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Original article

Alterations of lipid Profile and Minerals induced by Ketoprofen and the Potential role of *Ganoderma lucidum* in male Albino Rats

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Abstract
The non-steroidal anti-inflammatory drug called ketoprofen lowers fever and inflammation. Ganoderma lucidum has maintained its status as one of the most prominent medicinal herbs due to its association with health. This work aimed to find out alterations of lipid parameters and minerals induced by Ketoprofen and the potential role of Ganoderma lucidum. A total of twenty male albino rats were utilized in this study, randomly separated into four groups, with five rats in each group. Group 1: The standard laboratory conditions were maintained for the five members in the regular control group. Group 2: Ketoprofen group (KP); KP was administered orally by gavage every day in the morning within two weeks at a dosage of fifty milligrams per kg of body weight (BW). Group 3: Ganoderma lucidum (G.L) was given reishi mushroom by oral gavage every morning for four weeks in the morning at a dosage of three hundred milligrams per kilogram of BW. Group 4: A combination group (KP-G.L). For two weeks, animals received KP by swallowing at a dose of fifty milligrams per kilogram of BW. The animals were receiving G.L by oral gavage every morning for 4 weeks at a dose of 300 milligrams per Kg of BW following a two-week KP treatment. The KP had varying effects on the studied measurements. This led to significant changes, like a decline in overall body weight and folate, levels of ferritin, zinc (Zn), triglyceride (TG), and vitamin D (VD) levels. Meanwhile, it caused an increase in total cholesterol (TC), low-density lipoprotein (LDL), and high-density lipoprotein (HDL). The treatment also led to an increase in calcium (Ca) levels. As for the Ganoderma lucidum, the therapy led to a decrease in total weight without significant differences. It also caused a reduction in the level of ferritin and LDL. However, it caused a rise in levels of TC, TG, very low-density lipoprotein (VLDL), and HDL. And an increase in the level of Zn, Ca, and VD. In the combination group, all previous parameters were returned to close to normal values.
Keywords. Lipid Profile, Minerals, Ketoprofen, Ganoderma Lucidum, and Male Rats.

Introduction

The synthesis of ketoprofen (KP) dates back to 1968, and this drug falls within the broader category of propionic acid derivatives, which are characterized by their distinct pharmacological profile in biological systems. Compounds in this family have a triple efficacy: This derivative functions as a versatile therapeutic agent, manifesting significant efficacy in thermal regulation, inflammatory modulation, and nociceptive inhibition [1]. The drug acts as an inhibitor of cyclooxygenase (COX) enzymes, leading to the alleviation of inflammatory symptoms but with the potential to affect the body's normal functions [**Error! Reference source not found.**]. The mechanism of action of the drug relies on its easy access to the brain by traversing the blood – brain barrier (BBB). It specifically targets the hypothalamus, where it inhibits the production of prostaglandins (PGs), leading to the central effect of the drug, such as reducing fever [**Error! Reference source not found.**]. The drug is efficiently absorbed following oral or rectal administration and may also be administered via injection (intravenous or intramuscular) or transdermal. It is metabolized in the liver, and most of it is excreted through urine, with a small portion excreted through feces, and it does not accumulate in the body. Its side effects are primarily due to its suppression of COX enzymes [**Error! Reference source not found.**].

Selected compounds from the non-steroidal anti-inflammatory drug (NSAIDs) family have demonstrated strong dual efficacy: lipid reduction (by 80 percent for cholesterol) and anti-inflammatory effects by reducing oedema by seventy percent [**Error! Reference source not found.**]. Mushrooms have been companions to humanity, where their relationship with humans has intertwined between food and medicine **Error! Reference source not found.**]. Medicinal mushrooms are commonly formulated in biopharma ceutical applications as powders or liquid extracts, making them easy to incorporate into pharmaceutical products [**Error! Reference source not found.**]. Medicinal mushrooms have been used for centuries in various folk medicine traditions worldwide, with their long history witnessing widespread adoption, particularly in Asian cultures. The value of these fungi lies in their complex array of active chemical compounds, such as complex carbohydrates, polypeptides, peptides, terpenoids, essential micronutrients, and trace elements, which are attributed with multiple therapeutic potentials. These purported benefits include anti-cancer properties, anti-inflammatory and antioxidant effects, as well as cholesterol-lowering and blood sugar-regulating impacts, and the ability to modulate immune function [**Error! Reference source not found.Error! Reference source not found.**].

High pharmacological efficacy is exhibited by a myriad of compounds derived from medicinal mushrooms, which are increasingly recognized as critical sources for novel drug discovery in biological research, among which *Ganoderma lucidum (G.L)* stands out for its extensive role in disease prevention and treatment, as documented in traditional medical practices across many cultures. This mushroom has held a special place in traditional Chinese and Japanese medicine, where it has been used as a therapeutic agent. For a duration exceeding two millennia, these practices have been

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consistently integrated into traditional medicine, reflecting a long-standing historical precedent [**Error! Reference source not found.**, **Error! Reference source not found.**]. In the dose-response study, low doses of (*G.L*) did not show a significant effect on high density lipoprotein (HDL) levels, as the positive effect only becomes apparent when a higher dose, confirming the dose-dependent nature of this effect [**Error! Reference source not found.**]. Additional research has confirmed that the consumption of *G.L* has an effect on triglyceride (TG) levels in the blood, supporting its potential role in promoting a healthy lipid profile and metabolism [**Error! Reference source not found.**, **Error! Reference source not found.**]. Moreover, the extract raised serum insulin levels and significantly improved lipid status indicators in both healthy and diabetic mice, with these improvements being proportional to the administered dose [**Error! Reference source not found.**]. This work aimed to study alterations of lipid parameters and minerals induced by KP and the potential role of *Ganoderma lucidum* in male albino rats.

Methods

Tested compounds and animals

The reishi mushroom powder (*G.L*) imported from Malaysia was used, with the package containing 70 grams of the dried substance. Regarding the KP compound, it was administered in the form of capsules at a fixed dose of 50 mg/kg/day, and it was obtained through the European Egyptian Pharmaceutical Company (via local supply from accredited pharmacies). The study sample included twenty adult male albino laboratory rats, ranging in size between 170 and 200 grammes. These animals were obtained from the animal breeding facilities of the Zoology Department at the Faculty of Science, Omar Al-Mukhtar University. The rats were kept in room-temperature boxes, fed, and drank fresh water every day. Before the experiment and upon completion, the weight of each rat was measured and documented.

Ethical approval

The protocol received rigorous institutional oversight and was endorsed by two distinct entities: the Libyan National Committee for Biosafety and Bioethics, operating under the auspices of the Libyan Authority for Scientific Research, and the Bioethics Committee at Omar Al-Mukhtar University, Al-Bayda. Adherence to these institutional guidelines ensured that all investigative actions aligned with national bioethical standards (NBC:007. A. 24. 16).

Study design

Twenty male rats were partitioned into four distinct groups (n = 5 per group) using a randomized allocation protocol.

Group I

The normal control group, n = 5, was maintained under typical laboratory conditions.

Group II

Ketoprofen group (KP) n = 5; all received KP at a dose of fifty mg per kilogram of BW via feeding by mouth [**Error! Reference source not found.**] daily for 2 weeks in the early morning.

Group III

(G.L) n = 5; all received reishi mushroom at a dose of 300 milligrams per kilogram of body weight through oral feeding **Error! Reference source not found.**] each day for four weeks in the early hours of the morning.

Group IV

Combination group (KP-G.L), n = 5. The animals were given a dose of KP at a rate of 50/kg/ body weight orally daily for a two-week period. After the KP dose, the animal will be given a dose of G.L at a dose of 300 mg/kg body weight orally daily for four successive weeks. At the end of the study and 24 hours after the final administration, all animals were weighed and subsequently sacrificed, and blood specimens were taken by snipping a jugular vein to conduct biochemical tests.

Assay of a lipid profile

The total cholesterol level was determined using the **Error! Reference source not found.Error! Reference source not found.**] technique. TG was calculated using the [**Error! Reference source not found.**]. The [**Error! Reference source not found.**] The technique was used to test HDL. The calculation of (LDL) was performed based on the standard computational method, as defined in the Friedewald formula [**Error! Reference source not found.**].

Assay of electrolytes

To ensure the accuracy in estimating calcium concentration, the study relied on the chemical method previously described by using the methods of [**Error! Reference source not found.**]. The measurement of serum Zn concentration was conducted based on the analytical protocol established by using the methods of [**Error! Reference source not found.**]. The concentration of 25-hydroxyvitamin D was measured using the commercial (ELISA) technique from Roche Diagnostics (Mannheim, Germany) [**Error! Reference source not found.**]. An ELISA kit (CELL. BIOLABS, Inc., California, USA) was used to determine the content of folate [**Error! Reference source not found.Error! Reference source not**

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found.]. Ferritin, the necessary reagent kits for this test were provided by Roche Diagnostics (based in Mannheim, Germany) [**Error! Reference source not found.**].

Statistical Analysis

The statistical evaluation was conducted using Minitab software (version 17) and Graph Prism Pad; statistical significance was evaluated using ANOVA with Tukey's multiple comparisons test after confirming the normal distribution in the data, and $P < 0.01$ was considered appropriate for statistical significance.

Results

The data presented in the table revealed the effect of different treatments (KP*, G.L*, and their combination) on the weight dynamics of male rats. The untreated group recorded a statistically significant increase ($P < 0.01$) in final weight.

Table 1. Changes in the BW of male albino rats that were given KP, G.L, and their combination of treatments, when compared with the control group

Experimental groups	BW (gram)
	Final BW Mean ± SEM
Control	332 ± 15.89^{A}
KP	295 ± 12.10^{AB}
G.L	315 ± 7.38^{AB}
KP-*G.L*	259.5 ± 18.79^{B}

The data are presented as means ± SEM, with a sample size of 5 for each group. Significant differences (P < 0.01).

The data presented in (Table*2*) revealed the effect of different treatments (KP, G.L, and their combination) on cholesterol: It increased significantly in KP (59 ± 1.63) and KP-G.L (41.75 ± 3.97) and G.L (39.65 ± 0.47) compared to the control (21.3325 ± 1.03). While triglycerides (TG): G.L recorded a high increase (175.65 ± 5.39), the increases in KP (75 ± 8.15) and KP-G.L (84 ± 2.27) were not significant. HDL: Significantly increased ($P < 0.01$) in KP (40.325 ± 1.43), G.L (41.65 ± 1.43), and KP-G.L (40 ± 1.47) compared to the control (30.665 ± 0.85). LDL: Increased in KP (111 ± 1.47) compared to the control (83.3325 ± 4.40), while it decreased in G.L (68.65 ± 1.25) and KP-G.L (62 ± 3.24). VLDL: The G.L group recorded a significant increase (35 ± 1.08) compared to the control group (16.3325 ± 1.43), with relative stability in the other groups.

Table 2. The mean values of serum cholesterol, TG, HDL, and LDL, VLDL of male rats given KP, G.L, and their combined treatment in comparison to the control

Parameter	Experimental groups			
	Control Mean ± SEM	KP Mean ± SEM	*G.L* Mean ± SEM	KP-*G.L* Mean ± SEM
Cholesterol (mg/dl)	21.3325 ±1.03^{C}	59 ± 1.63^{A}	39.65 ±0.47^{B}	41.75 ±3.97^{B}
TG (mg/dl)	81 ± 7.22^{B}	75 ± 8.15^{B}	175.65 ± 5.39^{A}	84 ± 2.27^{B}
HDL (mg/dl)	30.665 ± 0.85^{B}	40.325±1.43^{A}	41.65 ±1.43^{A}	40 ± 1.47^{A}
LDL (mg/dl)	83.3325± 4.40^{B}	111 ± 1.47^{A}	68.65 ± 1.25^{C}	62 ± 3.24^{C}
VLDL (mg/dl)	16.3325± 1.43^{B}	15 ± 1.78^{B}	35 ± 1.08^{A}	16.25 ±0.85^{B}

The data are presented as means ± SEM, with a sample size of 5 for each group. Significant differences (P < 0.01) between averages in the same row are indicated by different elevated script letters (A, B, C & D), while averages with identical elevated script letters indicate no significant change (P < 0.01).

The data presented in (Table3) revealed the effect of different treatments (KP*, G.L*, and their combination) on the results of calcium levels, which showed a non-significant decrease in the KP group (7.925 ± 0.09) and the KP-G.L group (7.925 ± 0.05) compared to the control group (7.85 ± 0.06). In contrast, the G.L group recorded a statistically insignificant increase (7.975 ± 0.13). Data indicate a non-significant increase in VD levels for all groups compared to the control group (14.05 ± 1.86), where the KP group recorded a value of (10.15 ± 1.23), the G.L group recorded a value of (18 ± 3.08), while the KP-G.L group recorded a value of (13.75 ± 1.49). The results of serum ferritin levels show a decrease in the treated groups compared to the control group (4.93 ± 0.62), where the values were (3.78 ± 0.68) for the KP group, (3.58 ± 0.59) for the G.L group, and (2.6 ± 0.25) for the KP-G.L group. A significant decrease was also observed in the KP-G.L group compared to the G.L group. The results of folate levels show a significant decrease ($P < 0.01$) in the KP group (5.78 ± 1.03) compared to the control group (17.75 ± 0.85). While the G.L (13.13 ± 1.02) and KP-G.L (12.45 ± 0.79) groups recorded a non-significant decrease compared to the control rat group. The results of zinc (Zn) levels show a significant decrease ($P < 0.01$) in the KP group (73 ± 2.86) compared to the control group (80.67 ± 1.65). While the G.L (141 ± 2.27) and KP-G.L (142.75 ± 6.80) groups recorded a non-significant decrease compared to the control rat group.

Table 3. The mean mineral and vitamin values in male albino rats treated with KP and G.L, and their combined treatment, compared to the untreated group.

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Parameter	"Experimental groups"			
	Control Mean ± SEM	KP Mean ± SEM	*G.L* Mean ± SEM	KP-*G.L* Mean ± SEM
Ca (mg/dl)	7.85 ± 0.06^{A}	7.925±0.09^{A}	7.975 ± 0.13^{A}	7.925± 0.05^{A}
VD (ng/ml)	14.05±1.86^{A}	10.15±1.23^{A}	18 ± 3.08^{A}	13.75 ± 1.49^{A}
Ferritin (ng/ml)	4.93 ± 0.62^{A}	3.78 ± 0.68^{A}	3.58± 0.59^{A}	2.6 ± 0.25^{A}
Folate (ng/ml)	17.75 ± 0.85^{A}	5.78 ± 1.03^{C}	13.13±1.02^{B}	12.45 ± 0.79^{B}
Zinc (Ug/dl)	80.67±1.65^{B}	73 ± 2.86^{B}	141 ± 2.27^{A}	142.75± 6.80^{A}

The data are presented as means ± standard error of the mean (SEM), with a sample size of five for each group. significant differences (P<0.01) among means within the same row are indicated by different superscript letters (A, B, C, and D), whereas means sharing the same superscript letters indicate no significant difference (P<0.01).

Discussion

KP is primarily used for pain relief, while its role in mediating the metabolic process of energy production from fats remains unclear. Baseline body mass evaluations revealed marginal variations across the experimental groups at the study's inception. Although a reduction in BW was observed within the treated group by the end of the monitoring phase, this decline failed to achieve statistical significance compared to the control group. These findings are consistent with previous data from rodent models, where 5mg/kg induced weight loss, contrasting with the typical mean diurnal weight gain patterns documented over the five days [**Error! Reference source not found.**]. In terms of BW, the group that received *G.L* observed slightly lower values for weight in measurements, in contrast to the control group, but without significant differences. There were also no negative effects on the physiology or behaviors of the chickens fed a diet supplemented with wild *G.L* [**Error! Reference source not found.**,**Error! Reference source not found.**]. Multiple research studies have demonstrated that NSAIDs, which also exhibit cholesterol -lowering effects in both humans and experimental animals [**Error! Reference source not found.**]. The current results support the conclusions drawn by additional research that therapies lowering inflammation will return the profile of lipids to its normal state, which will result in a rise in HDL levels in the plasma and a decrease in TG levels. If the low LDL levels had first decreased, a therapy that decreases inflammation would also cause an increase in LDL levels [**Error! Reference source not found.**,**Error! Reference source not found.**].

The present results indicated a significant increase in cholesterol levels. Biosynthetic pathway of cholesterol is reportedly hindered by oxidized sterols derived from *G.L*, which exert their inhibitory effects by interfering with the transformation of acetate or mevalonate precursors [**Error! Reference source not found.**]. These results do not fully align, as there was a rise in cholesterol levels compared to the control. In alignment with the consensus established by Yuan *et al*. (2018) [**Error! Reference source not found.**] regarding the therapeutic efficacy of *G.L* extracts, current empirical data demonstrated a significant attenuation in LDL concentrations within the treated group relative to the control group. This observed hypolipidemic effect is likely mediated by the modulation of hepatic lipid metabolism; specifically, the extract appears to impede the elevation of LDL levels by suppressing the phosphorylation pathways of AMP (AMPK) and (ACC) within hepatocytes. A study using meta-analyses looked into the effect of *G.L* on lipid criteria in animal models. According to their results, *G.L* has reduced impacts on levels of lipid profiles. The results we obtained partially aligned with the findings of this study. While the results agreed on the significant decrease in LDL levels, they differed with regard to cholesterol, HDL-C, TG, and VLDL levels, where our research observed an increase in these parameters.

The present investigation found a non-significant reduction in Zn levels in the KP group relative to the untreated. Evidence from this research is consistent with documented observations wherein inadequate Zn levels significantly contribute to the development of gastroduodenal lesions during COX inhibitor therapy. Because of Zn deficiency in the animals used for research, injuries were amplified, and the resolution of ulcers was retarded [**Error! Reference source not found.**, **Error! Reference source not found.**]. Data derived from the present study demonstrate a statistically significant elevation in Zn concentrations within the group administered KP- *G.L* group relative to the control. As a prominent medicinal fungus, *G.L* remains a focal point of scientific inquiry due to its versatile bioactive constituents. Earlier investigations have scrutinized its efficacy as a bio-vehicle for trace element supplementation, such as selenium, Cu, and Zn. The current results reinforce the premise that *G.L* exhibits a high capacity for the bioaccumulation of minerals from its growth substrate. Consequently, the enriched fruiting bodies, in their desiccated form, represent a potent nutritional reservoir for delivering these essential micronutrients, thereby enhancing their overall dietary value [**Error! Reference source not found.**, **Error! Reference source not found.**]. In the experimental group treated with KP, a non-significant elevation in Ca concentrations was observed alongside a concomitant reduction in VD levels relative to the control. Substantial elevations in Ca and VD concentrations were observed in the group subjected to *G.L* treatment, surpassing the levels recorded in the control group. These findings align with prior research indicating that oral administration of *G.L* at a dosage of 292 mg/kg for a duration of five days induces a partial response in VD levels, as demonstrated in male albino rats. This correlation reinforces the physiological influence of *G.L* on mineral and vitamin homeostasis across different rodent models [**Error! Reference source not found.**].

In the present study, a discernible decrement in ferritin concentrations was observed in both the KP-treated and *G.L*-treated and KP-*G.L* groups relative to the untreated; nevertheless, these variations failed to reach the threshold of

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statistical significance.

Conclusion

The results obtained from the present study convincingly demonstrated that KP exposure resulted in varying degrees of biochemical parameters in rats. G. lucidum mushroom shows great potential as a dietary supplement that contributes to the prevention of disorders resulting from toxicity associated with the ketoprofen pathway (KP). This efficacy is attributed to its properties as a powerful antioxidant that neutralizes reactive oxygen species (ROS) and enhances the efficiency of enzymes responsible for detoxification, making it a promising therapeutic option for alleviating the damage caused by this pathway in both humans and animals.

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Conflicts of Interest

The authors declare no conflicts of interest.

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