other

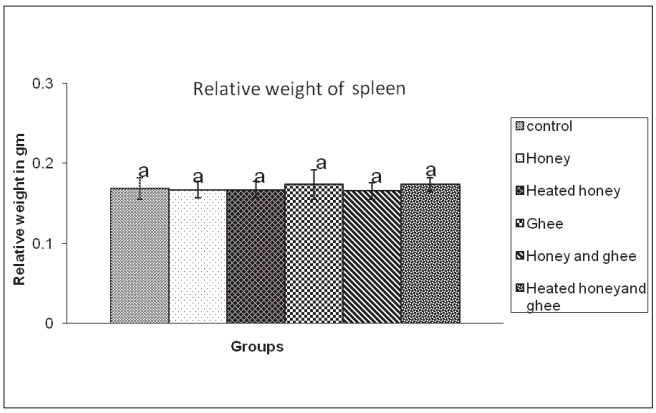

Figure 6: Effects of feeding of honey and ghee on relative weight of rat spleen. Values are mean ± SD of six rats; values bearing same superscript (a) are not significantly different from each other

respectively. The data also showed no change taking place in saturated to unsaturated fatty acid ratio by mixing honey with ghee.

Figures 1 and 2 give the weekly food intake and weight gain pattern, respectively, of rats fed with ghee and honey. The results show no change in food intake pattern of rats fed with ghee and honey samples. The relative organ weights, viz., liver, *Kidney*, heart and spleen were also not altered by the intake of honey and ghee samples either in raw or in heated forms [Figures 3–6].

Conclusion

The study has shown that heating of honey reduces the specific gravity with a subsequent raise in ash value, pH, HMF, browning, phenolics and antioxidant activity. The mixing of honey with ghee brings about enhancement in browning, antioxidants and specific gravity without altering the food consumption and organ weights of the rats. The study revealed that heated honey (>140°C) mixed with ghee produces HMF which may produce deleterious effects and act as a poison in due course (*Ushnam cha samagrutham madhu marayati*).

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हिन्दी सारांश

उष्णीकृत मधु, घी से संयुक्त मधु एवं उसके आहारशैली एवं भौतिक–रासायनिक गुणधर्मों का अध्ययन

ए.अन्नपुरानी, के.आर.अनिलाकुमार, फरहात आनुम, एन. अंजनेय मूर्ति, ए.एस.बावा.

मधु और घी यह दो भारतीय आहारपद्धति में अधिक प्रयुक्त होने वाले घटक हैं। आयुर्वेद के अनुसार गरम किया हुआ मधु और समान मात्रा में मिश्रीकृत घी और मधु दोनों स्वास्थ्य पर विपरीत परिणामकारक हैं। इसलिए प्रस्तुत अध्ययन इन संयोगों के भौतिक एवं रासायनिक गुणधर्मों के अभ्यास हेतु तथा अनेक चूहों में होने वाले परिणामों के अभ्यास हेतु किया गया। परिणाम स्वरूप यह देखा गया कि ६० द्र से. एवं १४० द्र से.पर उष्णीकृत मधु में हायड्रॉक्झेमिथाइल फ्युराल्डीहाइड की मात्रा में वृद्धि पायी गयी, जिसके कारण स्वास्थ्य पर विपरीत परिणाम मिल सकते हैं। उष्ण मधु और घी संयुक्त मधु का ६ सप्ताह तक चूहों को सेवन कराने पर आहार मात्रा, भारवृद्धि और अवयव भार वृद्धि पर कोई विपरीत परिणाम नहीं मिला।