# Blood Biochemical and Histopathological Investigation of Novel Herbal Formulas in High Fat Diet/Streptozotocin-Induced Hyperlipidemia and Hyperglycemia in Rats

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## Abstract

Polyherbal medicines are widely used as alternative treatment for various diseases including obesity and diabetes, as they are recognized as being less toxic compared to modern synthetic medicines. Nevertheless, the safety of herbal formulations has been widely debated. Therefore, this research aimed to investigate the effects of two novel herbal formulas on blood biochemistry and histopathology of liver and kidney of high-fat diet (HFD)/streptozotocin (STZ)-induced hyperglycemia/hyperlipidemia (HPG/HPL) in rats. Formula 1 (F1) consisted of *Dracaena cochinchinensis*, *Miliusa velutina*, *Emblica officinalis*, *Piper interruptum*, and *Albizia procera*, whereas Formula 2 (F2) consisted of *Cinnamomum bejolghota*, *Miliusa velutina*, *Acacia concinna*, *Ocimum gratissimum*, and *Albizia procera*. Rats in groups 1-3 were normal and fed a normal diet and orally received distilled water, F1 and F2, respectively. Rats in groups 4-9 were HPG/HPL induced rats and fed a high-fat diet for two months to induce hyperlipidemia and subsequently injected with 30 mg/kg of STZ to induce hyperglycemia. HPG/HPL-induced rats were then orally administered F1 and F2 at doses of 200 mg/kg daily for 30 days. These treatments were compared to the treatments of synthetic drugs, orlistat, metformin, and atorvastatin. After the treatment period, relative liver and kidney weights were assessed. Biochemical indices for liver function-including aspartate aminotransferase, alanine aminotransferase, alkaline phosphatase, total bilirubin and direct bilirubin-and kidney function-including blood urea nitrogen and creatinine-were evaluated. Additionally, hepatorenal histopathological alterations were examined. The results revealed that both F1 and F2, as well as the synthetic drugs, did not significantly alter relative liver weights compared to normal rats and HPG/HPL-induced rats. Moreover, the novel herbal formulas were able to attenuate all the biochemical parameters and structural changes in the liver and kidney of HPG/HPL-induced rat, except for F1, which caused an increase in aspartate aminotransferase level compared to normal and HPG/HPL-induced rats. Nevertheless, the novel herbal formulas had mild negative effects on liver functions in normal rats. In conclusion, the novel herbal formulas could restore liver and kidney function and structural integrity in HPG/HPL-induced rats but not in normal rats.

## Introduction

Medicinal plants have gained popularity, particularly during the COVID-19 pandemic. They are widely used to address various health condition, COVID-19, obesity, diabetes and cancers. However, excessive consumption of herbal medicines can lead to adverse effects, particularly on vital organs such as the liver and kidneys. Many modern herbal formulations focus on reducing fat and blood sugar levels, positioning them as potential alternatives to conventional medicine.. In 2019, obesity-related conditions were responsible for approximately 5 million deaths worldwide, with prevalence rates rising among both adults and children [1]. The liver and kidneys play essential roles in drug metabolism and elimination. However, excessive or inappropriate drug use can lead to inflammation and organ damage [2]. There have been reports of herbal plant toxicity affecting organ systems [3]. In response to these concerns, this study aims to develop an herbal formula with fat- and glucose-lowering effects using herbal extracts from *Dracaena cochinchinensis* (DC), *Miliusa velutina* (MV), *Emblica officinalis* (EO), *Piper interruptum* (PI), and *Albizia procera* (AP), *Cinnamomum bejolghota* (CB), *Acacia concinna* (AC), and *Ocimum gratissimum* (OG), and to evaluate its impact on liver and kidney tissues, as well as blood biochemistry. If proven safe, this formula could serve as a promising alternative for the treatment of diabetes and obesity in the future.

## Materials and Methods

### Herbal formulas

**Herbal formula 1** (F1) : DC + MV + EO + PI + AP (1:1:1:1:1) **Herbal formula 2** (F2) : CB + MV + AC + OG + AP (1:1:1:1:1)

### Experimental groups

Male Wistar rats

**1) Normal rats**

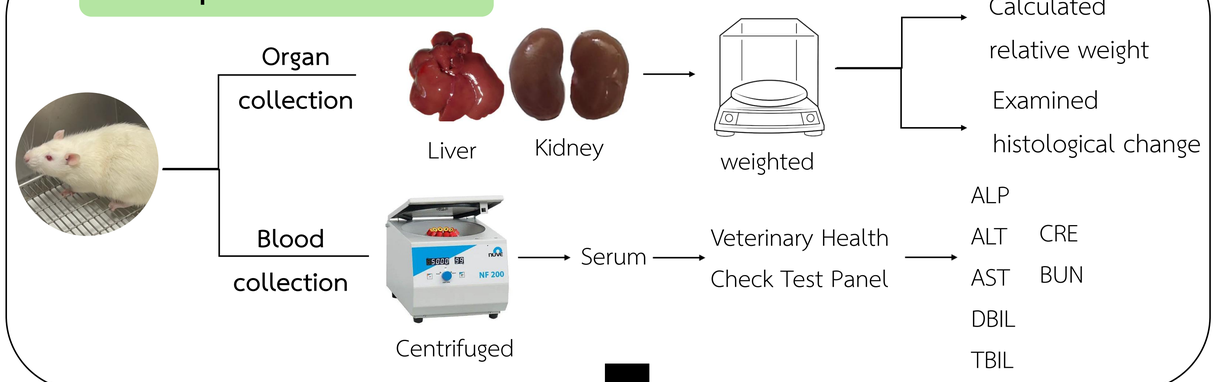
- Normal control (NC)_normal diet + distilled water
- Formula 1 group (F1)_normal diet + F1 at 200 mg/kg
- Formula 2 group (F2)_ normal diet + F2 at 200 mg/kg

**2) Obesity rats**

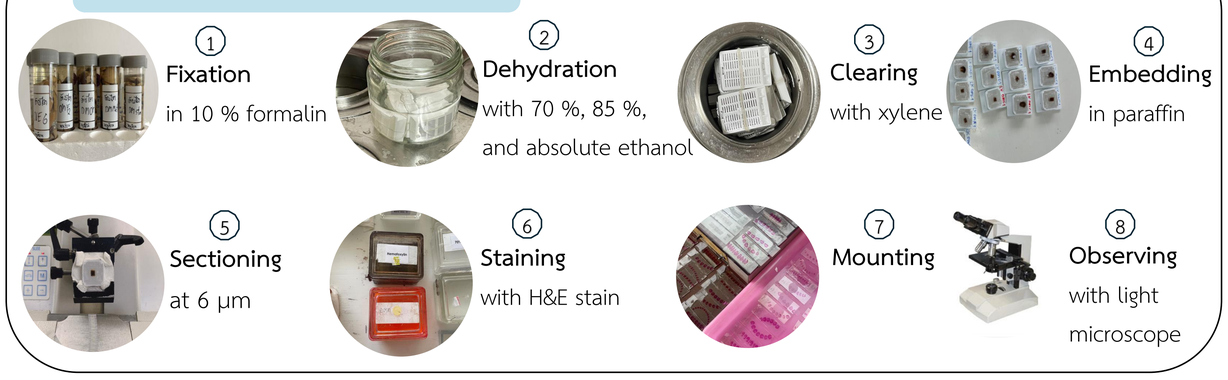
- Negative control (NEG)_high-fat diet/STZ + distilled water
- HLG/F1_high-fat diet/STZ + F1 at 200 mg/kg
- HLG/F2_high-fat diet/STZ + F2 at 200 mg/kg
- ATO_high-fat diet/STZ + atorvastatin 5 mg/kg
- MET_high-fat diet/STZ + metformin 250 mg/kg
- ORL_high-fat diet/STZ + orlistat 12 mg/kg

Note: HLG (high lipid and glucose)

### Sample collection




### Histological process




## Results and Discussion

**Table 1.** Relative weights of liver and kidney and blood biochemical parameters.

| Experimental groups | Relative weight (g/100g BW) | | Biochemical parameters | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | Liver | Kidney | ALP (U/L) | ALT (U/L) | AST (U/L) | DBIL (mg/dL) | TBIL (mg/dL) | CRE (mg/dL) | BUN (mg/dL) |
| NC | 2.72±0.17$^{ab}$ | 0.32±0.01$^{a}$ | 111.00±14.19$^{a}$ | 42.83±4.45$^{a}$ | 83.33±3.35$^{d}$ | 0.1±0.00 | 0.30±0.00$^{a}$ | 0.63±0.25 | 41.38±2.18$^{bcd}$ |
| NEG | 2.33±0.44$^{b}$ | 0.26±0.03$^{b}$ | 87.65±3.25$^{bcd}$ | 21.45±6.20$^{b}$ | 109.07±9.00$^{bcd}$ | 0.1±0.00 | 0.25±0.06$^{ab}$ | 0.50±0.00 | 49.57±8.02$^{ab}$ |
| HLG/F1 | 2.68±0.26$^{ab}$ | 0.31±0.02$^{a}$ | 102.98±21.97$^{ab}$ | 47.83±11.51$^{a}$ | 148.17±39.17$^{a}$ | 0.1±0.00 | 0.25±0.06$^{ab}$ | 0.58±0.10 | 51.77±7.28$^{a}$ |
| HLG/F2 | 2.45±0.27$^{ab}$ | 0.31±0.03$^{a}$ | 86.60±4.13$^{bcd}$ | 22.03±3..47$^{b}$ | 96.90±13.70$^{bcd}$ | 0.1±0.00 | 0.23±0.06$^{ab}$ | 0.53±0.05 | 36.84±2.69$^{cd}$ |
| ATO | 2.61±0.21$^{ab}$ | 0.30±0.02$^{a}$ | 92.85±13.18$^{bc}$ | 20.40±8.00$^{b}$ | 124.90±28.47$^{abc}$ | 0.1±0.00 | 0.23±0.05$^{ab}$ | 0.55±0.10 | 40.04±1.76$^{bcd}$ |
| MET | 2.51±0.11$^{ab}$ | 0.29±0.02$^{a}$ | 72.50±8.62$^{d}$ | 18.47±4.19$^{b}$ | 82.47±8.12$^{d}$ | 0.1±0.00 | 0.23±0.06$^{ab}$ | 0.53±0.05 | 33.08±5.07$^{d}$ |
| ORL | 2.85±0.23$^{a}$ | 0.30±0.01$^{a}$ | 88.60±6.28$^{bcd}$ | 25.97±2.72$^{b}$ | 112.05±23.7$^{bcd}$ | 0.1±0.00 | 0.20±0.00$^{b}$ | 0.50±0.00 | 46.08±6.34$^{abc}$ |
| F1 | 2.36±0.19$^{b}$ | 0.31±0.03$^{a}$ | 80.75±3.82$^{cd}$ | 21.98±3.87$^{b}$ | 128.58±16.38$^{ab}$ | 0.1±0.00 | 0.23±0.05$^{ab}$ | 0.63±0.25 | 41.72±6.29$^{bcd}$ |
| F2 | 2.41±0.13$^{b}$ | 0.33±0.03$^{a}$ | 77.25±6.27$^{cd}$ | 19.97±0.47$^{b}$ | 91.43±4.66$^{cd}$ | 0.1±0.00 | 0.20±0.00$^{b}$ | 0.57±0.12 | 44.82±9.23$^{abc}$ |

Values are expressed as mean ± S.D. The superscript letters indicate statistically significant differences ($p < 0.05$) between the experimental groups (Duncan's test).

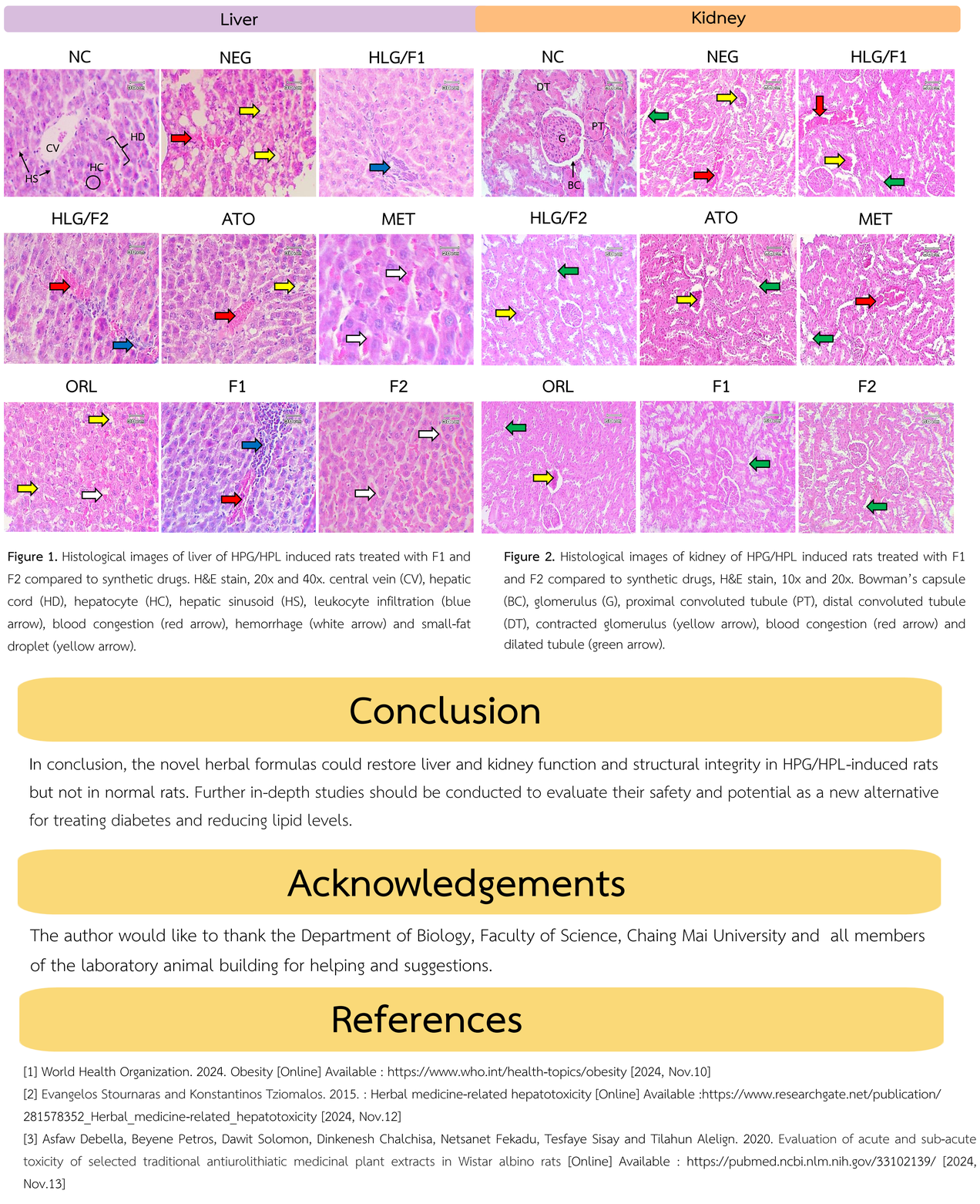



**Figure 1.** Histological images of liver of HPG/HPL induced rats treated with F1 and F2 compared to synthetic drugs. H&E stain, 20x and 40x. central vein (CV), hepatic cord (HD), hepatocyte (HC), hepatic sinusoid (HS), leukocyte infiltration (blue arrow), blood congestion (red arrow), hemorrhage (white arrow) and small-fat droplet (yellow arrow).

**Figure 2.** Histological images of kidney of HPG/HPL induced rats treated with F1 and F2 compared to synthetic drugs, H&E stain, 10x and 20x. Bowman's capsule (BC), glomerulus (G), proximal convoluted tubule (PT), distal convoluted tubule (DT), contracted glomerulus (yellow arrow), blood congestion (red arrow) and dilated tubule (green arrow).

## Conclusion

In conclusion, the novel herbal formulas could restore liver and kidney function and structural integrity in HPG/HPL-induced rats but not in normal rats. Further in-depth studies should be conducted to evaluate their safety and potential as a new alternative for treating diabetes and reducing lipid levels.

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