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Effect Of (+) And (-) Hydroxycitric Acid Sterio-Isomers Present In Natural Products In Counteracting Insulin Resistance

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Abstract:

Metabolic syndrome is a cluster of cardiovascular and metabolic risk factors that include impaired glucose metabolism and obesity. The use of nutraceuticals is an ideal choice for controlling this disorder. The aim of the present study is to investigate the effect (-) hydroxycitric acid present in garcinia fruit rind and (+) hydroxycitric acid present in hibiscus calyx on metabolic syndrome and compare it to that of metformin. Metabolic syndrome was induced in rats by ingestion of high fat high fructose (HFHF) diet for 90 days. Metformin (500 mg/Kg animal b.wt.), garcinia (1000 mg/Kg animal b.wt.) and hibiscus (250 mg/Kg animal b.wt) were orally administered throughout the last 30 days of the HFHF diet regimen. Both garcinia and hibiscus were effective in reducing serum blood glucose and insulin levels. The effect of garcinia on blood glucose was comparable to that of metformin, while the effect of hibiscus on insulin and HOMA-IR was comparable to that of metformin. Both were able to reduce serum leptin level. All treated groups showed a significant decrease in total cholesterol level. Only hibiscus was able to normalize liver function while garcinia failed to reduce the elevated liver function. As a conclusion we would recommend the use of hibiscus over garcinia to overcome the adverse effects of metabolic syndrome.

Key words: Metabolic syndrome; Hydroxycitric acid; Metformin; Garcinia; Hibiscus; Liver functions.

1. Introduction:

Obesity/central obesity, insulin resistance (IR), hypertension and circulating hypertriglyceridemia (dyslipidaemia) are the major features of metabolic syndrome [1]. Sedentary life style along with the ingestion of high quantities of carbohydrates and/or fats are considered as chief hazardous factors for developing metabolic syndrome, that contribute to the two central clinical features, i.e. central obesity and insulin resistance (IR). Imbalance between energy intake and energy expenditure results in excess body fat accumulation, as a consequence obesity occurs [2]. It appears to precede the development of the other metabolic syndrome causal factors. The world's population is facing an unprecedented increase in obesity rates. Obesity is most important public health problem that causes many diseases, including diabetes mellitus, dyslipidemia, hypertension, kidney diseases, some cancers, coronary heart disease and osteoarthritis (1). Not only are the developed countries facing worrying numbers of obese people, but also developing countries are reporting an increasing number of deaths related to obesity [3]. Worldwide, people

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having Type 2 Diabetes have reached more than 400 million, and ~2 billion are considered overweight or obese. By 2045, it is expected that more than 600 million people will suffer from type 2 diabetes [4].

One of the most common substitutional treatments, which have been used widely for weight loss, is herbs. The efficacy and safety of medicinal plants in dealing with obesity, dyslipidemia and diabetes mellitus have been widely investigated [5].

Garcinia cambogia fruit rind, known as Malabar tamarind, is commonly used in folk medicines to treat ulcers, diarrhea, hemorrhoids, tumors, fever and dysentery. It contains bioactive xanthones and benzophenones which are effective as anti-obesity, anti-diabetic, anti-platelet aggregation, anti-ulcer rand anti-tumor [6]. *Hibiscus sabdariffa* L. (roselle) family (Malvaceae) is a herbaceous plant, cultivated for leaf, fleshy calyx, seed or fibre [7]. It is commonly used in beverages and foods such as teas, jams, and jellies [8]. The antihypertensive effect of hibiscus extracts is attributed to the inhibition of angiotensin converting enzyme. Anthocyanins along protocatechuic acid and flavonol glycosides act synergistically to mediate this effect [9].

Metformin (N, N-dimethylbiguanide), a biguanide drug with a low toxicity profile, has been widely used in treating diabetes [10]. Metformin is a commonly used oral drug to treat type 2 diabetes and is considered well tolerated and safe with many years of clinical experience [11]. It is the first-line treatment and most prescribed anti-diabetes drug [12]. It has been used extensively in the management of type 2 diabetes for over 50 years, as a monotherapy or with other oral anti-diabetic drugs and insulin [13].

Garcinia, specially its main active ingredient hydroxycitric acid, is well known for its effect on reducing body weight [6]. The aim of the present study was to investigate the effect (-) hydroxycitric acid, present in garcinia fruit rind, and (+) hydroxycitric acid, present in hibiscus calyxes, on metabolic syndrome and to compare it to that of metformin.

2. Materials and methods:

Animals:

Adult male Wister albino rats weighing 120-140 g purchased from the animal house colony of the National Research Centre (Dokki, Giza, Egypt) were used. The animals were kept in the animal house under hygienic conditions. Animals were housed in controlled environmental conditions, at a constant temperature (25 ± 2 °C) and under a 12/12-h light/dark cycle. The protocol of this study complies with *the guide for Care and Use of Laboratory Animals* published by the US National Institutes of Health (NIH Publication No 85-23, revised 2011) and was approved by Ethics committee for Animals Experimentation at Faculty of Pharmacy, Cairo University at July 21st 2014 (Permit Number: PT 1178) and National Research Centre, Giza, Egypt at August 14th 2014 (Permit Number: 14:101). All efforts were made to minimize animal suffering and reduce the number of animals used.

Drugs:

Metformin powder was generously given as a gift from Cid Pharmaceutical Company. Garcinia capsules (500 mg Garcina Cambogia extract, 50% (-) hydroxycitric acid) were purchased from Paradise Herbs and Essentials, USA. Hibiscus Sabdarifa powdered calyxes was given as a gift from Sigma Pharmaceutical Industries and used to prepare water extract.

Standard chow diet: obtained from the animal house of the National Research Centre (Dokki, Giza, Egypt). High fat high fructose diet: A diet consisting of 24% W/W fat and 25% W/V fructose in drinking water was used for the induction of metabolic syndrome [14].

Experimental design:

The animals were housed in a temperature controlled room (20-22°C) with reversed 12 h light, dark cycles. After an adaptation period of 7 days, animals fed high fat high fructose diet for 60 days for the induction of metabolic syndrome. After 60 days of high fat high fructose diet, serum blood glucose, insulin, HOMA-IR, triglycerides and HDL were measured randomly to ensure the induction of metabolic syndrome. After induction of metabolic syndrome, animals were divided into 5 groups (6 rats each) and treated as follows: **Group 1:** Rats fed standard diet for 30 days and served as normal control group. **Group 2:** Rats fed high fat diet (24% W/W fat) combined with 25 % W/V fructose in drinking water for 30 days and served as metabolic syndrome group. **Group 3:** Rats receiving Metformin (500 mg/kg/day p.o) [15] and fed high fat diet (24% W/W fat) combined with 25% W/V fructose in drinking water for 30 days. **Group 4:** Rats receiving Garcinia (1000 mg/kg/day p.o) [16] and fed high fat diet (24% W/W fat) combined with 25% W/V fructose in drinking water for 30 days. **Group 5:** Rats

receiving Hibiscus (250 mg/kg/day p.o) [17] and fed high fat diet (24% W/W fat) combined with 25% W/V fructose in drinking water for 30 days. This is represented in figure 1.

After 30 days of treatment, rats were given no food for 12 h. and then blood samples (3ml) were collected from the retro-orbital plexus in dry centrifuge tubes and left to clot at room temperature. The samples were centrifuged at 1500 rpm for 10 min, and the clear supernatant was separated and used for the measurement of serum glucose, insulin, insulin resistance index (HOMA-IR), leptin, serum total lipid profile (triglyceride, total cholesterol, low density lipoprotein cholesterol (LDL), high density lipoprotein cholesterol (HDL), LDL/HDL ratio), serum liver function enzyme (Alanine transaminase (ALT) and Aspartate transaminase (AST)).

Percentage change of body weight gain was calculated as follows:% change of body weight of each rat = $\frac{\text{final weight initial weight}}{\text{Initial weight}} \times 100$

Biochemical Measurements:

Serum glucose [18], triglycerides [19], total cholesterol [20], LDL-cholesterol [21], HDL-cholesterol [22], ALT and AST [23] were determined using enzymatic colorimetric kits purchased from biodiagnostic. The serum insulin was determined using ELISA kit purchased from SPI BIO, whereas serum leptin was determined using ELISA kit purchased from RayBiotech. The procedures were performed according to the manufacturer's instructions.

Histopathological examinations:

Animals were sacrificed by cervical dislocation. The livers were isolated for histopathological examination. The liver samples were carefully removed, rinsed with ice-cold saline and immediately fixed in 10% formalin for 24 h. After fixation, specimens were trimmed using a scalpel to enable them to fit into an appropriately labeled tissue cassette. The samples were dehydrated by serial dilution of alcohol, cleared in xylenol and embedded in paraffin. Tissue specimens were cut into sections that can be placed on a slide. Tissues were stained using histochemical stains (haematoxylin and eosin) and examined under the light electric microscope.

Data analysis:

Data were expressed as mean ± standard deviation (S.D.). The data were analyzed using one-way ANOVA followed by performing Tukey test. GraphPad Prism software (version 7; GraphPad Software, Inc., San Diego, CA, USA) was used to perform the statistical analysis and to create the graphical presentations. The level of significance was fixed at $P < 0.05$ for all statistical tests. Data points were considered outliers only if they failed the Dixon test or if they were greater than four standard deviations from the mean. Finally, Mead's "Resource Equation" was used to ensure that sample sizes were sufficient to establish this statistically significant difference.

3. Results:

Figure 2 is quite revealing in several ways; it is apparent that rats with metabolic syndrome showed a significant increase in body weight gain, serum blood glucose, serum insulin and HOMA-IR, as compared to normal control group value. Treatment of animals with garcinia (1000 mg/Kg p.o.) and metformin (500 mg/Kg p.o) significantly decreased body weight gain after 30 days of treatment, as compared to metabolic syndrome group (43.42% and 40.71%, respectively). However, treatment with hibiscus (250 mg/kg, p.o.) didn't show any significant effect on body weight gain, as compared to metabolic syndrome group. All treated groups produced significant decrease in serum blood glucose level (74.92%. 75.14% and 84.38%, respectively), serum insulin level (26.84%, 60.67% and 30.4%, respectively) and HOMA-IR (20.55%, 45.03% and 25.58%, respectively) after 30 days of treatment, as compared to metabolic control group . Compared to metformin, garcinia (1000 mg/kg, p.o.) didn't show any significant effect on serum blood glucose, while hibiscus (250 mg/kg, p.o.) didn't show any significant effect on serum insulin and HOMA-IR.

In Figure 3, it is clear that rats with metabolic syndrome showed a significant increase in serum leptin, as compared to normal control group. Compared to metabolic syndrome group, all treated groups showed significant decrease in serum leptin level after 30 days of treatment (52.03%, 84.24% and 49.56%, respectively).

In Figure 4, it is clear that rats with metabolic syndrome showed a significant increase in serum triglycerides, total cholesterol, LDL and LDL/HDL ratio, while a significant decrease in HDL, as compared to normal control group . Compared to metabolic syndrome group, apparently metformin (500 mg/Kg p.o) showed a significant decrease in serum triglycerides level to 65.5% after 30 days of treatment. However, all treated groups showed significant decrease in serum total cholesterol level, as compared to metabolic syndrome control group (74.73%, 82.7% and 80.33%, respectively). It is obvious that only treatment of rats with metabolic syndrome with metformin (500 mg/kg p.o.) for 30

days results in a significant decrease in serum LDL level and a significant increase in serum HDL level, as compared to metabolic syndrome group (71.01% and 120.1%, respectively). Nevertheless, according to Figure 4, all treated groups showed significant decrease in serum LDL/HDL ratio after 30 days of treatment, as compared to metabolic syndrome group (59.83%, 79.76% and 81.18%, respectively)

Figure 5 showed that metabolic syndrome group showed a significant increase in ALT and AST levels, as compared to normal control group. Only treatment with metformin (500 mg/kg p.o.) for 30 days results in a significant decrease in serum ALT level, as compared to metabolic syndrome group (82.4%). However, the treatment of animals with metformin (500 mg/kg p.o.) and hibiscus (250 mg/kg p.o.) resulted in significant decrease in serum AST level after 30 days of treatment, as compared to metabolic syndrome group (80.24% and 68.75%, respectively).

For histopathology, sections from normal control liver showed normal liver architecture, with preserved central vein and hexagonal appearance of the hepatic lobules (Fig.6A). Nevertheless, the metabolic syndrome group showed extensive liver affection, dilated congested central veins (red arrow), cytoplasmic vacuolations specially zone 1and 2 (yellow arrows), and areas of necrosis along all zones, especially those located at zone 3 (blue arrows). Further examination revealed areas of fatty infiltration with attempts of nodules formation (Fig.6B). On the other hand, metformin (500 mg/Kg p.o.) showed central vein less dilated (red arrow), cytoplasmic vacuolation in the form of tiny vacuoles (yellow arrows) restricted to zone 3 "around central vein", and no necrosis but fatty globules scattered at various fields examined (green arrows) much improvement than control positive (Fig.6C). Additionally, garcinia (1000 mg/Kg p.o.) revealed dilated central vein (red arrow), extensive cytoplasmic vacuolation all over the three zones (yellow arrows) and necrosis (blue arrows) with a little improvement than control positive (Fig.6D). Moreover, hibiscus (250 mg/kg p.o.) absent fat infiltration, central vein is dilated, congested but with preserved architecture (Fig.6E).

4. Discussion:

Abdominal obesity, impaired glucose metabolism and dyslipidemia are three interconnected factors that eventually lead to what is so called "metabolic syndrome" {24]. Physical inactivity and diet rich in fats and carbohydrates are risk factors for central obesity and insulin resistance (IR). Obesity usually precedes the development of the other metabolic syndrome risk factors [1]. Several animal models have been widely used for the induction of metabolic syndrome. One of these models is high fat, high fructose diet [25]. In the present study high fat high fructose diet was used for the induction of metabolic syndrome. Diet consists of 24% W/W fat and 25% W/V fructose in drinking water [26].

The results of the present study showed that treatment with metformin (500 mg/Kg p.o.) for 30 days significantly decreased body weight of metabolic syndrome animals with significant decrease in serum blood glucose, insulin levels, HOMA index and leptin level, as compared to metabolic syndrome group. The findings of the current study are consistent with those of the previous study which showed that insulin sensitivity rises after metformin treatment as it decreases the hepatic insulin resistance, serum insulin level and HOMA index [27, 28, 29]. It was also reported that metformin decreased leptin levels, in adipose tissue as well as in serum levels [30]. In addition, the results of the present study showed that rats with metabolic syndrome treated with metformin (500 mg/Kg p.o.) for 30 days results in significant decrease in serum triglycerides level, total cholesterol and LDL level, as compared to rats with metabolic syndrome group. The previous studies also showed that metformin significantly decreased plasma triglycerides, total cholesterol and LDL concentrations and increased serum HDL level [27, 31, 32]. This could be attributed to the effect of metformin in increasing free fatty acid esterification and inhibition of lipolysis in adipose tissue [28]. In this study, metformin (500 mg/Kg p.o.) was found to reduce serum ALT and AST. This shows that metformin treatment may improve liver functions, which comes in agreement with previous studies which showed that metformin decreased serum ALT and AST levels [29, 33].

The anti-obesity effect of garcinia is attributed to the combined actions of several mechanisms including suppressing de novo fatty acid biosynthesis and increasing energy expenditure, which consequently decreases body fat accumulation and promotes weight loss in experimental animals [34]. The previous studies showed that the hyperglycemia in high fat diet is improved with garcinia by declining insulin resistance. Further, garcinia promotes glycogenesis and lipid oxidation. It could be used to

manage diabetes, by increasing metabolic pathway via rising glucose oxidation through improving insulin action [16, 35]. In the present study, garcinia was investigated to highlight its beneficial effects on high fat high fructose induced metabolic syndrome. Metformin was used as a standard anti-diabetic drug [15].

The current study showed that garcinia (1000 mg/Kg) had produced significant reduction of body weight gain after 30 days of treatment, as compared to metabolic syndrome group. Serum blood glucose, insulin level, insulin resistance index and leptin were significantly decreased as compared to metabolic syndrome group. The effect of garcinia on blood glucose was comparable to that of metformin. Moreover, garicinia (1000 mg/kg) was found to cause significant decrease in serum total cholesterol and LDL level, as compared to metabolic syndrome value. These results match those observed in earlier study that showed that garcinia had reduced serum level of these parameters in high fat fed animals and high sucrose fed animals [34]. The present study also showed that treatment of rats with metabolic syndrome with garicinia (1000 mg/kg) had not caused any significant effect on serum triglycerides and HDL levels, as compared to metabolic syndrome group, which comes in harmony with another study that showed that administration of garcinia to high fat diet fed mice did not produce any significant effect on these parameters [36].

On the other hand, the results of the present study showed that rats with metabolic syndrome treated with garcinia (1000 mg/Kg 30 days) showed a significant increase in serum ALT and AST level, compared to normal control group. Moreover, the previous study also showed that mice supplemented with garcinia had impaired liver function shown by the increase of plasma ALT and AST, indicating that garcinia may promotes liver damage in mice fed high fat diet. Several studies have shown that products containing hydroxycitric acid have potential hepatotoxicity [36].

It is interesting to note that treatment of rats with metabolic syndrome with hibiscus (250 mg/kg p.o.) leads to a significant decrease in serum glucose level insulin level and HOMA-IR, as compared to metabolic syndrome . The effect on insulin and HOMA-IR level was comparable to that of metformin. Former investigators reported that hibiscus has a hypoglycemic effect [36, 37]. Other studies stated that hibiscus has anti-insulin resistance properties and may have antdiabetic activity due to restoration of insulin level [27, 30, 38, 39].

Furthermore, hibiscus resulted in a significant decrease in serum leptin level, as compared to metabolic syndrome group. The previous studies have shown the effect of hibiscus in lowering seum leptin level [40]. It was previously reported by Chang *et al.,* 2014 [41] that anthocyanin in hibiscus had decreased serum leptin level in diet-induced obesity in mice. Moreover, Boix-Castejón *et al.,* 2018 [42] designated that the polyphenols in hibiscus are capable of normalizing leptin expression in obese subjects.

The data of the present study showed that hibiscus (250 mg/Kg p.o,) results in a significant decrease in serum total cholesterol level with no effect on triglycerides level, as compared to metabolic syndrome. The previous studies showed that hibiscus extract had improved the lipid profile picture of hyperlipidemic animal models [43]. This effect is attributed to the hibiscus effect in reducing Cholesterol biosynthesis by inhibiting Hydroxymethylglutaryl-coenzyme A reductase (HMG-CoA reductase) [44]. From our study it is clear that hibiscus is effective in reducing serum total cholesterol which is in harmony with previous studies.

Present investigation showed that hibiscus resulted in a significant decrease in serum AST level, compared to metabolic syndrome group and normalized ALT level. This finding is in agreement with the previous studies that showed that hibiscus has hepatoprotective effect [17].

5. Conclusion:

In conclusion, and from the results of the present study, it is clear that garcinia and hibiscus are effective in decreasing serum blood glucose and insulin levels in rats with metabolic syndrome. The effect of garcinia on glucose and the effect of hibiscus on insulin are comparable to that of metformin. Garcicnia failed to reduce the increased liver functions while hibiscus results in a significant decrease in liver functions.

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8. Conflict of Interest:

The authors declare that they have no conflicts of interest.

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Figure 1

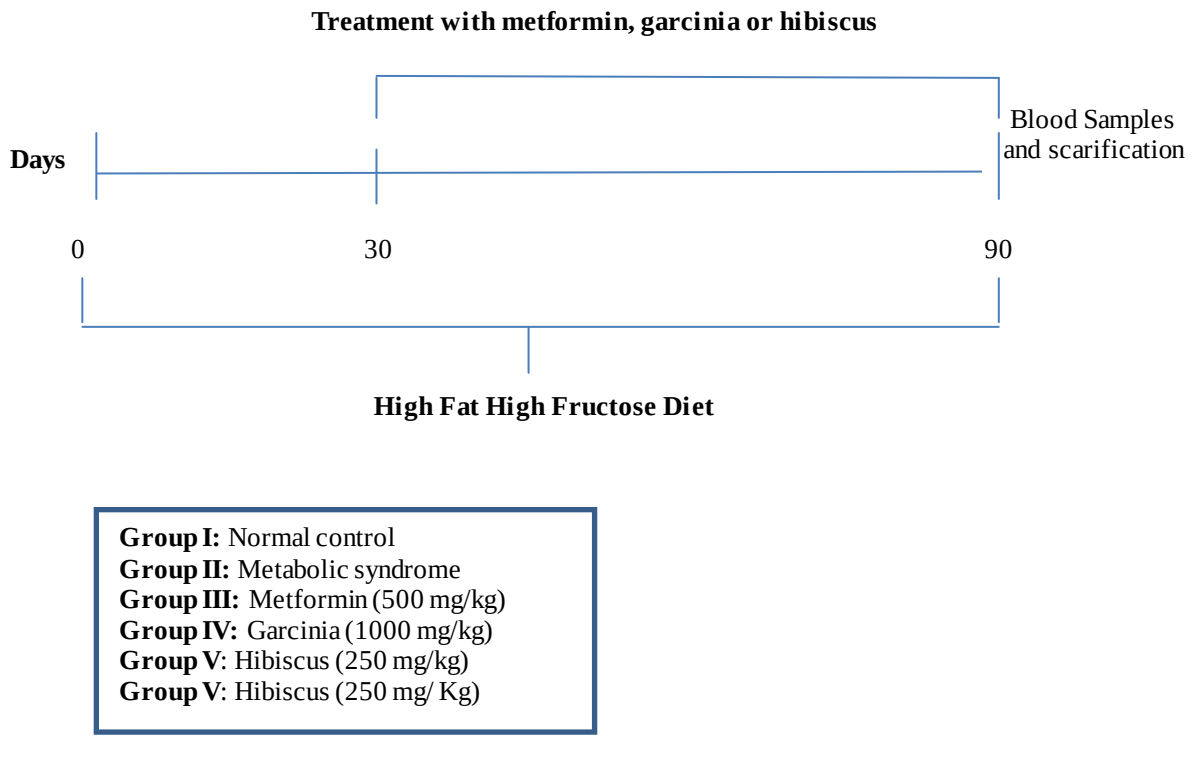
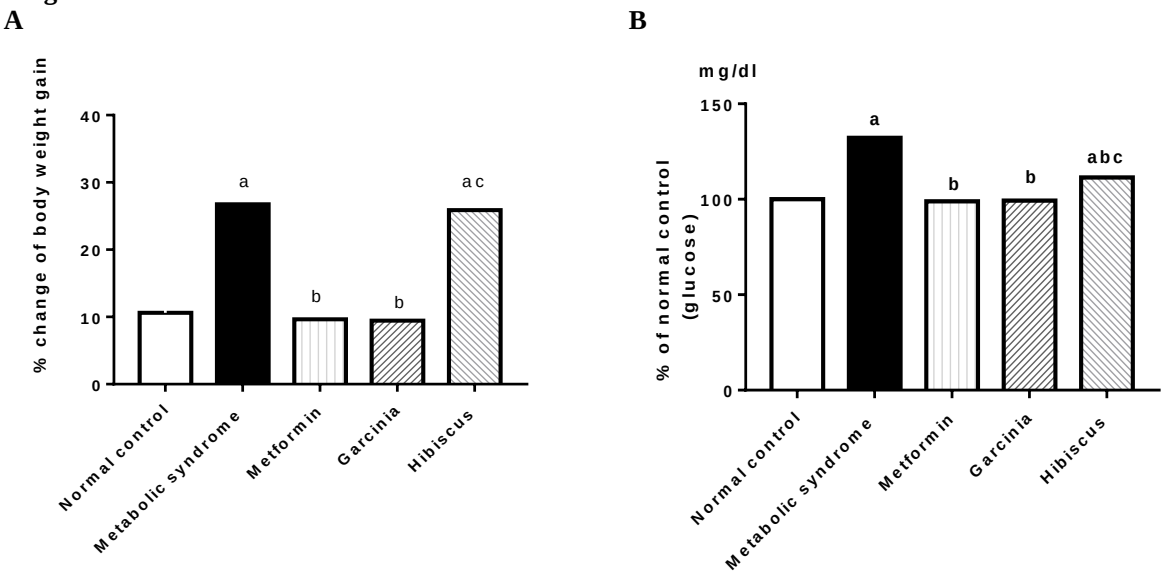


Figure 2



D

C

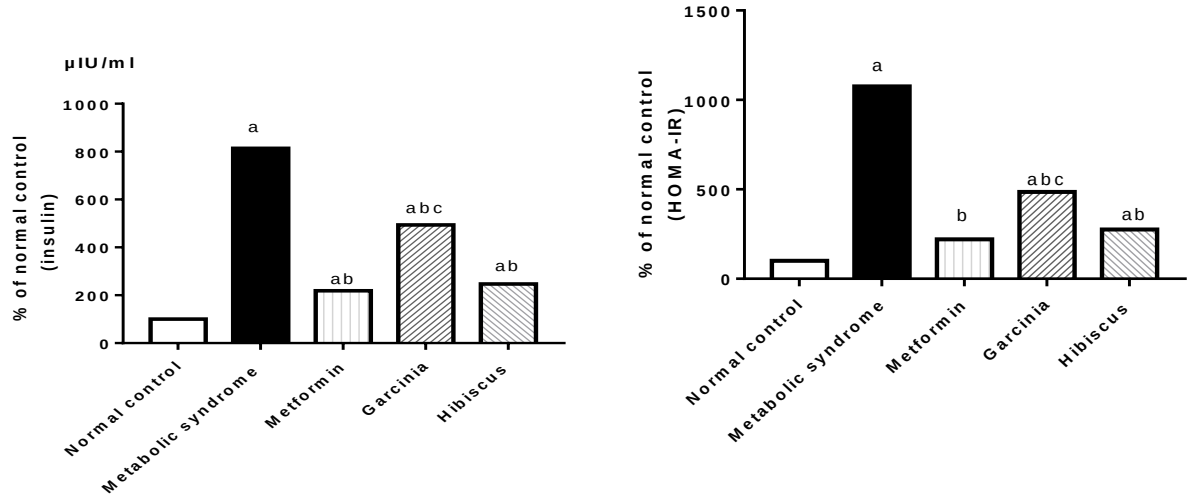

µIU/ml
% of normal control (insulin)
1000
800
600
400
200
0
a
ab
abc
ab
Normal control
Metabolic syndrome
Metformin
Garcinia
Hibiscus
% of normal control (HOMA-IR)
1500
1000
500
0
a
b
abc
ab
Normal control
Metabolic syndrome
Metformin
Garcinia
Hibiscus

Figure 3

A

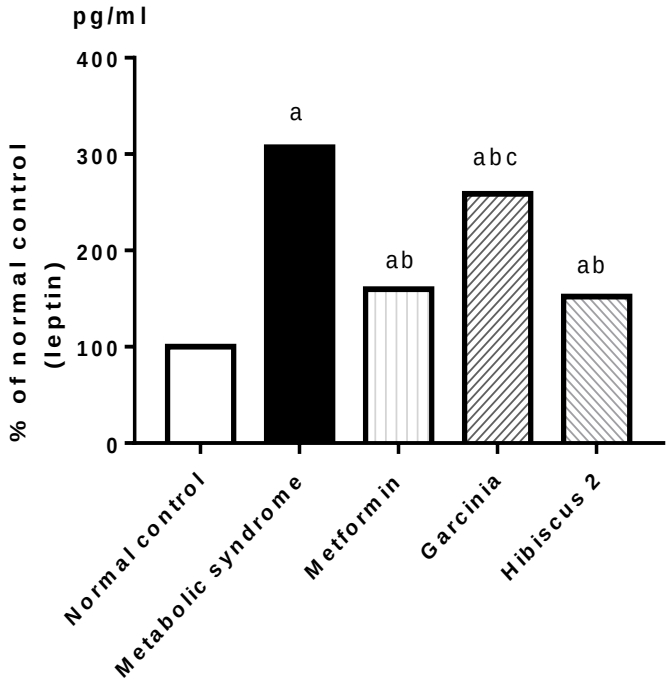
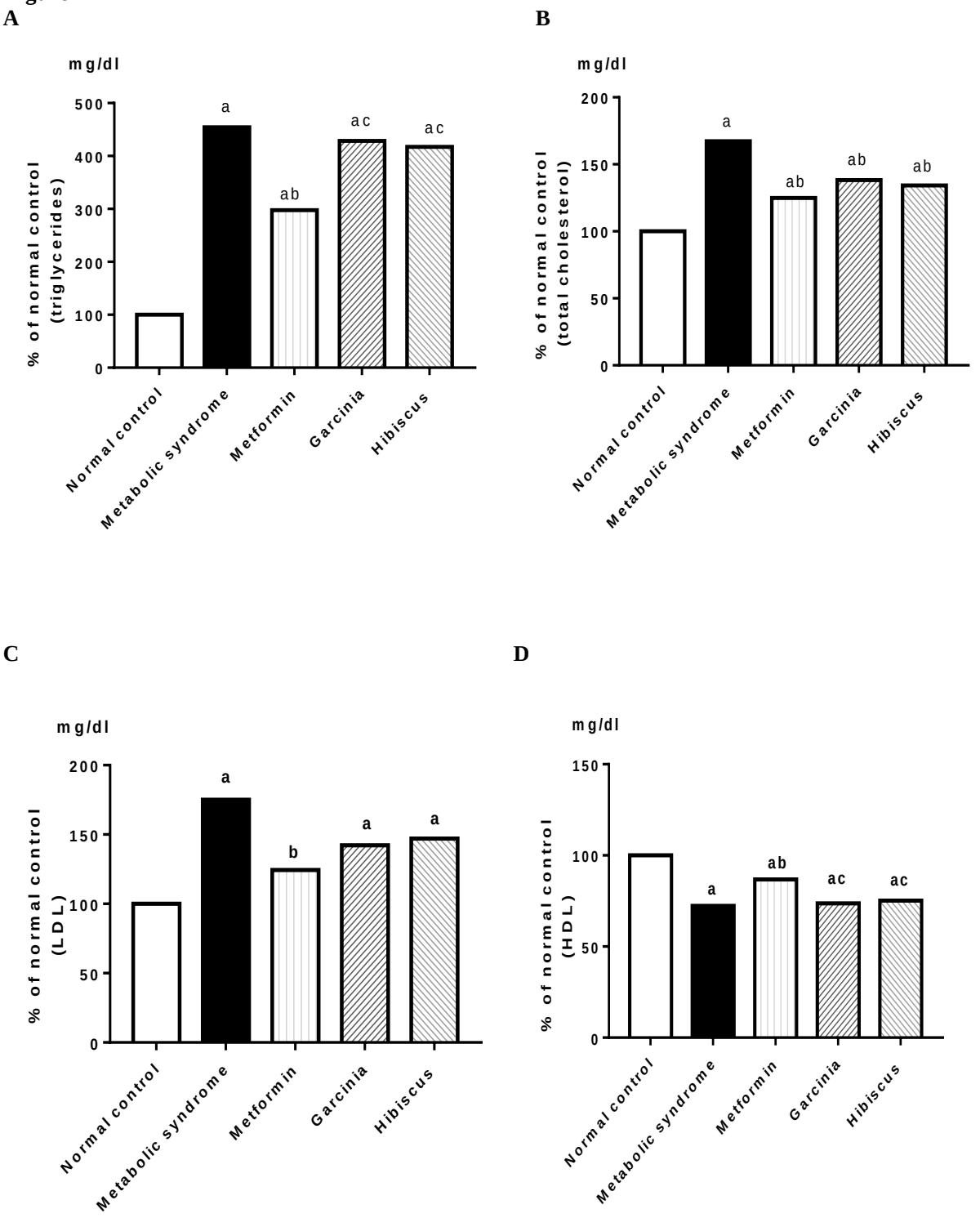

pg/ml
% of normal control (leptin)
400
300
200
100
0
a
ab
abc
ab
Normal control
Metabolic syndrome
Metformin
Garcinia
Hibiscus 2

Figure 4


A
mg/dl
% of normal control (triglycerides)
500
400
300
200
100
0
a
ab
ac
ac
Normal control
Metabolic syndrome
Metformin
Garcinia
Hibiscus
B
mg/dl
% of normal control (total cholesterol)
200
150
100
50
0
a
ab
ab
ab
Normal control
Metabolic syndrome
Metformin
Garcinia
Hibiscus
C
mg/dl
% of normal control (LDL)
200
150
100
50
0
a
b
a
a
Normal control
Metabolic syndrome
Metformin
Garcinia
Hibiscus
D
mg/dl
% of normal control (HDL)
150
100
50
0
a
ab
ac
ac
Normal control
Metabolic syndrome
Metformin
Garcinia
Hibiscus

E

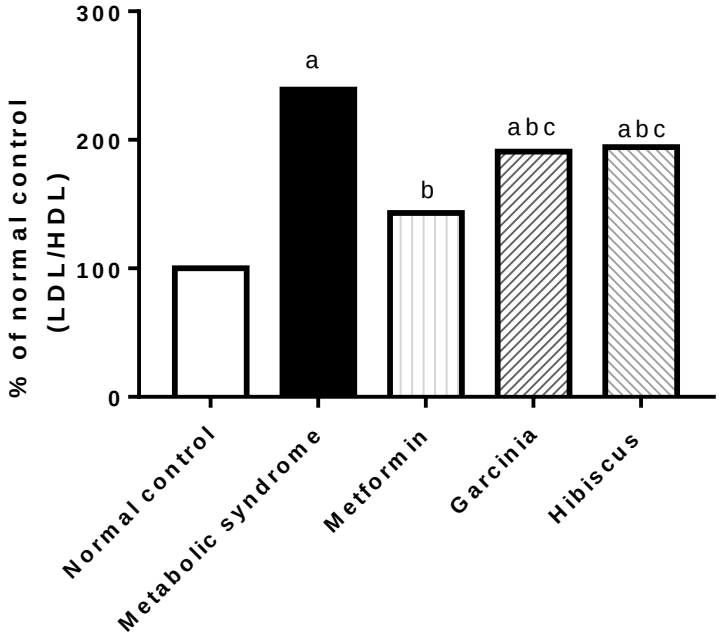
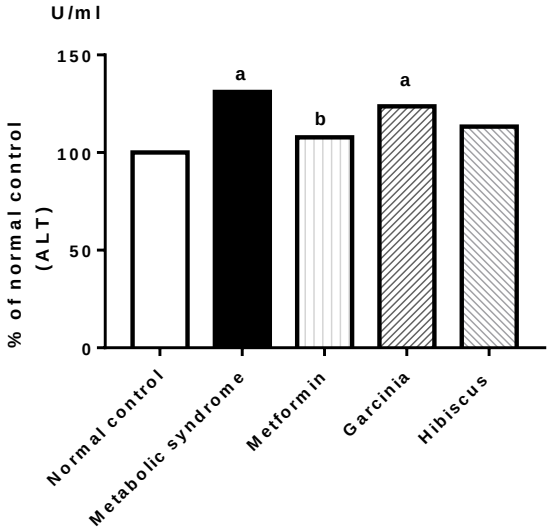

300
200
100
0
% of normal control (LDL/HDL)
a
b
abc
abc
Normal control
Metabolic syndrome
Metformin
Garcinia
Hibiscus

Figure 5

A


U/ml
150
100
50
0
% of normal control (ALT)
a
b
a
Normal control
Metabolic syndrome
Metformin
Garcinia
Hibiscus

B

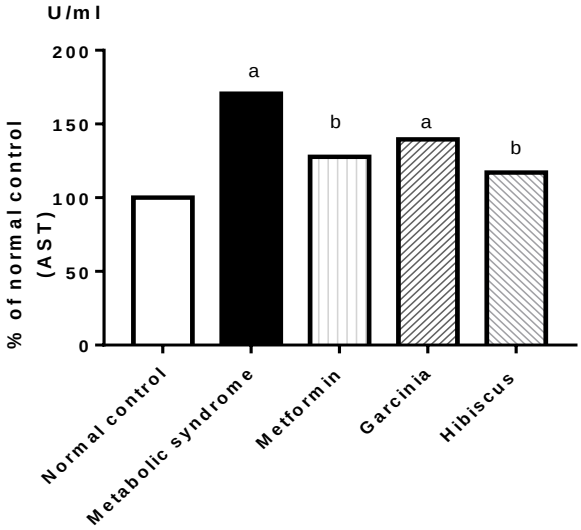

U/ml
200
150
100
50
0
% of normal control (AST)
a
b
a
b
Normal control
Metabolic syndrome
Metformin
Garcinia
Hibiscus

Figure 6
Liver specimens(X100 magnification)

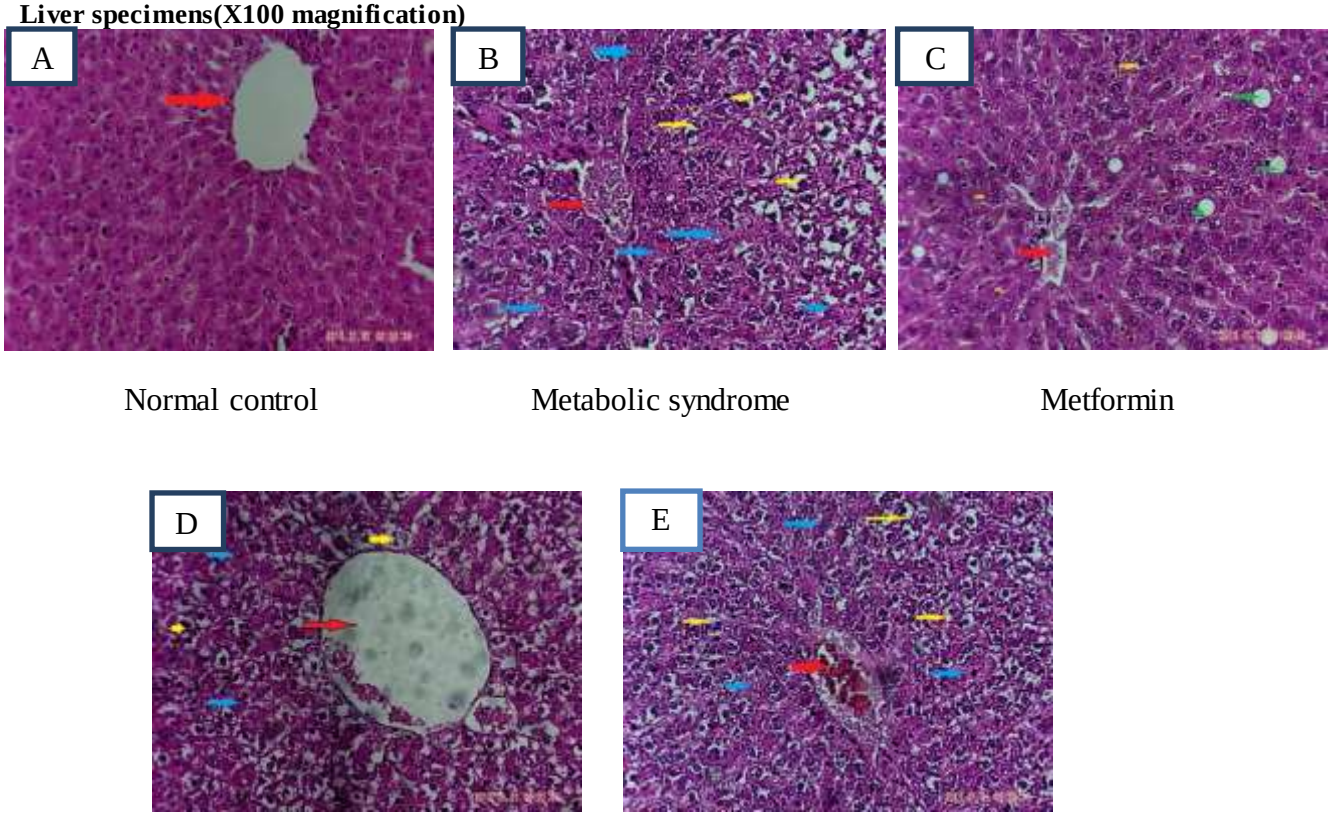


Figure Captions

FIGURE. 1. Schematic representation of the experimental design.

FIGURE. 2. The effect of metformin, garcinia and hibiscus on body weight (A) serum glucose (B), insulin (C) and insulin index (HOMA-IR) (D) after 30 days of treatment (A) Each bar with vertical line represents the mean of experiments ± S.D. (n=6).[a] vs. normal control group, [b] vs. metabolic syndrome group and [c] vs. metformin group (Statistical analysis was performed out by one-way ANOVA followed by Tukey's test, the criterion for statistical significance was set at P<0.05 level).

FIGURE. 3. The effect of metformin, garcinia and hibiscus on serum leptin (A) after 30 days of treatment. Each bar with vertical line represents the mean of experiments ± S.D. (n=6).[a] vs. normal control group, [b] vs. metabolic syndrome group and [c] vs. metformin group (Statistical analysis was performed out by one-way ANOVA followed by Tukey's test, the criterion for statistical significance was set at P<0.05 level).

FIGURE. 4. The effect of metformin, garcinia and hibiscus on serum triglycerides (A), total cholesterol (B), low density lipoprotein (LDL) level (C), high density lipoprotein (HDL) level (D) and LDL/HDL ratio (E), after 30 days of treatment. Each bar with vertical line represents the mean of experiments ± S.D. (n=6).[a] vs. normal control group, [b] vs. metabolic syndrome group and [c] vs. metformin group (Statistical analysis was performed out by one-way ANOVA followed by Tukey's test, the criterion for statistical significance was set at P<0.05 level).

FIGURE 5. The effect of metformin, garcinia and hibiscus on serum alanine aminotransferase (ALT) level (A) and aspartate aminotransferase (AST) level (B) after 30 days of treatment. Each bar with vertical line represents the mean of experiments ± S.D. (n=6).[a] vs. normal control group, [b] vs. metabolic syndrome group and [c] vs. metformin group (Statistical analysis was performed out by one-way ANOVA followed by Tukey's test, the criterion for statistical significance was set at P<0.05 level).

FIGURE 6. Liver section of normal control (A), metabolic syndrome (B), metformin (C), garcinia (D) and hibiscus (D).