Protective Effect of Nimboide against High Fat Diet-induced Obesity in Rats via Nrf2/HO-1 Pathway

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Abstract: Current time obesity is the major challenges globally and the incidence of the obesity has raised dramatically in current years. The obesity enhanced the various metabolic diseases such as diabetes, cardiac, cancer and steatohepatitis. Natural drug having the long history to ameliorate the obesity and its related metabolic disorder. In this experimental study, we scrutinized the anti-obesity effect of nimboide against high fat diet (HFD) induced obesity in rats. Wistar rats were divided into 5 groups and each group contains 10 rats. The body weight, tissue weight was estimated at regular time. Carbohydrate, lipid, hepatic, inflammatory cytokines, antioxidant and inflammatory parameters were estimated. The mRNA expression was also estimated. Nimboide treated groups significantly ($p < 0.001$) suppressed the body weight at dose dependent manner. Nimboide significantly ($p < 0.001$) reduced the hepatic parameters and altered the antioxidant parameters such as thiobarbituric acid reactive substances (TBARS), glutathione (GSH), catalase (CAT), glutathione peroxidase (GPx), superoxide mutase (SOD), glutathione S transferase (GST); decreased the level of inflammatory cytokines (IL-1β, IL-6, TNF-α). Nimboide suppressed the mRNA expression of glucose-6-phosphatase HO-1 and nuclear factor erythroid-2 related factor-2 (Nrf2). Collectively, we can say that nimboide having the capability to suppressed the HFD induced obesity via Nrf2/HO-1 pathway.

Key words: antiobesity, nimboide, antioxidant, anti-inflammatory, Nrf2/HO-1 pathway

1 Introduction
Excessive intake of dietary fat increased the chance to develop the obesity, which has currently exhibited the alarming proportions globally due to increase the risks for human health and wellbeing¹. Excessive consumption of fat and expansion of chronic obesity in the body lead to develop the various metabolic dysfunctions such as expansion of insulin resistance (IR) and finally induces the diabetes mellitus (type II)²–⁴. Some evidence suggest that the genetic predisposition has a role in obesity and diabetes, dietary intake linked with the IR pathogenesis especially high intake of dietary fats⁵. As per the previous reports that boost the body mass index (BMI) 1 kg/m², enhance the chance for the development of type 2 diabetes mellitus (T2DM) (20%)⁶. However, the obesity incidence enhances the susceptibility to hypertension (more than 3.5 times) and study suggest that the hypertensive patient (approximately 60-70%) having the obesity⁷.⁸ Moreover, the obesity increases the kidney disease risk factor, when the BMI increases 5 kg/M². As per the reports, the patient having the kidney disease along with the obesity, increase the 60% of mortality⁹. During the COVID-19 pandemic, the report suggested the increases evidence of obesity, increases the risk factor to develop the more complication of COVID-19. As per the report of Public Health England, BMI 35-45 could enhance the chance of dying due to the COVID 19 (40%) and when the BMI reach above the 40 could enhance the risk factor 90%⁹,¹⁰. During the pandemic COVID-19, the obese patient having the higher risk and

Abbreviations: TBARS; Thiobarbituric acid reactive substances, GSH; Glutathione, CAT; Catalase, GPx; Glutathione peroxidase, SOD; Superoxide mutase, GST; Glutathione S transferase, TIIIDM; Type 2 diabetes mellitus (TIIIDM), SD; Sprague Dawley, TC; Total cholesterol, LDL; Low density lipoprotein, HDL; High density lipoprotein, TG; Triacylglycerols, NEFA; Non-esterified fatty acids, TP; Total protein, HCT; Hematocrit, WBC; White blood cell, Hb; Haemoglobin, RBC; Red blood carpules, MCV; Mean cell volume, LYM; Lymphocytes, PLT; Platelet, MCHV; Mean corpuscular hemoglobin concentration, NQO1; NAD(P)H quinone oxidoreductase 1, FFAs; Free fatty acids

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more chance for the death. Previous studies already proved the link between the HFD and oxidative stress. Long term taken the high saturated fat diet play an important role as inducer for the oxidative stress and suppressed the endogenous antioxidant defence system. During the increase level of oxidative stress, enhances the level of lipid peroxidative (LPO) in the hepatic tissue and the circulation. Report suggests that the oxidative stress pathogenesis of metabolic derangements leading the obesity, insulin resistance and T2DM. It is proved that increase the oxidative stress precedes the expansion of obesity and metabolic dysregulation there are induced by an HFD.

Natural product having the long history to treat the various disease due to less side effects and more protective effect. Recent years natural product gain more attraction for researcher due to effective therapies against the various human diseases. Various research investigation have been directed and scrutinize for the biological effects. Aza-dirachta indica A. is very popular tree in China. The bark, flower, leaves, root, seed and bark of the plant used against the diseases and also used in the industrial products. The leaves of the plant commonly used for the treatment of diabetes, reducing fever and eczema. The bark of the plant used as toothbrush and its root help to heal the disease and mosquito repellent. Nimbolide is the active phyto-constituent of plant and commonly used as anti-diabetic, antimalarial, antitumor, antiulcer, anti-inflammatory and hepatoprotective. Due to its antidiabetic and anti-inflammatory and antioxidant effect of nimbolide, in this investigation scrutinize the anti-obesity effect of nimbolide against HFD induced obesity in rats and explore the possible mechanism.

2 Materials and Methods

2.1 Animals

Sprague Dawley (SD) rats sex-male, weight 200 ± 20 g; aged 3 weeks were used for the current experimental study. The rats were kept in the standard laboratory condition such as maintained the temperature 20 ± 5°C; relative humidity 65% with 12/12 h dark/light. The rats were kept in the laboratory condition for 7 days before the experimental study.

2.2 High fat diet

After the acclimatization, the rats were received the HFD (mention in Table 1) during the whole animal experiment. The normal control group rats received the standard diet throughout the experimental period. Throughout the whole animal protocol, the rats were received the water ad libitum.

<table>
<thead>
<tr>
<th>Ingredients</th>
<th>Control diet</th>
<th>High fat diet</th>
</tr>
</thead>
<tbody>
<tr>
<td>Yellow corn</td>
<td>70.24</td>
<td>29.50</td>
</tr>
<tr>
<td>Soybean</td>
<td>8.80</td>
<td>3.70</td>
</tr>
<tr>
<td>Corn gluten</td>
<td>5.00</td>
<td>2.1</td>
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<tr>
<td>Wheat bran</td>
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<td>1.68</td>
</tr>
<tr>
<td>Vegetable oil</td>
<td>4.74</td>
<td>1.99</td>
</tr>
<tr>
<td>Sucrose</td>
<td>0.0</td>
<td>5.14</td>
</tr>
<tr>
<td>Cellulose</td>
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</tr>
<tr>
<td>Animal Fat</td>
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</tr>
<tr>
<td>Lysine</td>
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<td>0.39</td>
</tr>
<tr>
<td>Methionine</td>
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<td>0.0</td>
</tr>
<tr>
<td>Dicalcium phosphate</td>
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<td>0.42</td>
</tr>
<tr>
<td>Ground limestone</td>
<td>0.82</td>
<td>0.0</td>
</tr>
<tr>
<td>Premix</td>
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<td>0.13</td>
</tr>
<tr>
<td>Common Salt</td>
<td>0.0</td>
<td>1.13</td>
</tr>
<tr>
<td>Total</td>
<td>100 mg</td>
<td>100 mg</td>
</tr>
</tbody>
</table>

2.3 Experimental group

The rats were divided into different groups and each group contains 10 rats as follows

- Group I: normal control
- Group II: HFD control
- Group III: HFD + Nimbolide (5 mg/kg)
- Group IV: HFD + Nimbolide (10 mg/kg)
- Group V: HFD + Nimbolide (15 mg/kg), respectively.

The body weight of all group rats were estimated at regular time. After the 10 weeks, all group rats were fasted overnight (16 h), before the sacrificed. Ketamine (90 mg/kg) and xylazine (10 mg/kg) were used for anesthetized the all-group rats. The blood samples were collected from all group rats via puncturing the retro orbital and centrifuged at 15,000 rpm at 4°C for 15 min. The serum sample were separated out and kept in the sterile cryovials at −80°C for further biochemical analysis.

2.4 Organ weight

After sacrificed the rodent, immediately removed the organs such as kidney, heart, pancreas, aorta, brain and liver within 30 min and the organ washed with the ice-cold phosphate buffered saline to remove the excessive blood clot. After that, the absorbent paper was used for remove the remaining fluids before weighing on electronic balance for getting the constant weight. The ratio of organs/hepatic and renal) to final body weight was also estimated.

2.5 Antioxidant parameters

The antioxidant parameters such as MDA, TAC, enzymatic antioxidant parameters GSH, GPx, SOD and CAT were estimated using the previous reported method with...
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minor modification\textsuperscript{6,11,24}.

2.6 Lipid profile

Total cholesterol (TC), low density lipoprotein (LDL), high density lipoprotein (HDL) and triacylglycerols (TG) were analyzed using the colorimetric assay via following the manufacture instruction (Bioclin, Belo Horizonte, Brazil). The very low density lipoprotein (VLDL) was estimated using the following formula

\[ VLDL = \frac{Triacylglycerol}{5} \]

2.7 Non-esterified fatty acids (NEFA)

The NEFA level was estimated in the serum using the manufacture protocol (Elabscience calorimetric kit, USA).

2.8 Hepatic parameters

Alanine transaminase (ALT), albumin (ALB), aspartate transaminase (AST) and alkaline phosphatase (ALP) was estimated using the ELISA kits following the manufacture protocol Nanjing Jiancheng Bioengineering Institute, Nanjing, China.

2.9 Renal parameters

The level of total protein (TP), creatinine and albumin were determined using the previous reported method with minor modification\textsuperscript{23,24}.

2.10 Hematological parameters

Hematological parameters such as hematocrit (HCT), white blood cell (WBC), haemoglobin (Hb), red blood carpules (RBC), mean cell volume (MCV), lymphocytes (LYM), platelet (PLT) and mean corpuscular hemoglobin concentration (MCHV) were determined using readymade diagnostic reagent kits (Sunlong Biotech Co., Ltd. Zhejiang, China).

2.11 mRNA expression

The total RNA was isolated from the DNA using the Trizol (Invitrogen, Carlsbad, USA) using the manufacture protocol. PrimeScript RT reagent kit was used for synthesized the complementary DNA (cDNA) with gDNA Eraser. 7300 real time PCR detection system was used for conducting the RT-PCR (Applied Biosystems, CA, USA). 1 \( \mu \)g total RNA was used as templates with corresponding gene primers (Table 2). The mRNA expression of insulin receptor, Nrf2, phosphoenolpyruvate carboxykinase, glucose-6-phosphate and HO-1 were estimated using the qRT-PCR. Hypoxanthine guanine phosphoribosyltransferase (HPRT1) was used as the internal gene. The data were presented as relative expression levels and were calculated via comparative Ct method (\( \Delta \Delta Ct \)).

2.12 Statistical analysis

The data of the current study was analyzed using the GraphPad Prism Software (GraphPad Prism 8, St Louis, USA). One way ANOVA with Dunnett’s test was used for comparison between the groups. All the result of this experimental study was presented as mean \( \pm \) SEM. \( P < 0.05 \) was considered as the significant.

3 Results

3.1 Water intake, body weight and organ weight

Table 3 exhibited the water intake of all group rats. Normal rats showed the normal pattern for the water consumption throughout the experimental period. Obese rats demonstrated the increased water intake. Nimbole treatment significantly \( (p < 0.001) \) suppressed the water intake. Nimbole (15 mg/kg) treated rats showed the water intake pattern similar to the normal control rats. During the obesity, increased the body weight commonly observed. HFD induced obese rats exhibited the increased

<table>
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<td>HO-1</td>
<td>GGTGTCAGGGGAAGGCTTTA</td>
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<tr>
<td>2</td>
<td>G6Pase</td>
<td>ACTCTCGCTATCTTCTGGA</td>
<td>CACAGCAATGCGAGACAGAC</td>
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<td>3</td>
<td>Nrf2</td>
<td>TGTAGATGACCAGTGAGTCGC</td>
<td>TGTCTGCTGATGCGCTGCTT</td>
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Table 2 List of primer.

<table>
<thead>
<tr>
<th>S. No</th>
<th>Parameters</th>
<th>NC</th>
<th>HFD</th>
<th>HFD+NB (5 mg/kg)</th>
<th>HFD+NB (10 mg/kg)</th>
<th>HFD+NB (15 mg/kg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Water intake</td>
<td>40.05 ( \pm ) 1.93</td>
<td>53.45 ( \pm ) 1.89</td>
<td>50.4 ( \pm ) 1.80*</td>
<td>45.94 ( \pm ) 1.92**</td>
<td>41.03 ( \pm ) 1.82***</td>
</tr>
</tbody>
</table>

All the values are expressed as mean SD for six animals. Significant levels are *\( p < 0.05 \), **\( p < 0.01 \), ***\( p < 0.001 \) when compared with DMBA control group.
body weight (Fig. 1a) and suppressed the kidney relative weight (Fig. 1c) and hepatic relative weight (Fig. 1d). Nimboide treated rats significantly \( p < 0.001 \) suppressed the body weight and increased the kidney relative weight and hepatic relative weight.

Figure 1b showed the feed intake of all group rats. HFD induced rats exhibited the increased feed intake and nimboide treated group rats exhibited the suppression of the feed intake and nimboide (15 mg/kg) treated rats exhibited the feed intake similar to the normal patter.

3.2 Glucose and NEFA

Figure 2a demonstrated glucose level of all group rats. HFD induced obese rats demonstrated the increased glucose level and suggest the impairment of the glucose adsorption. Nimboide treatment significantly \( p < 0.001 \) suppressed the glucose level and 15 mg/kg treated group rats bring the glucose level almost near to the normal level.

The level of NEFA was considerably boosted in the HFD induced obesity rats. The similar result was observed in the HFD induced obesity rats. Nimboide treated rats significantly \( p < 0.001 \) suppressed the level of NEFA (Fig. 2b).

3.3 Lipid parameters

The alteration of lipid parameters is commonly observed during the obesity. In this experimental study, HFD induced obese rats exhibited the boosted level of TC, TG, LDL, VLDL and reduced level of HDL. Nimboide treatment significantly \( p < 0.001 \) altered the lipid parameters (Fig. 3).

3.4 Resistin, HFABP and leptin

HFD induced obese rats showed the boosted level of resistin, HFABP and leptin as compared to other group rats.

![Fig. 1](image1.png)

**Fig. 1** Showed the effect of nimboide on the body weight, relative organ and fed intake of HFD induced obesity in rats. a: body weight, b: fed intake, c: kidney relative weight and d: hepatic relative weight. Statically significant different are presented via asterisks. Where *\( p < 0.05 \), **\( p < 0.01 \) and ***\( p < 0.001 \) was considered significant, more significant and extreme significant. Each group contain the 10 rats.

![Fig. 2](image2.png)

**Fig. 2** Showed the effect of nimboide on the glucose level of HFD induced obesity in rats. Statically significant different are presented via asterisks. a: glucose, b: NEFA, Where *\( p < 0.05 \), **\( p < 0.01 \) and ***\( p < 0.001 \) was considered significant, more significant and extreme significant. Each group contain the 10 rats.
Nimbolide treatment significantly \((p < 0.001)\) reduced the level of resistin (Fig. 4a), HFABP (Fig. 4b) and leptin (Fig. 4c).

3.5 Hepatic parameter

During the obesity, the alteration of hepatic parameter is commonly observed due to deposition of fat in the hepatic tissue. HFD induced obese rats showed the increased level of AST (Fig. 5a), ALT (Fig. 5b) and ALP (Fig. 5c) and nimbolide treatment significantly \((p < 0.001)\) decreased the level of hepatic parameter.

3.6 Non-hepatic parameter

HFD induced obese rats showed the suppressed level of albumin, TP and increased level of creatinine as compared to other treated or non-treated rats. Nimbolide treated rats significantly \((p < 0.001)\) increased the level of albumin (Fig. 6a), TP (Fig. 6b) and reduced the level of creatinine (Fig. 6c).

3.7 Antioxidant parameters

HFD induced obese rats showed the increased level of MDA and suppressed level of CAT, SOD, GPx, GSH, TAC. Nimbolide treatment significantly \((p < 0.001)\) decreased the level of MDA and increased the level of CAT, SOD, GPx, GSH, TAC.

Fig. 3 Showed the effect of nimbolide on the lipid parameters of HFD induced obesity in rats. Statically significant different are presented via asterisks. Where * \(p < 0.05\), ** \(p < 0.01\) and *** \(p < 0.001\) was considered significant, more significant and extreme significant. Each group contain the 10 rats.

Fig. 4 Showed the effect of nimbolide on the resistin, HFABP and leptin of HFD induced obesity in rats. a: resistin, b: HFABP and c: leptin. Statically significant different are presented via asterisks. Where * \(p < 0.05\), ** \(p < 0.01\) and *** \(p < 0.001\) was considered significant, more significant and extreme significant. Each group contain the 10 rats.
level of MDA (Fig. 7a) and boosted the level of CAT (Fig. 7b), SOD (Fig. 7c), GPx (Fig. 7d), GSH (Fig. 7e), TAC (Fig. 7f).

3.8 Inflammatory cytokines
HFD induced obese rats demonstrated the increased level of inflammatory cytokines such as TNF-α (Fig. 8a), IL-1β (Fig. 8b) and IL-6 (Fig. 8c). Nimbolide treatment significantly \( p < 0.001 \) suppressed the level of inflammatory cytokines at dose dependent manner.

3.9 Hematological parameter
Table 4 showed the effect of nimbolide on the HFD induced obese rats. HFD induced rats showed the modulated level of Hb, RBC, MCV, PCV, MCH, MCHC, lymphocytes, neutrophils, basophils, monocytes and nimbolide treatment altered the level of hematological parameters at dose dependent manner.

3.10 mRNA expression
Figure 9 exhibited the mRNA expression level of all group rats. HFD induced group rats exhibited the reduced mRNA expression of HO-1, Nrf2 and increased expression of G6Pase. Nimbolide treatment significantly \( p < 0.001 \) boosted the expression of HO-1, Nrf2 and suppressed the expression of G6Pase.
Discussion
Metabolic syndrome is a multifactorial condition of fasting hyperglycaemia, insulin resistance, dyslipidaemia, hypertension and obesity\textsuperscript{25, 26}. Previous research suggests that presence of metabolic syndrome enhance the risk for the development of the type 2 diabetes mellitus (T2DM)\textsuperscript{24}. HFD proficiently induced the metabolic syndrome which further showed via significant changes in the body characteristics, insulin resistance, dyslipidaemia and fasting hyperglycaemia\textsuperscript{13, 27}. Previous study showed the HFD induced obesity is a valid animal model for the determination of protective effect of tested drug against the obesity\textsuperscript{28}. Due to limitation of the available treatment for the obesity, in this experimental study we try to explore the anti-obesity effect of nimbole (active phyto-constituent of Azadirachta indica) against the HFD induced obesity in the rats and explore the underlying mechanism.

In this experimental study, we fed the rats with the HFD (presented in Table 1) and HFD group rats exhibited the increased body weight as well as boosted the relative fat mass due to consumption of high dietary fat content. The body weight of HFD group rats boosted possibly due to taken the high fat mass. HFD rats treated with the nimbole significantly reduced the body weight. This result...
showed the ability of nimbolide to suppress the fat mass as well as body weight. Previous research showed the similar result and provide the strength of our experimental study.

The hematological parameters were altered during the obesity. In this study, the level of Hb, RBC, MCV, PCV, MCHC, MCH, WBC, eosinophils, basophiles and monocytes were altered. The previous study exhibited the similar result. HFD group rats exhibited the increased level of neutrophil count and suppressed level of lymphocytes which in occurred due to the fact that obesity can start the production of glucocorticoids as well as IL-6 that play an important role in bone marrow granulopoiesis. They further boost the neutrophils mobilization from bone marrow and also induces the prolongation of their intravascular half-life. Previous study suggests that lymphocytopenia is commonly observed in the obese rats due to systemic inflammatory response due to suppression of T-cells in thymus, peripheral blood and spleen. Furthermore, the production of the corticosteroids increases during the obesity that further shown the increase production of lymphocytopenia. Targeting the lymphopenia and neutrophilia is the best approaches to treated the obesity. Both parameters can be attributed for their antioxidant and anti-

Table 4  The effect of nimbolide on the hematological parameters against HFD induced obesity in rats.

<table>
<thead>
<tr>
<th>S. No</th>
<th>Parameters</th>
<th>NC</th>
<th>HFD</th>
<th>HFD+NB (5 mg/kg)</th>
<th>HFD+NB (10 mg/kg)</th>
<th>HFD+NB (15 mg/kg)</th>
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<tr>
<td>1</td>
<td>Hb (g/dL)</td>
<td>13.43 ± 1.56</td>
<td>8.45 ± 1.39</td>
<td>9.04 ± 1.34*</td>
<td>11.89 ± 2.64**</td>
<td>13.02 ± 2.03***</td>
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<td>2</td>
<td>RBC (10^6/µL)</td>
<td>6.56 ± 1.03</td>
<td>2.45 ± 1.01</td>
<td>3.04 ± 1.15*</td>
<td>4.56 ± 1.48**</td>
<td>6.32 ± 1.83***</td>
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<td>3</td>
<td>WBC (10^3/µL)</td>
<td>7.34 ± 1.94</td>
<td>13.28 ± 2.98</td>
<td>11.04 ± 2.73*</td>
<td>9.49 ± 2.04**</td>
<td>7.87 ± 1.90***</td>
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<td>4</td>
<td>MCV (fL)</td>
<td>80.3 ± 3.45</td>
<td>95.83 ± 4.83</td>
<td>93.91 ± 4.89*</td>
<td>90.03 ± 3.82**</td>
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<tr>
<td>5</td>
<td>PCV (%)</td>
<td>48.31 ± 2.94</td>
<td>43.12 ± 3.21</td>
<td>44.09 ± 3.84*</td>
<td>45.81 ± 3.82**</td>
<td>47.08 ± 2.80***</td>
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<tr>
<td>6</td>
<td>MCH (pg)</td>
<td>30.23 ± 2.83</td>
<td>36.84 ± 2.89</td>
<td>35.41 ± 1.89*</td>
<td>33.04 ± 2.04**</td>
<td>31.04 ± 1.89***</td>
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<tr>
<td>7</td>
<td>MCHC (g/dL)</td>
<td>29.34 ± 2.04</td>
<td>32.40 ± 2.93</td>
<td>32 ± 2.03ns</td>
<td>31.34 ± 1.89**</td>
<td>30.23 ± 1.89***</td>
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<tr>
<td>8</td>
<td>Lymphocytes (10^3/µL)</td>
<td>60.64 ± 3.09</td>
<td>22.01 ± 2.34</td>
<td>27.43 ± 2.84**</td>
<td>38.04 ± 3.01***</td>
<td>57.3 ± 3.79***</td>
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<tr>
<td>9</td>
<td>Neutrophils (10^3/µL)</td>
<td>25.03 ± 1.89</td>
<td>65.02 ± 3.24</td>
<td>59.30 ± 3.03*</td>
<td>43.05 ± 2.89**</td>
<td>28.03 ± 3.06***</td>
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<td>Basophils (10^3/µL)</td>
<td>0.21 ± 0.08</td>
<td>0.32 ± 0.09</td>
<td>0.31 ± 0.05ns</td>
<td>0.30 ± 0.04*</td>
<td>0.27 ± 0.07**</td>
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<tr>
<td>11</td>
<td>Monocytes (10^3/µL)</td>
<td>3.78 ± 0.45</td>
<td>3.42 ± 0.65</td>
<td>3.49 ± 0.78*</td>
<td>3.58 ± 0.98**</td>
<td>3.75 ± 0.87***</td>
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<tr>
<td>12</td>
<td>Eosinophils (10^3/µL)</td>
<td>1.86 ± 0.15</td>
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<td>1.76 ± 0.13ns</td>
<td>1.8 ± 0.12**</td>
<td>1.84 ± 0.13***</td>
</tr>
</tbody>
</table>

All the values are expressed as mean SD for six animals. Significant levels are *p < 0.05, **p < 0.01, ***p < 0.001 when compared with DMBA control group.

Fig. 9 Showed the effect of nimbolide on the mRNA expression of HFD induced obesity in rats. a: HO-1, b: Nrf2 and c: G6Pase. Statically significant different are presented via asterisks. Where *p < 0.05, **p < 0.01 and ***p < 0.001 was considered significant, more significant and extreme significant. Each group contain the 10 rats.

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obesity effect that can suppress the production of oxidative stress via corticosteroids and cytokines. Nimbolide treatment considerably modulated the haematological parameters at dose dependent.

The current investigation has showed the alteration of lipid profile in the HFD rats that suggesting the dyslipidaemia along with the considerably boosting the glucose level. Previous investigation exhibited the similar types of results. Obesity is the hallmark of the dyslipidaemia and increased the level of TGs along with the increased level of LDL and suppressed the level of HDL. The dyslipidaemia mainly attributed to enhanced the NEFA level that resultant excessive spill over due to HFD. Previous research suggests that the NEFA can affect the insulin signaling pathway, suppress the glucose uptake taken by muscle, increased TAGs synthesis and finally induces the gluconeogenesis in the liver. All the alteration commonly observed during the hyperglycemia in HFD group and nimbolide modulated the all parameters and suggesting the anti-obesity effect. It is well known that oxidative stress plays an important role in the hyperglycemia and obesity or both. Oxidative stress starts the production of free radicals that can delay glucose utilization via peripheral tissue and insulin function.

Previous study suggest that the increased fat mass led to increase the adipokines production include resistin, leptin and inflammatory cytokines. The observed hyperleptinemia, as well as the observed decrease in food intake, are suggestive of leptin resistance, a situation that resulted in decreased energy expenditure. Resistin is directly responsible for the expansion of insulin resistance. In this study, we have observed that HFD induced rats exhibited the increase level of resistin and leptin and nimbolide treatment considerably suppressed the resistin and leptin. This result coincided with the previous result. However, NEFA can act not only as a source of energy, but it can also play a considerable role in regulation of intracellular protein kinases such as JNK and pKC, which resultant its induces the dysfunction in insulin signaling. IL-6 and TNF-α are considered adipokines marker and both parameter level boosted during the obesity. In this experimental study, we observed the increased level of inflammatory cytokines includes IL-6, TNF-α, IL-1β which can function to boost the inflammatory reaction as well as boosted the obesity. During the obesity, start the accumulation of fat in the tissue due to dysfunctional of lipid metabolism such as lipolysis which in turn boost the production and secretion of free fatty acids (FFAs). Increased FFAs level can boost the inflammatory cytokines and expansion of insulin resistance. Additionally, reduction of intracellular antioxidant in fat tissue, boosted the production of reactive oxygen species (ROS) which further induced the oxidative stress. The increased oxidative stress enhanced the production of insulin resistance and inflammatory cytokines. Enhancing the NEFA in the obese rat potentially enhanced the inflammatory cytokines and oxidative stress, due to induces the electrophysiologically remodelling in the cardiac muscle. In this study, HFD group rats exhibited the increased level of lipid peroxidation, glucose and reduced level of endogenous antioxidant enzymes and nimbolide treated rats significantly suppressed the level of glucose and altered the lipid parameters. Nimbolide exhibited these effects due to suppressing the adipokines as well as lipid metabolism and its treatment restores the antioxidant enzymes and suppressed the inflammatory cytokines.

Previous study suggest that Nrf2 affect the various antioxidant protein expression includes NAD(P)H quinone oxidoreductase 1 (NQO1), glutathione peroxidase and HO-1 (phase II detoxifying enzyme). In this experimental study, we scrutinized the anti-obesity effect of nimbolide against HFD induced obesity in rats via alteration of Nrf2/HO-1 pathway. The result suggests that nimbolide considerably boosted the expression of Nrf2 and HO-1 and previous research suggest that the Nrf2/HO-1 pathway play an important role in induction of obesity and oxidative stress. Definitely, nimbolide administration increased the HO-1 and Nrf2 mRNA expression in hepatic tissue.

5 Conclusion

The HFD induced obesity is related with the metabolic perturbations that can be arise due to induction of inflammatory reaction and oxidative stress which further exhibited the effect on the kidney, endothelial blood vessels and liver function. In this study, nimbolide considerably reduced the body weight, relative liver, kidney and feed intake. Nimbolide also suppressed the blood glucose level along with the alteration of hepatic and non-hepatic parameters. Nimbolide treatment significantly altered the lipid and antioxidant parameters along with suppression of inflammatory cytokines. The result suggests that the nimbolide having the beneficial effect in the treatment and prevention of obesity, oxidative stress, dyslipidemia, gluconeogenesis and inflammation induced by HFD via Nrf2/HO-1 pathway. These findings point to the possibility of using nimbolide to prevent and treat metabolic syndrome.

Author Contribution

Lin Zhang performed research, Yujun Li Daqing Sun and Feng Bai contributed analytic tools, analyzed data. Feng Bai designed research. All authors wrote the manuscript.
and corrected the proof.

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