Ameliorating Effect of Maca Extract and Ashwagandha on the Thyroid and Reproductive Hormones in Obese Rats.

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Abstract:

This study aimed to investigate the ameliorating effect of maca and ashwagandha and their correlation extract on the thyroid and reproductive hormones. Forty male rats were divided into two main groups (n=5), the first main group (control -) was fed on a standard diet only, and the main subgroups were fed on a high-fat diet to induce obesity for two weeks, then divided into 7 equal subgroups divided to (control +) and 6 groups an administrator orally with 500 and 1000 mg/ kg of ashwagandha, maca and their mixture of extract for 6 weeks. At the end of the experiment serum lipid profile, glucose, liver, and kidney function were determined also, and serum of MDA, TSH, thyroid, and testosterone hormone levels were determined in addition, histopathology examinations for liver and kidney were done. The results indicated that administrated with ashwagandha, maca, and their mixture extract recorded a significant decrease in body weight, serum ALT, MDA, cholesterol, triglyceride, LDL-c, and VLDL-c when compared to the (control+) group, also recorded a high significant decrease in serum creatinine, urea and glucose in all administrated groups, in addition to results recorded a high considerable increase in serum HDL-c, AST and CAT, T3, T4, TSH and serum testosterone level in tested groups compared with (control+) group. Also, results indicated enhancements histological examinations of the liver and kidney but with different degrees in all administrated groups, where the strongest effect showed in correlation extract groups. We can conclude that administrated with maca and ashwagandha extract improves thyroid and reproductive function by ameliorating thyroid, testosterone hormones and preventing liver and kidney functions.

Key Words : Testosterone – Obesity – Ashwagandha – Maca - thyroid.

التأثير المحتمل لمستخلص الماكا والأشواجندا على هرمونات الغدة الدرقية والتناسلية في فئران التجارب المصابة بالسمنة.

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المستخلص:

هدفت الدراسة الحالية إلي التحقق من التأثير المحتمل لمستخلص الماكا والأشوجندا وخليطهما علي هرمونات الغدة الدرقية والتناسلية علي فئران التجارب المصابة بالسمنة، أجريت الدراسة علي٤٠ فأر من فئران التجارب، تم تقسيم الفئران عشوائيا إلي مجموعتين رئيسيتين، المجموعة الأولي (المجموعة الضابطة السالبة) تغذت علي الغذاء القياسي فقط، المجموعة التجربية الثانية تم تغذيتها علي غذاء مرتفع في نسبة الدهون لمدة أسبوعين لإستحداث السمنة، ثم تم تقسيمها إلي ٧ مجموعات متساوية (المجموعة الضابطة الموجبة و ٦ مجموعات تجريبية تم تجريعها بـ ٥٠٠، ١٠٠٠ ملجم /كجم من مستخلص الأشوجندا ،الماكا وخليطهما لمدة ٦ أسابيع، بعد الإنتهاء من التجربة، تم قياس دهون الدم ، وظائف الكبد، الكلي، مستوي هرمونات الغدة الدرقية والهرمون المحفز للغدة الدرقية، وكذلك تم الفحص الهستولوجي للكبد والكلي، وقد وجد من نتائج الدراسة أن المعاملة بمستخلص الأشوجندا والماكا أدي إلي إنخفاض معنويا في مستوي الكلوليسترول الكلي، VLDL–C والجليسريدات الثلاث مقارنة بالمجموعة الضابطه الموجبة، كما سجلت النتائج إرتفاعا معنويا في مستوي HDL هرمون T_3, T_4, TSH مقارنة بالمجموعة الضابطة الموجبة، كما أظهرت النتائج تحسنا معنويا في وظائف الكبد والكلي في المجموعات المعاملة بالمستخلصات المختلفة أكدها الفحص الهستولوجي مقارنة بالمجموعة الضابطة، ولهذا فقد خلصت هذه الدراسة إلي أن المعاملة بمستخلصات الماكا والأشوجندا تحسن من إفراز هرمونات الغدة الدرقية والتناسلية وتقلل من حدوث الأجهاد التأكسدي.

الكلمات المفتاحية : توستستيرون – سمنة - أشوجندا= ماكا - الغده الدرقيه.

1. Introduction:

Obesity is a chronic retrograde condition impacting a rapidly increasing number of people worldwide, which characterized by multifactorial and excessive fat accumulation, often relapsing and difficult to treat chronic disease which associated with mortality and morbidity, ranging from premature death to chronic conditions such as cardiovascular, diabetes and malignancies, which may severely compromise patients life expectancy as mentioned by **Hruby et al., (2016)** and **Yang et al., (2022).**

Ezzati (2024) reported that age standardized prevalence of obesity increased from 1990 to 2022. A high body mass index is responsible for over 120 million adult person-years lost each year **(Lobstein, 2023). Shekar and Popkin (2021)** mentioned that overweight or obesity are effected by a number of factors linked to changing food systems, the resultant shifts in food consumption, eating behaviors, reduced energy expenditure changes in technology, lifestyles in all phases of life, early life undernutrition and reduced linear growth.

The relation between obesity, hypogonadism and hypothyroidism was becoming a hot topic, meanwhile **Hamilton et al. (2011)** reported that testosterone play a critical role in the regulation of metabolism, and shown that low testosterone levels may lead to obesity. Also thyroid hormone, helps regulate numerous other organs through the blood, these hormones normally act in the body to regulate energy use, infant and childhood development **(Sanyal and Raychaudhuri, 2016).**

Kumar et al. (2023) reported that medicinal plants have been the primary source of medicines for humans since ancient times, and currently around eighty per cent of world's population depends on traditional medical system. World Health Organization has mentioned that the international market value for herbal products is approximately equivalent to $6.2 billion till the date and is believed to reach up to $5 trillion at the end of 2050**.**

Ashwagandha *Withania somnifera L*. is native to parts of Asia, including India, China, Myanmar, the Middle East, Southern Europe, and Africa. **(Lim and Barnes, 2024). Mukherjee et al,. (2021)** mentioned that traditional use of ashwagandha showed by use the dried root or whole plant, and are prepared in various forms, including decoction, paste, medicated oils,

clarified-butter/ghee-based preparation and others for internal or external use. Contemporary preparations in the global market are usually solid dose forms (tablets, typically capsules, powders and gummies), formulated mainly as single- and sometimes, multi-ingredient products (with other herbal and non-herbal ingredients). Ashwagandha appears to be a safe herb and subacute toxicity trials revealed no harm.

despite that**, Mellado-García et al. (2016)** studied that A 90-day oral administrated of three doses of Ashwagandha in rats to detect any potential harm. The results showed brain, heart, lung, stomach, liver, kidneys, spleen, ovaries and testis were all normal in all rats

Maca *Lepidium meyenii* known as "Peruvian ginseng," is an annual herbaceous plant of the Brassicaceae family **(Gonzales et al., 2006).** Maca using as a medicinal herb and vegetable root for its physical and psychological effects since 2000 years ago, it is considered a food supplement, not a drug, and it is available in many countries in different colors white, yellow, red and black, traditionally used for aphrodisiac and fertility-enhancing properties in males and females as well as for improving menopausal symptoms **(Gonzales et al., 2003 & Srikugan et al., 2011).** Maca is traditionally consumed fresh and after boiled in water or milk and can be made into juices, cocktails, alcoholic beverages and Maca coffee **(Gonzales et al., 2005** and **Campos et al., 2013).** Maca is prescribed because of its supposed properties to decrease anxiety, depression, and stress (**Gonzales et al., 2002). Rubio et al (2011) and Gonzales et al., (2015)** reported that, Maca is useful for treating sexual dysfunction, osteoporosis, benign prostatic hyperplasia, memory and learning, depression and anxiety**.**

Wan et al., (2018) investigated the prevention and amelioration capacity of the aqueous extract of black Maca (AEM) on high fat and fructose diet induced metabolism disorder for 20 weeks, the results revealed that 32 bioactive compounds in Maca involved in metabolism disorder. Also found daily AEM supplementation presented with the beneficial effects of improved hyperlipidemia, hyperinsulinemia, insulin resistance and hepatic steatosis.

Fei et al., (2020) investigated the effects of aqueous extract of Maca on energy metabolism and immunoregulation in spleen defcient mice, reslts found inhibited loss of body weight and immune organ atrophy caused by cyclophosphamide.

The present study, aimed to investigate the ameliorating effect of Maca extract, Ashwagandha and their correlation on the thyroid and reproductive hormones in obesity rats.

2. Materials and methods:

2.1Materials:

2.1.1. Plant and animals:

The dried Ashwagandha and Maca roots were obtained from Medical Plant Company (Harraz), Bab Alkhalq, Cairo, Egypt. The plant roots were ground to a fine powder using a high-speed mixer (Moulinex-LM2428EG), then kept in a bottle and saved at room temperature until used in preparing extraction.

Forty male of Wister Albino rats weighing 170±5 g were obtained from Helwan farm for experimental animals, Cairo, Egypt.

Ethical Approval:

Ethical Approval of the current study was approved by Sohag University ethical guidelines for animal care and use in scientific research under registration number Soh18-3-7/2024-01.

2.2. Methods:

2.2.1. Plant extraction:

The aqueous extracts of the two plants were prepared according to the traditional method according to **Fei et al. (2020)**. The dried plant powder (500 g) of each plant was placed in a recipient with 2 liters of water and boiled for 2 hr. The final preparation of both extracts was left to cool at room temperature and filtered, afterward added 500ml of water to each filter residue, and the two solutions were boiled again for 2 h. The two filtrates of each plant were combined and concentrated to 500 ml. The concentrate contains 1000 mg/ml from each plant. Ashwagandha and Maca extracts were placed in small vials and frozen at (−20◦C) until use. Before the experiments, the concentrate for

two plants were dissolved in an aqueous solution to concentrations of 500 mg/ml.

2.2.2 Experimental design:

Forty male rats Wister Albino rats initially weighing 170±5 g, were randomlydivided into two main groups. The first group : the negative control group (control -) fed a standard diet. Another group was fed a high-fat diet (10% corn oils and 20% tallow) to induce obesity for 2 weeks, the main subgroups were divided into 7 subgroups (n=5) (1) fed in slandered diet (positive control group), (2 and 3) feed in slandered diet and were administrator orally with 500 and 1000 mg/ kg of ashwagandha roots extract, (4 and 5) feed slandered diet and orally administration with 500 and 1000 mg/kg of Maca extract, (6 and 7) feed slandered diet and administration orally with mixture of Maca and Ashwagandha extract 500 and 1000 mg/kg (1:1) for 6 weeks. Initial and final body weight were recorded, and body mass index was recorded at the end of the experiment.

After 6 weeks of administration, animals were weighed, fasted overnight and anesthetized with diethyl ether, blood samples were withdrawn from the retro orbital venous plexus, and calculated to evaluate the following biochemical parameter:

-Lipid profile.

Liver Functions: Alanine aminotransferase (ALT), Aspartate aminotransferase (AST), Malondialdehyde (MDA) and Catalase (CAT).

- Kidney functions urea and creatinine level.

-Glucose level.

-Thyroid hormones: (T3 and T4), Thyroid stimulating hormone (TSH), and testosterone hormone.

Animals were rapidly sacrificed after blood collection, the liver and kidney specimens were fixed in 10% neutral buffer formalin, then trimmed, washed in water, dehydrated in ascending grades of ethyl alcohol, cleared in xylene, and embedded in paraffin. Thin sections (4-6µ) were processed and stained with Hematoxylin & Eosin stain for histology analyses according to **(Bancroft, 2008).**

2.2.3. Biochemical analysis:

- Serum total cholesterol and triglycerides were determined according to **Allain (1974), Fossati and prencipe (1980)** respectively, HDL and LDL were assayed according to **Abcam, (2019),** VLDL-c was calculated mg/dl according to Lee and Nieman, (1996).
- Serum MDA, CAT, ALT, and AST levels were determined according to **Ohkawa et al. (1979), Aebi (1984), Young (1990), and Sherwin (1984)** respectively.
- Determination of kidney functions: serum urea, creatinine and glucose level were assayed according to **Young (1990), Tietz (2006)** and **Carroll (1970)** respectively.
- Serum T_3, T_4, TSH and testosterone hormone were determined according to **Berbel et al. (2010); Barker, (1984); Burge and Patel (1977)** and **Sun et al., (1989)** respectively.
- The estimation of **Body Mass index (BMI)** according to **Rabiu et al. (2017) as** fallowing equation: BMI= Weight (g) / Length (cm^2) .

2.2.4. Statistical analysis:

All analyses were performed using the statistical package for social science (SPSS 20 software). All data were expressed as mean±SE. Statistical analysis was performed using one-way analysis of variance (ANOVA) followed by Duncan's multiple test. Statistical significance was considered at $P<0.01$.

3. Results and discussion:

3.1 Effect of Maca and Ashwagandha extract on body weight and body weight gain:

The results in Table (1) illustrated the effect of Maca and Ashwagandha extract on body weight, the results recorded a significant decrease in body weight in all groups administrated by maca and ashwagandha extract compared to the control (+) group and the initial body weight, this decrease was statically significant at P<0.01 in groups administrated with 500 and 1000 mg/Kg mixture of Maca and Ashwagandha extract.

Also, results showed a decrease in body mass index in all groups administrated by maca and ashwagandha extract, meanwhile, body mass index was recorded as 0.48 in control (+) and 0.37 Mixture extract group 1000mg/kg.

These results are in agreement with **Groh et al. (2017)** who reported that maca root contains nearly all of the essential amino acids and an abundance of others, along with crucial muscle-building nutrients like iron, potassium, and calcium which are effective for muscle growth and healthy weight gain.

Also, **Elhassaneen et al. (2023)** found lower body weight gain, improve serum lipid profile, decreased liver functions, improve neurological disorders, and positively manipulated obesity in obesity rats fed diets containing 4, 6, and 8 g/100g diet) of Ashwagandha roots for two months.

Table (1): Effect of Maca and Ashwagandha extract on body weight.

Parameters / Groups	Initial body weight	Final body weight	Chang in body weight	Body mass index
Control (-)	169.0±3.05^{a}	٢٠٥,٣±١,٧٦^{b}	+36.3	٠,٤٥±٠,٠١^{ab}
Control (+)	167.3 ±2.33 ^{a}	٢٢٣,٧±١,٩ ^{a}	+56.4	٠,٤٨±٠,٠٢ ^{a}
Ashwagandha 500mg/kg	١٦٧,٣ ±٢,٨٤ ^{a}	٢٠٣,٧±٢,٧ ^{bc}	+36.4	٠,٤١±٠,٠٢ ^{bc}
Ashwagandha 1000mg/kg	١٦٦,٣ ±١,٨٥ ^{a}	٢٠٢,٣±١,٩ ^{bc}	+36	٠,٤١±٠,٠١ ^{bc}
Maca 500mg/kg	١٦٨,٣ ±٠,٩ ^{a}	٢٠٠,٣±٠,٩ ^{bc}	+32	٠,٣٩±٠,٠١ ^{c}
Maca1000mg/kg	١٦٨,٧ ±١,٢ ^{a}	١٩٩,٧±٢,٩ ^{bc}	+31	٠,٣٩±٠,٠١ ^{c}
Mixture extract 500mg/kg	١٦٨,٧ ±٣,٣ ^{a}	١٩٥,٣±٢,٦ ^{cd}	+26.6	٠,٣٩±٠,٠١ ^{c}
Mixture extract 1000mg/kg	١٦٧,٣ ±١,٨٦ ^{a}	١٨٩,٧±٥,٢ ^{d}	+22.4	٠,٣٧±٠,٠٠٤ ^{c}

Values are mean ± ES. Values in the same column sharing the same, superscript letters are not statistically significantly different ($P \leq 0.01$).

3.2 Effect of Maca extract and Ashwagandha on liver functions:

The results in Table (2) illustrated the effect of Maca and Ashwagandha extract on liver functions, from such data it could be noticed that increase in

ALT and AST activities in the control (+) group compared to the normal control group. Administrated with Maca, Ashwagandha and their mixture extract led to a considerable ($p<0.01$) reduce the levels of activity of these enzymes compared with the control (+) group.

The enhanced in ALT and AST activities may be due to Maca and Ashwagandha content of phenolic compounds could lower liver serum enzyme activity through many suggested effects, including blocking the hepatocellular uptake of bile acids, improving the antioxidant capacity of the liver, diminishing the bilirubin concentration, reducing the damage to hepatocytes, and acting as scavengers of reactive oxygen *species* **(Elmaadawy et al., 2016; Sayed-Ahmed et al., 2020; Ali, 2021 and Elhassaneen et al., 2021).** These results agree with the results obtained by **Sultana et al. (2012)** who found Ashwagandha root extract restored serum AST, and ALT toward normal levels in gentamicin-intoxicated rats. Also, **Khateib and Diab (2024)** showed significant decreases in the indicators for kidney and liver function, lipid profiles and serum glucose levels in in male rats fed on maca powder and at 5% of the diet for 28 days.

Table (2): Effect of Maca and Ashwagandha extract on liver functions.

Parameters / Groups	**ALT** (u/ml)	**AST** (u/ml)
Control (-)	31.56±0.60 ^{f}	44.59 ±0.53 ^{f}
Control (+)	123.21±1.39 ^{a}	146.18±1.23 ^{a}
Ashwagandha 500mg/kg	108.46±1.74 ^{b}	121.49±0.49 ^{b}
Ashwagandha 1000mg/kg	86.41±1.12 ^{c}	100.41±0.96 ^{c}
Maca 500mg/kg	93.69±1.23 ^{c}	108.96±2.02 ^{c}
Maca1000mg/kg	77.39±1.13 ^{d}	84.04±2.27 ^{d}
Mixture extract 500mg/kg	59.73±0.75 ^{e}	70.09±1.25 ^{d}
Mixture extract 1000mg/kg	47.29±0.60 ^{e}	55.73±1.48 ^{e}

Values are mean ± ES. Values in the same column sharing the same, superscript letters are not statistically significantly different ($P\leq 0.01$).

3.3 Effect of Maca and Ashwagandha extract on serum MDA and CAT concentrations:

The obtained results demonstrated in Table (3) revealed that high-fat diet-induced obesity in rats exhibited a significant increase in serum MDA and decrease in serum CAT concentrations when compared with normal control groups, administrated with Maca, Ashwagandha and their mixture extract led to a significant decrease in serum MDA compared with (control +) group the best results was performed in group administrated with 10% mixture of Maca and Ashwagandha extract, also results found significant increase elevated serum CAT in all administrated groups meanwhile recorded the best results in 10% Maca and Ashwagandha mixture.

Mishra (2000) and Singh et al. (2010) reported that ashwagandha root contains sitoindosides and withaferin antioxidant activity by enhancing the free radical scavenging enzymes such as superoxide dismutase and catalase. Also decrease in serum MDA in the positive control group may be due to a decrease in thyroid hormone, meanwhile, **Baskol et al. (2007) and Lakshmi et al. (2013)** reported that oxidative stress in hypothyroid subjects' increase MDA level.

These results agree with **Abdel-Wahhab et al. (2019)** who found significant decrees in MDA, TSH, T3, and T4 after one month of treatment of ashwagandha methanolic extract on hypothyroidism modeled rats. Also, **Mohamed et al. (2024),** feeding rats on maca roots reduced the elevation of blood glucose and lipids, enhanced insulin resistance and liver function, and successfully restored the state of oxidative stress and inflammation. This effect might be brought about by the ballast chemicals found in maca; dietary fiber, particularly insoluble fiber, retains water and gels. They are essentially not absorbed by the circulatory system since they are not broken down by the body. They affect the glycemic response, lower cholesterol, increase satiety, and increase fecal bulk. Thus, fiber can be used to treat diseases like diabetes.

Table (3): Effect of Maca and Ashwagandha extract on serum MDA and CAT concentrations.

Parameters / Groups	MDA(n. mol/mg/ protein)	CAT (u/mg/ protein)
Control (-)	0.79±0.01 [f]	5.16±0.05 [a]
Control (+)	4.27±0.14 [a]	0.93±0.01 [f]
Ashwagandha 500mg/kg	3.22±0.10 [b]	1.7±0.06 [ed]
Ashwagandha 1000mg/kg	2.84±0.10 [cb]	2.08±0.05 [d]
Maca 500mg/kg	3.08±0.04 [b]	1.87±0.02 [d]
Maca1000mg/kg	2.46±0.05 [d]	2.55±0.05 [c]
Mixture extract 500mg/kg	2.11±0.03 [e]	2.83±0.04 [c]
Mixture extract 1000mg/kg	1.48±0.03 [e]	4.18±0.20 [b]

Values are mean ± ES. Values in the same column sharing the same, superscript letters are not statistically significantly different ($P \leq 0.01$).

3.4 Effect of Maca and Ashwagandha extract on serum lipid profiles:

The obtained data presented in Table (4) revealed that high-fat diet-induced obesity in rats caused a significant increase in serum cholesterol, triglyceride, LDL, and VLDL, and a significant decrease in serum HDL in the (control +) group compared to (the control -) group, in contrast results found significant increase in serum HDL and significant decrease in serum cholesterol, triglyceride, LDL and VLDL in all groups administrated with Maca, Ashwagandha and their mixture extract compared to the (control +) group.

Meanwhile, **Tafuri et al. (2019)** reported that Maca contains important fatty acids, macacids, macaridin, alkaloids, glucosinolates, and phenolics compounds that effects on lipid, mineral, and antioxidant metabolisms. A dry Maca contains 10.2% proteins, 59% carbohydrates, 2.2% lipids, 8.5% fiber, 40.1% free fatty acids (linoleic, palmitic, and oleic acids), and 52.7% saturated fatty acids and unsaturated fatty acids according to **(Gonzales, 2012).** Also **Li**

et al., (2017) stated that Maca contains 13.42% proteins, 1.42 oils, and 3.41% ash, and contains 0.14, 1.24, and 0.20 mg/g Maca respectively from Maca amide, glucosinolate, and Alkaloid.

In addition, **Lee and Chang, (2018)** found that the highest K and Ca content in Maca roots was recorded at 30.7 and 2.25mg/g respectively, also the previous studies reported the importance of K and Ca in dietary intake for a beneficial effect on the coronary heart disease by decreasing blood pressure and maintaining an adequate Na+ to K+ ratio, also Ca required for vascular contraction **(Weaver, 2009; Institute of Medicine, 2011).** Also, **Ali, (2021)** suggested that some of the useful effects of dietary intake of Ashwagandha roots on triglyceride levels are attributable to the reduction of stress oxidative and lipid peroxidation.

Table (4): Effect of Maca and Ashwagandha extract on lipid profile.

Parameters / Groups	Cholesterol (mg/dl)	Triglyceride (mg/dl)	HDL (mg/dl)	LDL (mg/dl)	VLDL (mg/dl)
Control (-)	64.29±1.18 ^{f}	81.18±0.62 ^{f}	38.77±0.61 ^{a}	12.56±0.68 ^{f}	16.24±0.12 ^{f}
Control (+)	178.99±0.92^{a}	205.91±1.52^{a}	3.63±1.39 ^{f}	70.90±0.69^{a}	41.18±0.30^{a}
Ashwagandha 500mg/kg	154.73±3.04^{b}	177.62±1.65^{b}	11.13±0.28^{e}	57.35±1.01^{b}	35.52±0.33^{b}
Ashwagandha 1000mg/kg	131.18±0.70^{c}	150.70±0.66^{d}	14.85±0.33^{d}	40.75±0.26^{c}	30.14±0.13^{c}
Maca 500mg/kg	141.94±0.79^{b}	162.50±1.51^{c}	13.88±0.07^{d}	49.70±0.52^{b}	32.50±0.30^{c}
Maca1000mg/kg	115.83±0.40^{c}	144.88±0.69^{d}	18.33±0.24^{c}	37.32±0.84^{c}	28.98±0.14^{d}
Mixture extract 500mg/kg	107.23±3.34^{d}	125.21±2.38^{e}	21.10±0.71^{c}	29.69±0.51^{d}	25.58±0.13^{d}
Mixture extract 1000mg/kg	86.20±0.70^{e}	102.66±1.66^{e}	29.66±0.83^{b}	19.74±0.40^{e}	20.53±0.33^{e}

Values are mean ± ES. Values in the same column sharing the same, superscript letters are not statistically significantly different ($P \leq 0.01$).

3.5 Effect of Maca and Ashwagandha extract on kidney function and serum glucose level:

Table (5) shows the effect of different Maca and Ashwagandha extract administrations on kidney function. In comparison with the (control -) group, a high fat diet recorded significantly increased levels of urea and creatinine. Administration with Maca and Ashwagandha extract improve urea and creatinine level towards normal values, especially in groups administered with the mixture of Maca and Ashwagandha extract. Blood glucose increased significantly in the (control +) group, administrated with Maca and Ashwagandha extract succeeded in ameliorating significantly the serum of glucose compared with the (control -) group.

Improve in kidney function may be due to Maca and Ashwagandha content of flavonoids and phenolic compounds, which can remove excess ammonia, uric acid urea, non-protein, creatinine, and nitrogen and offer protection against hyper-ammonemic and nephrotoxic conditions according to **Harikrishnan et al. (2008).** Also**, Anwer et al., (2008)** reported that Ashwagandha contains bioactive components such as withaferin A, withanolide B, withanolide A, and withanoside IV, contributing to its anti-diabetic properties.

These results were correlated with the results suggested by **Shimmi et al. (2011)** who observed decrease serum urea and creatinine level in nephrotoxicity rats administrated with Ashwagandha. **Abdel-Wahhab et al. (2019)** reported that Ashwagandha extract and the reference drug thyroxine had no effects on serum AST, ALT, urea and creatinine among the different groups. Also, **Khateib and Diab (2021)** revealed that oral administration of ashwagandha extract for 28 days caused significant increases in HDL and catalase, but decreases in serum glucose level, cholesterol, triglyceride, LDL, VLDL, MDA, kidney function, and liver function markers. Also **Sabry and Mahmod, (2023)** found a decrease liver enzymes and kidney functions in rats fed ashwagandha powder (100-200 and 400mg/kg) compared to the control group.

Table (5): Effect of Maca and Ashwagandha extract on kidney function and serum glucose level.

Parameters / Groups	Creatinine (mg/dl)	Urea (nmol/ml)	Glucose (mg/dl)
Control (-)	2.51^{f}±1.36	1.43^{f}±0.11	90.17^{f}±4.78
Control (+)	19.19^{a}±0.07	15.37^{a}±0.15	409.51^{a}±16.16
Ashwagandha 500mg/kg	15.27^{b}±0.14	11.93^{b}±0.48	320.09^{b}±3.22
Ashwagandha 1000mg/kg	12.43^{c}±0.17	9.69^{c}±0.14	282.28^{cb}±1.68
Maca 500mg/kg	12.91^{c}±0.36	10.94^{b}±0.04	298.08^{b}±1.33
Maca1000mg/kg	10.91^{d}±0.34	8.50^{c}±0.35	201.89^{d}±1.92
Mixture extract 500mg/kg	8.46^{d}±0.07	6.27^{d}±0.48	179.63^{d} ±2.92
Mixture extract 1000mg/kg	6.27^{e}±0.15	4.71^{e}±0.16	129.84^{e}±2.29

Values are mean ± ES. Values in the same column sharing the same, superscript letters are not statistically significantly different ($P \leq 0.01$).

3.6 Effect of Maca and Ashwagandha extract on thyroid and testosterone hormones:

The effect of maca and ashwagandha extract on thyroid and testosterone hormones were presented in Table (6) meanwhile results found significant decrease in serum T3, T4, TSH and testosterone hormone level in groups feed high fat diet compared to normal control group. In contrast results found administration with maca and ashwagandha extracts led to increment in all serum hormone level this increment was statistically significant ($P \leq 0.01$) in groups administered by the mixture of maca and ashwagandha extracts.

Kelly and Jones (2015) reported that testosterone deficiency is associated with energy imbalance, impaired glucose control, reduced insulin sensitivity, and dyslipidemia, and low testosterone levels are associated with reduced lean mass in males and increased fat mass (particularly central adiposity), these morphological features are linked to metabolic dysfunction. Also**, Liu et al. (2015) and Khalifa et al. (2022)** pointed out that many feed Additives plants had bioactive such as (alkaloids, flavonoids, glycosides,

mucilages, saponins, tannins, phenol, phenolic acids, coumarin, terpenes, essential oils, lectins, and polypeptides) used to improve productive and reproductive performance. **El-Sheikh et al., (2019)** mentioned that Maca contains specific compounds that are responsible for Maca's ability to support hormonal balance, these compounds called glucosinolates which can affect fertility for both men and women. **Hayashi et al. (2024)** reported that Ashwagandha elevated thyroid hormone is caused by stimulation to the thyroid gland, and Ashwagandha root extract might act both as a prothyroidic agent and as an antiperoxidative agent.

These results agreed with the results obtained by **Zheng et al. (2002)** who observed that Maca extracts used as powder and capsule for their enhancer the fertility and physical activity. **Ohta et al. (2016)** showed that increases serum testosterone and enhances the steroidogenic ability of cultured Leydig cells in male rats feeding maca from 6 to 8 weeks when compared with controls. Also, **Abdel-Wahhab et al. (2019)** found that ashwagandha extract treatment thyroid function by ameliorating thyroid hormones and preventing oxidative stress in animal models of hypothyroidism after 1 month from treatments with ashwagandha extract. And **Alhahawachee et al. (2023)** showed the ameliorating effect of Ashwagandha extract in male rats after early postnatal hypothyroidism.

Table (6): Effect of Maca and Ashwagandha extract on thyroid and testosterone hormones.

Parameters / Groups	T_3 pmol/L	T_4 pmol/L	TSH µlU/ml	Testosterone ng/ml
Control (-)	$24.00^{a}\pm0.35$	$57.80^{a}\pm0.75$	$18.55^{a}\pm0.10$	$27.57^{a}\pm0.08$
Control (+)	$7.08^{f}\pm0.45$	$8.92^{f}\pm0.17$	$2.26^{f}\pm0.15$	$4.80^{f}\pm0.11$
Ashwagandha 500mg/kg	$9.79^{e}\pm0.12$	$13.49^{e}\pm0.23$	$5.19^{e}\pm0.31$	$8.66^{e}\pm0.21$
Ashwagandha 1000mg/kg	$12.07^{d}\pm0.09$	$17.09^{e}\pm0.47$	$9.11^{d}\pm0.03$	$13.16^{d}\pm0.08$
Maca 500mg/kg	$12.97^{d}\pm0.14$	$23.29^{d}\pm0.36$	$7.87^{e}\pm0.15$	$11.06^{d}\pm0.08$
Maca1000mg/kg	$14.63^{c}\pm0.14$	$30.69^{c}\pm0.56$	$10.58^{d}\pm0.09$	$16.23^{c}\pm0.05$
Mixture extract 500mg/kg	$16.44^{c}\pm0.40$	$35.49^{c}\pm0.26$	$13.24^{c}\pm0.09$	$19.27^{c}\pm0.13$

Mixture extract 1000mg/kg	19.01^{b}±0.60	41.42^{b}±0.40	16.03^{b}±0.07	22.49^{b}±0.59

Values are mean ± ES. Values in the same column sharing the same, superscript letters are not statistically significantly different ($P \leq 0.01$).

3.7 Effect of Maca and Ashwagandha extract on liver and kidney tissue:

The liver is an essential metabolic organ, maca and ashwagandha as a herbal plant may have free radical scavenging activity and thereby can be used for the prevention and treatment of liver and kidney damage. In this study the results illustrated the liver and kidney section examination on rats under a light microscope, meanwhile, results showed a slight enhancement in the picture of the liver and kidney section in all groups administrated with maca and ashwagandha extract, while the force enhanced showed in group administrated with 500 and 1000mg /kg from the mixture maca and ashwagandha extract

These findings were in accordance with the study of **Shimmi et al. (2011)** who observed the nephroprotective effect of Ashwagandha root against gentamicin-induced nephrotoxicity in Wistar albino rats, and also reported that herbal plants such as Ashwagandha (*Withania somnifera*) may have free radical scavenging activity thereby can be used for the prevention and treatment of kidney damage. Also, **(Gencoglu, 2020)** showed the protective and regulatory effectiveness of Maca on the levels of the antibodies in the liver, and fat tissues in rats when fed with a normal or high-fat diet. **Rasheed et al. (2021)** showed pretreatment with Ashwagandha was the preservation of renal architecture compared to the group treated with Cisplatin for 7 days of induced nephrotoxicity in rats.

Control group (-)

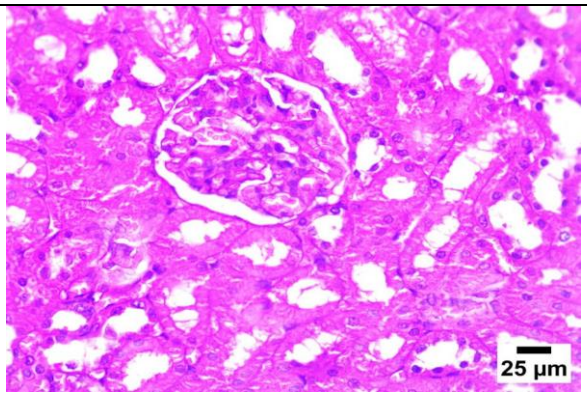

Fig. 1 Photomicrograph showing normal histological structure of renal tubules and glomeruli (H&E stain).

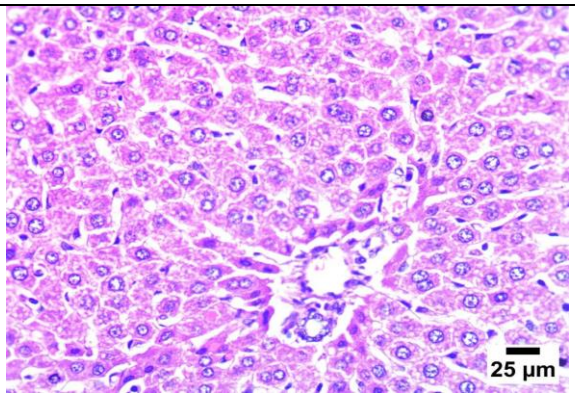

Fig. 2 Photomicrograph showing the normal histological structure of portal area and hepatocytes (H&E stain).

Control group (+)

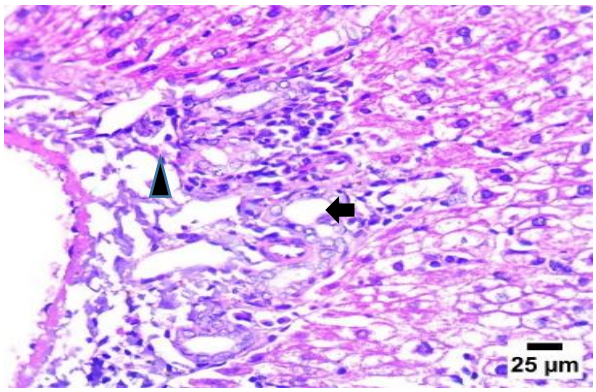

Fig.3 Photomicrograph showing edema with mononuclear inflammatory cells infiltration in the portal area (arrowhead). Also newly formed bile ductulus were detected (black arrow) in addition to ballooning hepatocytes which were recognized as swollen hepatocytes with rarefied cytoplasm (blue arrow) (H&E stain).

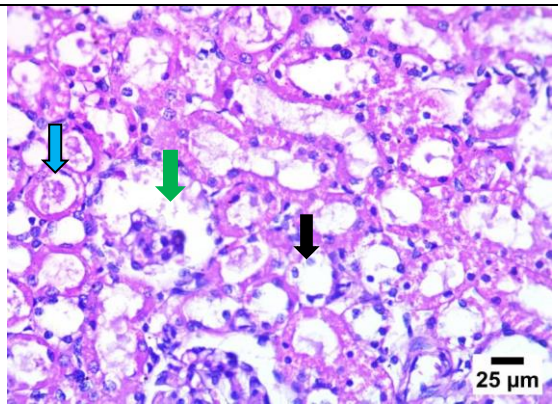

Fig.4 Photomicrograph, some renal tubules showing necrobiotic changes in the epithelium lining (black arrow) others revealing renal cast formation in their lumen (blue arrow), in addition to that some glomeruli showing shrinkage of glomerular tuft (green arrow) (H&E stain)

Ashwagandha 500mg/kg

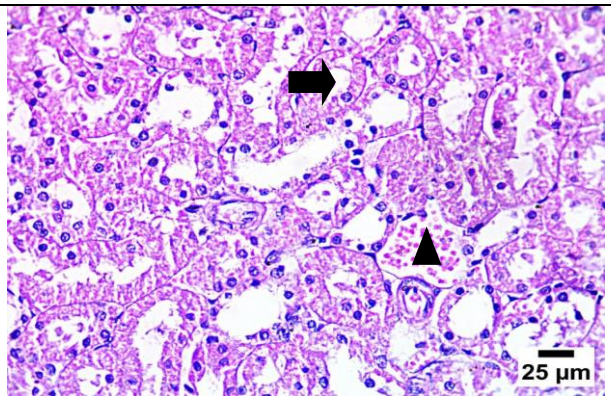

Fig.5 Photomicrograph showing mild necrobiotic changes in the epithelium lining renal tubules (black arrow) in addition to mild congestion of interstitial renal blood vessels (arrowhead) (H&E stain)

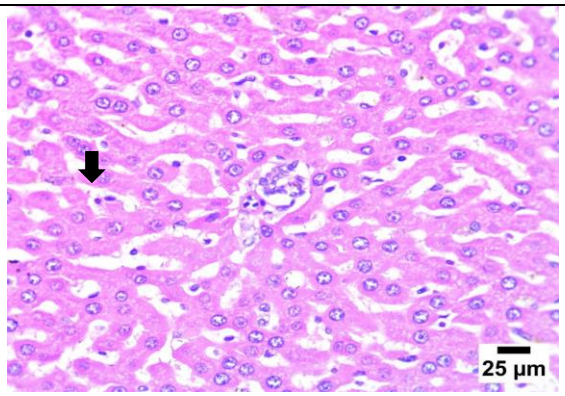

Fig. 6 Photomicrograph showing normal portal area, mild dilatation of hepatic sinusoids with mild activation of Kupffer cells (arrow), also normal hepatocytes were detected (H&E stain)

Ashwagandha 1000mg/kg

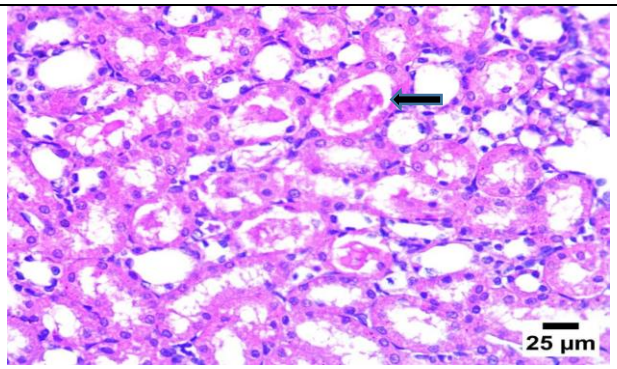

Fig.7 Photomicrograph Most renal tubules reveal normal epithelium lining, but others showing renal cast formation in their lumen (arrow) (H&E stain).

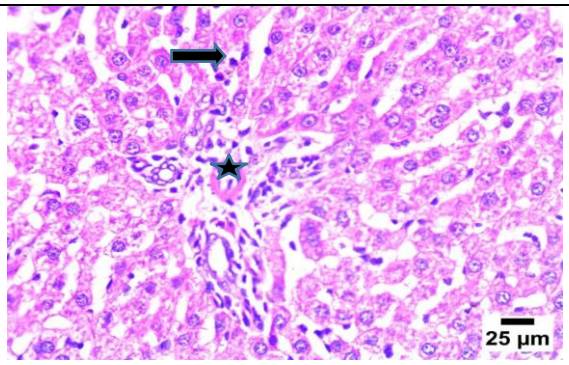

Fig. 8 Photomicrograph showing mild mononuclear inflammatory cells infiltration in the portal area (star), dilatation of hepatic sinusoids with mild activation of Kupffer cell and mild leucosis (arrow) (H&E stain).

Maca 500 mg/kg

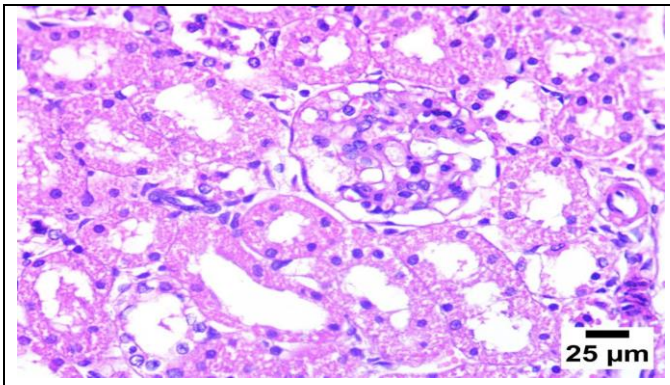

Fig.9 Photomicrograph showing the majority of renal tubules with normal epithelium lining also no marked pathological changes were detected in glomeruli (H&E stain)

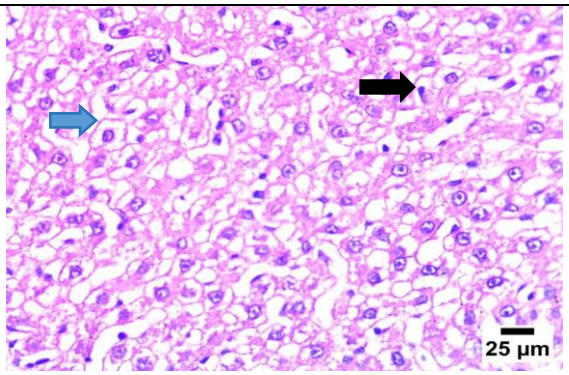

Fig. 10 Photomicrograph showing mild dilatation of hepatic sinusoids with mild activation of Kupffer cell (black arrow) in addition to vacuolar degeneration of hepatocytes (blue arrow) (H&E stain)

Maca 1000 mg/kg

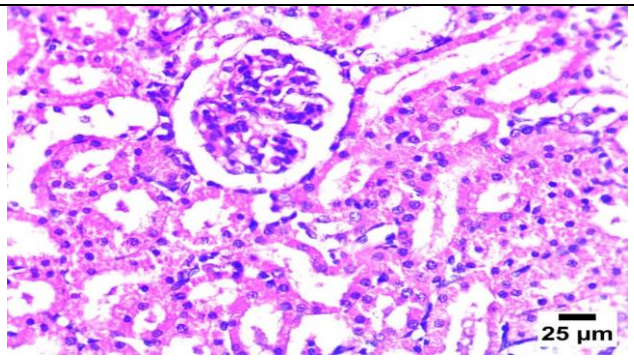

Fig.11 Photomicrograph showing no marked pathological changes in the epithelium lining renal tubules and renal glomeruli (H&E stain)

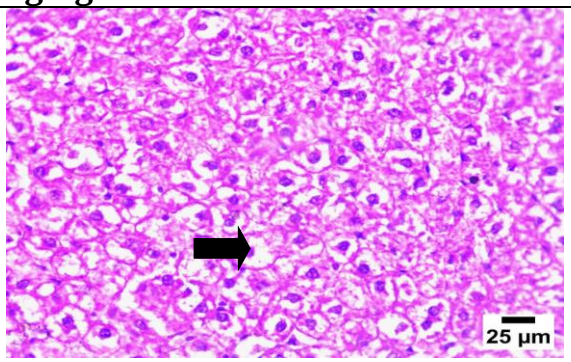

Fig. 12 photomicrograph showing vacuolar degenerative changes in hepatocytes (arrow) (H&E stain)

Mixture extract 500mg/kg

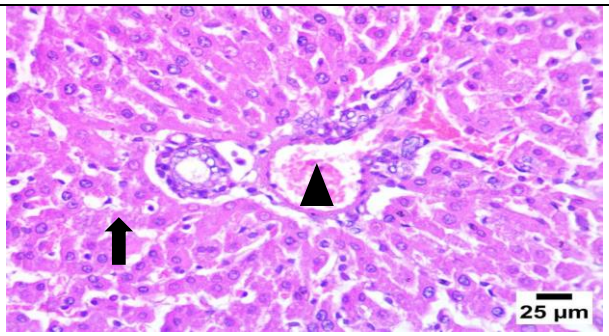

Fig.13 Photomicrograph showing mild

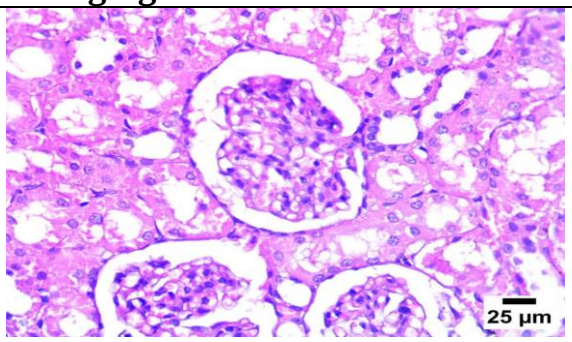

Fig.14 photomicrograph showing, no

congestion of portal blood vessels (arrowhead), mild dilatation of hepatic sinusoids (arrow) with marked improvement in hepatocytes (H&E stain).	marked pathological changes in epithelium lining renal tubules and glomeruli (H&E stain).
Mixture extract 1000mg/kg	
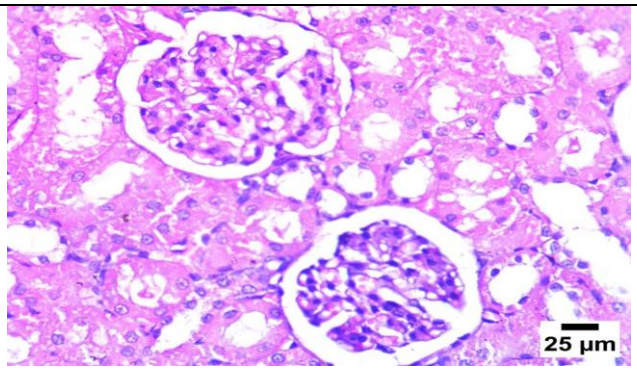	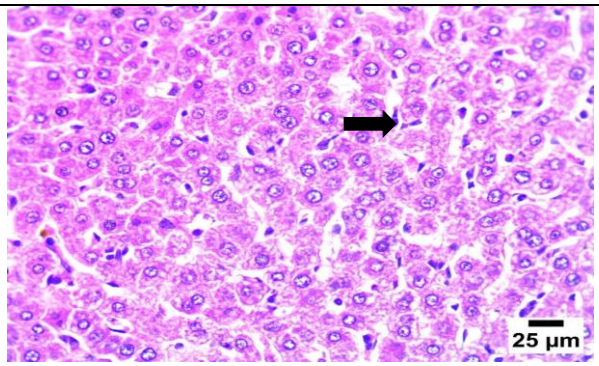
Fig.15 Photomicrograph revealing, the majority of renal tubules with normal epithelium lining and no marked pathological changes in renal glomeruli (H&E stain).	**Fig.16** Photomicrograph revealing mild dilatation of hepatic sinusoids with mild activation of Kupffer cells (arrow) (H&E stain)

Conclusion:

The results of the current study found that the administrated of ashwagandha, maca extract and their mixture had an effective in reducing body weight and body fats, which in turn to a reduction in the body mass index. Also, these extracts improving the secretion of thyroid and testosterone hormones. Furthermore, Ashwagandha and Maca extracts improves liver and kidney functions, through the active compounds found in this plant which able to act as antioxidants and prevent a lot of diseases such as obesity, diabetes, heart disease and oxidative stress

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