

## EFFECT OF FEEDING OF HONEY MIXED WITH GHEE AND ITS HEATED FORMS ON HEPATOTOXICITY, ANTIOXIDANT ENZYMES AND LIPID PROFILE OF RATS

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### ABSTRACT

Honey and ghee are widely used in our diet. Ayurveda emphasized that heated honey and honey mixed with equal amount of ghee produce deleterious effects that eventually lead to death. This study reveals their effect on multicomponent antioxidant system in rat liver. Six groups of rats were maintained for 6 weeks as Control; Honey incorporated diet; Heated honey incorporated diet; Ghee incorporated diet; Honey with ghee incorporated diet and heated honey with heated ghee incorporated diet. Results revealed a significant raise in serum alkaline phosphatase, uric acid, hepatic glutathione S-transferase, glucose-6-phosphate dehydrogenase and  $\gamma$ -glutamyl transpeptidase with associated increase in serum conjugated dienes, hydroperoxides and malondialdehyde of rats fed with honey mixed ghee and heated honey mixed with heated ghee. Hence, it is concluded that consumption of honey with equal amount of ghee and its mixture in the heated forms raises the detoxifying enzymes and the levels of lipid peroxides in rat liver.

**Key words:** Hepatotoxicity, Antioxidant, Lipid profile, glutathione transferase, alkaline phosphatase, phosphate dehydrogenase, conjugated dienes, hydroperoxides

### 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 & brahmacharya) for a healthy life. 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 leads to number of diseases including sterility, herpes, eye diseases, skin eruptions, ascites, fistula, leprosy, sprue, edema, fever, rhinitis, fetal distress and may even cause death<sup>1</sup>. Honey is a sweet, sticky, yellowish fluid made by bees from nectar. Ghee is the Indian name for clarified butter fat, and is usually prepared from cow's milk, buffalo's milk or from mixed milks. 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 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. At 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 amount of ghee produce deleterious effect in the body to the extent of causing death<sup>2</sup>. A recent study on the physico-chemical characteristics of heated honey and honey mixed with ghee revealed the production of hydroxymethyl furfural<sup>3</sup>. Hence a study has been conducted in order to evaluate the toxic effects, if any, on consumption of honey, heated honey, ghee, honey mixed with equal amount of ghee and heated honey mixed with heated ghee in rat models.

### MATERIALS AND METHODS

#### Materials

All the biochemicals used in these investigations were of highest purity and procured from Sigma Company, USA; Merck, Germany; Sisco Research Laboratory, Mumbai; Across Organics, Mumbai; Spectrochem, Mumbai and S.D. Fine Chemicals, Mumbai.

Unprocessed natural honey (raw) was procured from Madikeri forest office, Karnataka, India. The honey samples were heated to 60°C and 140°C. Cow's ghee was procured from Salem, Tamilnadu, India. Honey and ghee mixture: Cow's ghee mixed with equal quantity of unprocessed honey.

Heated cow's ghee mixed with equal quantity of 140°C heated

unprocessed honey.

#### Experimental protocol

Male Wistar Rats was divided into six groups of six rats each. Group I: fed with normal pellet diet served as a control. Group-II: fed with honey along with control diet. Group-III: fed with heated (140°C) honey with control diet. Group-IV: fed with ghee with control diet. Group-V: fed with honey mixed ghee with control. Group-VI: fed with heated- honey (140°C) mixed with heated ghee with control diet. The dose of honey and ghee was decided as prescribed in Ayurvedic literature. As per Ayurvedic practice, for 70 kg men, the normal dose of honey recommended was 48g/day (1 pala matra) and recommended dose of ghee was 30 ml/ day (hraseeyasi matra). Accordingly, for the Wistar rats of 150g body weight the dose of honey was fixed at 102 mg of honey /day and that of ghee was calculated to be 64 $\mu$ l /day.

The animals were kept at ambient temperature and exposed to light-dark cycle of 12 hours each. The left over diet was collected, dried and weighed to determine the food intake. Weekly food intake and weight gain was monitored. After the completion of feeding for 6 weeks, the rats were sacrificed under mild anesthesia (sodium pentobarbitone, 50 mg/ kg body weight i.p.) and blood was removed through cardiac puncture and liver, kidney, heart, brain and colon were quickly excised. Clearance of experimental design by the Institutional Ethical committee for rats was taken.

#### Biochemical assays

The activity of serum transaminases was determined by Reitman and Frankel's method as mentioned by Tietz<sup>4</sup>. The quantitative determination of serum creatinine was done by modified Jaffe's Kinetic method as described by Bowers *et al*<sup>5</sup>. The determination of serum proteins and alkaline phosphatase were done by Lowry's<sup>6</sup> and McComb and Bower's methods<sup>7</sup>. The quantitative determination of cholesterol in serum was done as described by Allain *et al*<sup>8</sup>. For the determination of serum HDL and LDL cholesterol the method described by O'Brien *et al*<sup>9</sup> was followed. The quantitative determination of urea in serum was done by Modified Berthelot methodology as described by Searcy *et al*<sup>10</sup>. The quantitative determination of uric acid and triglycerides and glucose in serum was done by the method explained by Fossati *et al*<sup>11</sup>, Jacobs *et al*<sup>12</sup> and Trinder *et al*<sup>13</sup> respectively.

Thiobarbituric acid reactive substances (TBARS) as malondialdehyde was assayed in liver homogenate as reported<sup>14</sup>.

The lipid residue was dissolved in cyclohexane and the amount of conjugated dienes (CD) produced was estimated as described<sup>15</sup>. The upper layer of the same assay was utilized for the estimation of hydroperoxides (HP) according to the prescribed procedure<sup>15</sup>.

Glutathione peroxidase (GSH-Px) and Catalase activity in liver was determined by the method of Weiss *et al*<sup>16</sup> and Cohen *et al*<sup>17</sup> respectively in the supernatant of liver homogenate. Hepatic glutathione S-transferase (GST) activity was determined by the procedure of Habig *et al*<sup>18</sup> in the supernatant from liver homogenate. Superoxide dismutase (SOD) was measured by the inhibition of cytochrome C reduction mediated via superoxide anions generated by xanthine-xanthine oxidase and monitored at 550nm as described by Suttle NF<sup>19</sup>. One unit of SOD was defined as the amount required for inhibiting the reduction of cytochrome C by 50%.  $\gamma$ -glutamyltranspeptidase (GGT) was estimated by the method of Meister *et al*<sup>20</sup>. Protein in tissues was determined according to Lowry *et al*<sup>6</sup>.

#### Statistical analysis

Statistical analysis was carried out using one way analysis of variance (ANOVA).

### RESULTS

#### Hepatoprotective biochemical parameters and serum lipid profile

Figs. 1 to 6 give the effect of feeding of honey and ghee for six weeks on rat SGOT, SGPT, serum creatinine, total proteins and albumin and globulin levels. There was no significant difference in SGOT, SGPT and creatinine in the five groups of rats fed with honey, heated honey, ghee, honey mixed ghee and heated-honey mixed with ghee. There was no significant difference in total proteins of honey and ghee fed rats but there was a significant increase ( $p < 0.05$ ) in total proteins of rats fed with heated honey, honey mixed with ghee and heated honey mixed with heated ghee. At the same time, there was no significant difference in serum albumin and globulin of rats fed with honey, heated honey and ghee. Serum albumin and globulin of rats fed with honey mixed ghee and heated honey mixed with heated ghee were enhanced significantly.

Table 1 shows the effect of feeding of honey, heated honey, ghee, honey mixed ghee and heated honey mixed heated ghee for six weeks on rat serum alkaline phosphatase (ALP), total cholesterol, triglycerides, HDL and LDL cholesterol levels. There was no significant difference in serum ALP in honey, heated honey and ghee fed rats. At the same time, there was a significant difference ( $p < 0.05$ ) in ALP of rats fed with honey mixed ghee and heated honey mixed heated ghee. There was no significant difference in serum cholesterol and triglycerides in rats fed with honey, heated honey, ghee and honey mixed ghee. However, there was a significant increase in serum triglycerides in rats fed with heated honey mixed heated ghee. However, the LDL cholesterol in rats fed with heated honey mixed with heated ghee showed significant decrease ( $p < 0.05$ ) as compared to other groups.

Table 2 depicts the effects of feeding of honey, heated honey, ghee, honey mixed with ghee and heated- honey mixed heated ghee on rat serum urea- B, uric acid, total and direct bilirubin and glucose. These results show that there was no significant difference in serum urea- B in rats fed with honey, heated honey and ghee. However, the urea-B levels in the rats fed with honey mixed with ghee and heated honey mixed with heated ghee was found to be significantly decreased as compared to control groups. There was a significant increase ( $p < 0.05$ ) in uric acid levels of rats fed with honey mixed ghee and heated honey mixed with heated ghee. However, there was no significant difference in bilirubin and glucose levels in rats fed with honey, heated honey, ghee, honey mixed ghee and heated honey mixed heated ghee.

#### Hepatic lipid peroxides

Table 3 show the effects of feeding of honey, heated honey, ghee, honey mixed ghee and heated- honey mixed heated- ghee on hepatic lipid peroxides of rats. There was a significant increase in hepatic TBARS, hydroperoxides and conjugate dienes of rats fed with honey mixed with ghee and heated- honey mixed with heated-ghee. At the same time, there was no significant difference in these parameters in rats fed with honey, heated honey and ghee per se.

#### Hepatic detoxifying and antioxidant enzymes

Table 4 describes the effect of feeding of honey, heated- honey, ghee, honey mixed with ghee and heated- honey mixed heated- ghee on detoxifying enzymes of liver in rats. There was a significant increase in GST and GGT of rats fed with honey mixed with ghee and heated- honey mixed with heated-ghee. A significant increase in the hepatic G-6-PD activity was also noted probably to regulate the GSH availability for the GSH-related redox reactions.

Table 5 shows the effect of feeding of honey and ghee on hepatic antioxidant enzymes of rats. The SOD, catalase, GSH-Px and GSSG-reductase activities were not changed in rats fed with honey mixed with ghee and heated- honey mixed with heated- ghee.

#### Histopathological analysis of the tissues

Micro section of liver of all groups of rats showed normal architecture with hepatic lobules and hepatocytes arranged in sheets and cords with central veins was normal. Micro section of kidney of all groups of rats showed normal cortex and medulla with normal glomerulus's and collecting tubules. Micro section of brain in all groups showed normal texture of cerebral cortex and cerebellum where as heart tissue showed cardiac muscle arranged concentrically. The intestinal tissues of all groups of rats on micro section showed normal mucosal lining epithelium with sub mucosa and serosal layer and also lymphoid aggregates (Payer's patches)

### DISCUSSION

In Ayurveda, Acharya Charaka has quoted that heated honey and honey mixed with equal ghee produce deleterious effect in the body and may eventually cause death. Hence we were interested to evaluate the changes in antioxidant system, if any, on consumption of heated honey, honey mixed with equal amount of ghee and heated- honey mixed with heated -ghee in rat models.

There was a significant increase in total proteins of rats fed with heated honey, honey mixed with ghee and heated- honey mixed with heated -ghee. At the same time, there was no significant difference in serum albumin and globulin of rats fed with honey, heated honey and ghee. Serum albumin and globulin of the rats fed with honey mixed ghee and heated- honey mixed with heated- ghee was enhanced significantly. These significant changes observed may probably be due to the early signs of liver cirrhosis<sup>21</sup>. The elevation in ALP observed in honey mixed ghee and heated honey mixed ghee may probably be due to the early signs of biliary tract obstruction<sup>7</sup>.

The hypertriglyceridemia observed in rats fed with heated- honey mixed heated- ghee may possibly be due to hyperlipidemia or nephritis<sup>12</sup>. The LDL cholesterol in rats fed with heated honey mixed with heated ghee shows significant decrease ( $p < 0.05$ ) as compared to other groups. Further studies are warranted to elucidate this observed change in LDL. The observed increase in uric acid levels of rats fed with honey mixed ghee and heated- honey mixed with heated -ghee may possibly be due to early signs of impaired renal functions<sup>11</sup>.

As honey is rich in phenolics and flavonoids, the consumption of the same has resulted in the decreased hepatic TBARS levels in rats fed with honey and heated- honey. The significantly increased TBARS levels, conjugated dienes and hydroperoxides in the liver of rats fed with heated- honey mixed with heated-ghee might be a part of the molecular mechanism of cell injury. This might in turn lead to generation of lipid hydroperoxides which can decompose to yield a

range of cytotoxic products, most of which was aldehyde, as exemplified by MDA, 4-hydroxyl nonenal, etc.<sup>22</sup>

The study showed increase in GST, G-6-PD and GGT of rats fed with honey mixed with ghee and heated- honey mixed with heated-ghee probably due to toxicity. Enhancement of GST has been shown to increase the ability for detoxification of some carcinogens<sup>23</sup>. The cytosolic GST catalyzes conjugation reaction of GSH and electrophilic substance, and therefore, as an integral role to play in the detoxification of electrophilic toxicants<sup>24</sup>. The high level of GGT found in fetal and neoplastic liver has made it an attractive putative marker for the hepatic neoplasia<sup>25-27</sup>. The significantly enhanced GGT was an indicative of toxicity because significant increase in hepatic GGT activity was reported in inflammation and the enzyme was also an effective biochemical marker for immunotoxicity of xenobiotics<sup>28</sup>. The raise in G-6-PD activity might lead to elevate the HMP shunt pathway and thereby increase the level of NADPH, which in turn helps to maintain GSH- related redox reactions.

Superoxide dismutase is responsible for removal of superoxide radicals<sup>29</sup>, thus it may contribute to the modulation of redox state of liver cells. GSH redox system and the associated enzymes viz. GSH-Px, GSSG-reductase and G-6-PDH generally regulate the GSH level and play key role in free radical and peroxide metabolism and was partly responsible for cellular protection<sup>30</sup>. The enhanced detoxifying enzymes could contribute towards the removal of lipid peroxides in the tissues of rats fed with honey mixed with ghee.

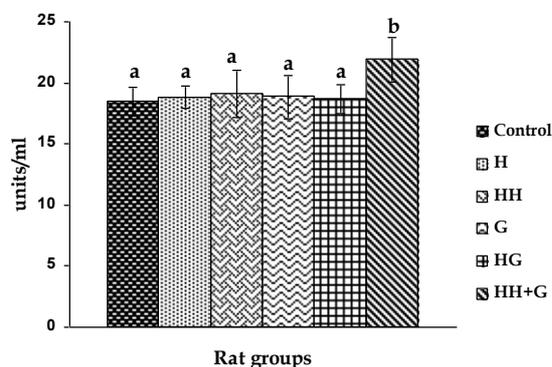
White (1994) proposed the HMF level to be a reliable heating/storage index in honey<sup>31</sup>. The normal values of HMF must not exceed 80 mg / kg of honey coming from tropical regions with ambient temperature. In our earlier work<sup>3</sup> we observed significant increase in browning in heated 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) are acting as non-nutrient antioxidants<sup>32</sup>. 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<sup>33</sup>.

In view of this, it is not advisable to consume heated- honey and honey mixed with equal quantities of ghee. As a result, the use of heated- honey based products warrants safety evaluation. Hence, it is concluded that consumption of honey with equal amount of ghee and its mixture in the heated forms modifies hepatic detoxification system and the levels of lipid peroxides in rat liver.

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**Fig 1: Effect of feeding of honey and ghee on serum GOT**  
(H- Honey, HH- Heated honey, G- Ghee, HG- Honey with ghee, HH+G- Heated honey with heated ghee)

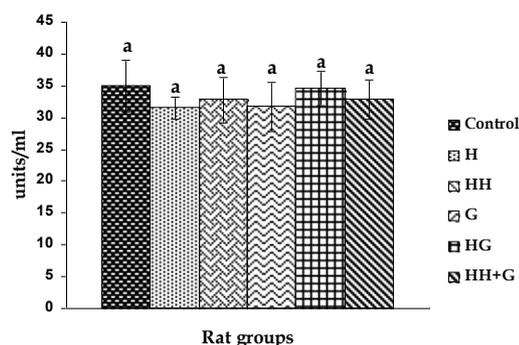


Fig 2: Effect of feeding of honey and ghee on serum GPT

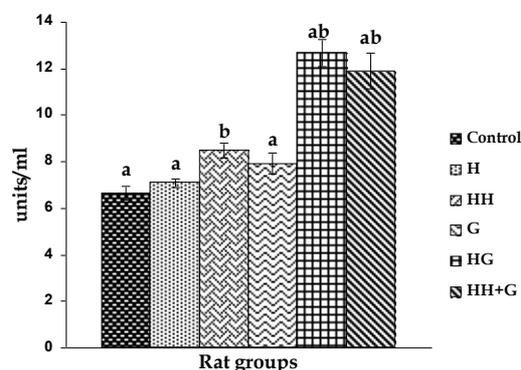


Fig 4: Effect of feeding of honey and ghee on serum protein

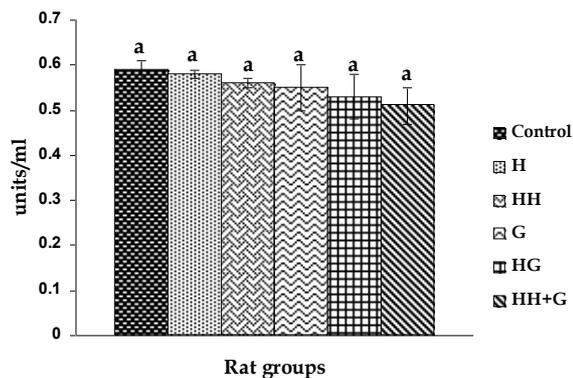


Fig 3: Effect of feeding of honey and ghee on serum creatinine

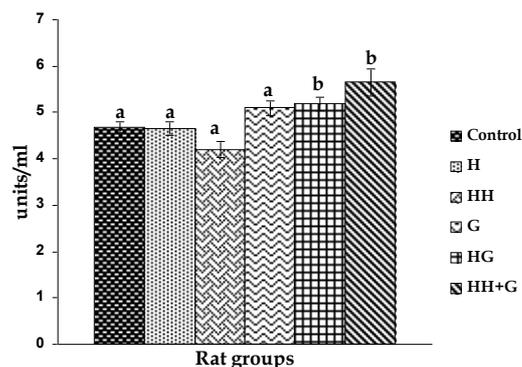


Fig 5: Effect of feeding of honey and ghee on serum albumin

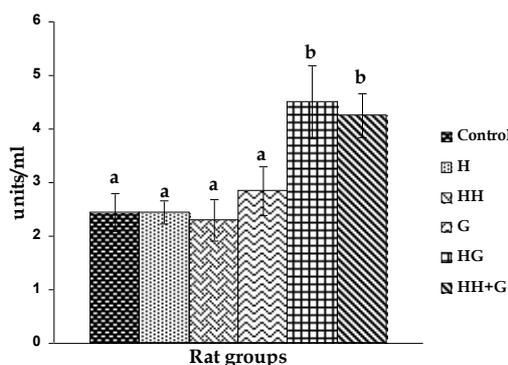


Fig 6: Effect of feeding of honey and ghee on serum globulin

Table 1: Effects of Feeding of Honey and Ghee on rat Serum Alkaline Phosphatase and Lipid components

Rat Group	Alkaline phosphatase units/L	Total cholesterol mg/dl	Triglycerides mg/dl	HDL mg/dl	LDL mg/dl
Control	15.09 ± 1.59 <sup>a</sup>	173.20 ± 14.5 <sup>a</sup>	112.95 ± 13.60 <sup>a</sup>	54.40 ± 7.3 <sup>a</sup>	96.21 ± 8.17 <sup>a</sup>
Honey	16.08 ± 1.71 <sup>a</sup>	182.78 ± 17.8 <sup>a</sup>	117.40 ± 11.40 <sup>a</sup>	54.65 ± 2.6 <sup>a</sup>	104.6 ± 8.42 <sup>a</sup>
Heated- honey	14.01 ± 1.91 <sup>a</sup>	182.48 ± 15.4 <sup>a</sup>	119.90 ± 09.25 <sup>a</sup>	45.92 ± 3.9 <sup>a</sup>	112.55 ± 8.18 <sup>a</sup>
Ghee	15.18 ± 1.31 <sup>a</sup>	179.11 ± 15.5 <sup>a</sup>	111.24 ± 12.80 <sup>a</sup>	45.93 ± 2.3 <sup>a</sup>	101.9 ± 8.01 <sup>a</sup>
Honey and ghee	18.18 ± 0.11 <sup>b</sup>	178.22 ± 17.4 <sup>a</sup>	117.11 ± 11.50 <sup>a</sup>	48.91 ± 4.2 <sup>a</sup>	105.9 ± 8.32 <sup>a</sup>
Heated- honey and Ghee	19.20 ± 0.14 <sup>b</sup>	179.21 ± 16.1 <sup>a</sup>	218.98 ± 14.30 <sup>b</sup>	46.83 ± 4.9 <sup>a</sup>	78.58 ± 8.13 <sup>b</sup>

Values are Mean ± SD of 6 rats. Values bearing different superscripts in the same column were significantly different (p < 0.05)

**Table 2: Effects of Feeding of Honey and Ghee on rat Serum Urea- B, Uric acid, total Bilirubin, Direct Bilirubin and Glucose levels**

Rat Group	Urea B mg/dl	Uric acid mg/dl	Total Bilirubin mg/dl	Direct Bilirubin mg/dl	Glucose mg/dl
Control	32.15 ± 1.09 <sup>a</sup>	4.50 ± 0.37 <sup>a</sup>	0.73 ± 0.16 <sup>a</sup>	0.46 ± 0.03 <sup>a</sup>	87.98 ± 9.2 <sup>a</sup>
Honey	35.22 ± 2.33 <sup>a</sup>	5.03 ± 0.29 <sup>a</sup>	0.78 ± 0.12 <sup>a</sup>	0.42 ± 0.08 <sup>a</sup>	87.51 ± 8.9 <sup>a</sup>
Heated- honey	35.25 ± 6.07 <sup>a</sup>	4.88 ± 0.31 <sup>a</sup>	0.76 ± 0.08 <sup>a</sup>	0.41 ± 0.03 <sup>a</sup>	85.35 ± 8.5 <sup>a</sup>
Ghee	35.47 ± 5.50 <sup>a</sup>	5.03 ± 0.23 <sup>a</sup>	0.82 ± 0.07 <sup>a</sup>	0.47 ± 0.09 <sup>a</sup>	97.89 ± 9.3 <sup>a</sup>
Honey & ghee	33.26 ± 2.30 <sup>a</sup>	6.47 ± 0.29 <sup>b</sup>	0.78 ± 0.07 <sup>a</sup>	0.41 ± 0.01 <sup>a</sup>	80.78 ± 7.9 <sup>a</sup>
Heated- honey & ghee	33.63 ± 1.74 <sup>a</sup>	6.53 ± 0.50 <sup>b</sup>	0.79 ± 0.06 <sup>a</sup>	0.45 ± 0.08 <sup>a</sup>	89.23 ± 8.3 <sup>a</sup>

Values were Mean ± SD of 6 rats.  
Values bearing different superscripts in the same column were significantly different (p < 0.05)

**Table 3: Effects of Feeding of Honey and Ghee on Hepatic lipid peroxides**

Rat Group	TBARS n moles/g	Hydroperoxides x10 <sup>3</sup> moles/g	Conjugated dienes x10 <sup>-5</sup> moles/g
Control	32.26 ± 2.55 <sup>a</sup>	11.71 ± 0.09 <sup>a</sup>	51.17 ± 5.2 <sup>a</sup>
Honey	27.56 ± 1.35 <sup>b</sup>	11.99 ± 1.40 <sup>a</sup>	51.48 ± 5.1 <sup>a</sup>
Heated- honey	26.01 ± 1.90 <sup>b</sup>	11.11 ± 1.10 <sup>a</sup>	52.46 ± 5.0 <sup>a</sup>
Ghee	34.69 ± 1.09 <sup>a</sup>	10.15 ± 1.24 <sup>a</sup>	56.27 ± 4.2 <sup>a</sup>
Honey & ghee	37.05 ± 1.01 <sup>b</sup>	15.29 ± 1.49 <sup>b</sup>	66.13 ± 5.9 <sup>b</sup>
Heated- honey & ghee	38.99 ± 2.33 <sup>b</sup>	16.21 ± 1.90 <sup>b</sup>	72.56 ± 5.6 <sup>b</sup>

Values are Mean ± SD of 6 rats.  
Values bearing different superscripts in the same column were significantly different (p < 0.05)

**Table 4: Effects of Feeding of Honey and Ghee on Hepatic detoxifying enzymes**

Rat Group	GSSG-Red x 10 <sup>-2</sup> σ	G-6- PD x 10 <sup>-2</sup> ω	GSTx10 <sup>2</sup> Ω	GGT γ
Control	33.43 ± 1.3 <sup>a</sup>	06.62 ± 1.20 <sup>a</sup>	1.28 ± 0.06 <sup>a</sup>	1.35 ± 1.27 <sup>a</sup>
Honey	33.43 ± 1.8 <sup>a</sup>	11.04 ± 1.23 <sup>b</sup>	1.13 ± 0.00 <sup>a</sup>	1.38 ± 0.19 <sup>a</sup>
Heated- honey	31.05 ± 2.3 <sup>a</sup>	10.23 ± 0.08 <sup>b</sup>	1.72 ± 0.01 <sup>a</sup>	1.29 ± 0.10 <sup>a</sup>
Ghee	32.95 ± 2.3 <sup>a</sup>	11.54 ± 0.44 <sup>b</sup>	1.45 ± 0.01 <sup>a</sup>	1.30 ± 0.18 <sup>a</sup>
Honey and ghee	31.89 ± 2.9 <sup>a</sup>	12.01 ± 0.33 <sup>b</sup>	1.83 ± 0.02 <sup>ab</sup>	1.89 ± 0.12 <sup>b</sup>
Heated- honey and Ghee	30.98 ± 2.8 <sup>a</sup>	12.09 ± 0.91 <sup>b</sup>	1.88 ± 0.02 <sup>ab</sup>	1.99 ± 0.11 <sup>b</sup>

Values are Mean ± SD of 6 rats. Values bearing different superscripts in the same column were significantly different (p < 0.05)  
 -mmoles NADP produced / min / mg protein  
 ω - μmoles NADP reduced / min / mg protein  
 Ω - mmoles conjugate formed / min / mg protein  
 γ - nmoles p- nitroanilide released / min / mg protein

**Table 5: Effects of Feeding of Honey and Ghee on Hepatic antioxidant enzymes**

Rat Groups	Catalase ω	SOD δ	GSH-Px
Control	0.169 ± 0.059 <sup>a</sup>	14.11 ± 1.12 <sup>a</sup>	127.44 ± 1.27 <sup>a</sup>
Honey	0.179 ± 0.077 <sup>a</sup>	14.02 ± 0.06 <sup>a</sup>	127.39 ± 1.28 <sup>a</sup>
Heated- honey	0.125 ± 0.041 <sup>a</sup>	14.05 ± 0.03 <sup>a</sup>	120.76 ± 1.21 <sup>a</sup>
Ghee	0.119 ± 0.074 <sup>a</sup>	14.07 ± 0.07 <sup>a</sup>	128.65 ± 1.21 <sup>a</sup>
Honey & ghee	0.132 ± 0.084 <sup>a</sup>	13.52 ± 1.16 <sup>a</sup>	123.00 ± 1.22 <sup>a</sup>
Heated- honey & ghee	0.133 ± 0.041 <sup>a</sup>	13.88 ± 1.05 <sup>a</sup>	123.01 ± 1.23 <sup>a</sup>

ω - ΔA / min / mg proteins  
 δ - Units / min / mg proteins  
 m moles NADP produced / min / mg protein  
 Values are Mean ± SD of 6 rats.  
 Values bearing different superscripts in the same column were significantly different (p < 0.05)

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