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Research Article

ANTI-OBESITY POTENTIAL OF POTASH ALUM: PHARMACOLOGICAL AND BIOCHEMICAL APPROACH

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ABSTRACT

The objective of present study was to evaluate the pharmacological and biochemical role of Potash Alum as an anti-obesity agent in Wistar rats fed on high fat diet (HFD). Animals were fed on HFD (58% fat) with or without Potash Alum for 24 weeks. Results revealed that oral intake of Potash Alum exhibited significant reduction in body weight, food intake, serum triglycerides (TGs), total cholesterol (TCs) and high density lipoproteins (HDL) whereas simultaneously increased the dry weight of feces, total lipids in feces, compared to HFD fed control. The levels of blood hemoglobin and glucose were also assessed and there were no significant changes in these parameters. We suggest that the inhibitory effects of potash alum on obesity, might be attributed to the inhibition of lipid absorption through the inhibition of pancreatic lipase.

Keywords: Obesity, Potash alum, Pharmacological, Biochemical parameters.

INTRODUCTION

The prevalence of overweight and obesity is increasing at an alarming rate and it is a manifestation of the epidemics of a sedentary lifestyle and excessive calorie intake[1]. Body mass index (BMI) are used to measure healthy weight (BMI between 20 and 25), overweight (BMI greater than 25) and obese (BMI greater than 27)[2]. Globally, 1.6 billion people are overweight (Body Mass Index, BMI, between 25 and 30) and 400 million are obese (BMI above 30)[3]. The pervasiveness of obesity continues to increase in the major affluent societies[4]. The incidence of obesity in the developed countries is an epidemic[5]. The World Health Organization has recognized the obesity epidemic as one of the top 10 global health problems[6]. It is estimated that in many countries 2–8% of the total health cost is spent on obesity[7]. Overweight and obesity are now the major contributors to cardiovascular disease, type II diabetes, sleep apnoea, musculoskeletal disorders and some types of cancers along with psycho-social disorders[8]. The current approach of treatment for obesity includes behavioral therapy aimed at modifying eating-related activities[9], exercise to increase caloric expenditure[10] and following specific dietary regimen to lower calorie and fat intake[11]. Pharmacological treatments are generally considered as an adjunct to this core therapy. Current pharmacological interventions have displayed limited efficacy and unpleasant side-effects of the drug linked obesity treatment[12-13]. Even when combined with diet and exercise, patients taking these weight-loss drugs typically lose 10–15% of their body weight, plateau at 4–6 months and then begin to regain weight[14].

Most of the medicinal therapy is of organic composition. Life on the earth is carbon based due to its properties of forming bond with itself and other atoms. Lesser work has been done on the inorganic salts by the researchers in the modern medical therapy. Therapeutic efficacy of inorganic salt aluminum and its compound has been evaluated in many diseases[15-18]. Of which Potash Alum has been used in the treatment of neoplasm in vivo without causing its side effects and thus increasing the life expectancy[19]. Moreover, the alum has been used in the ayurvedic preparation of Himalya "styplon" which is used in the treatment of bleeding gums, bleeding heamorrhoids, epistaxis, abnormal uterine bleeding, hematuria, and hemoptysis[20]. In homeopathic system of medicine, Potash Alums are used in the treatment of asthma and dementia [21]. Latest trend of research on potash alum is mainly aimed at infectious diseases either the viral or bacterial [22-23]. On the basis of existing literatures and research work, the present study is aimed for pharmacological evaluation of the Potash Alums on the high fat fed induced obesity.

MATERIALS AND METHODS

Animals

Healthy male Wistar rats weighing 100-150g were procured from central animal house facility of Siddhartha Institute of Pharmacy, Dehradun, India. All animals were housed at ambient temperature (22 ± 1°C) and relative humidity (55 ± 5°C) with standard diet and water provided ad *libitium*. Studies conducted were approved by the Institutional Animal Ethical Committee (1435/PO/a/11/CPCSEA) of Siddhartha Institute of Pharmacy, Dehradun, India.

The animals were divided into 6 groups (n=6). Group I was the normal untreated control rats and received normal diet daily for 24 weeks. Group II, disease control rats, received HFD during whole study. Group III, prophylactic group, received Potash Alum + HFD during whole experimental protocol, Group IV, therapeutic group received HFD + Potash Alum during experiment, Group V received HFD for 15 days then kept on normal diet and administered Potash Alum during the study and Group VI Alum control received Potash Alum with normal diet during whole experiment.

Induction of obesity in rats

Healthy Wistar rats showing normal body weights in the range of 150-200 gm were selected. They were fed with HFD (composition of HFD shown **Table 1**) for two weeks prior to Potash alum treatment. After two weeks the body weights were measured and Potash Alum was administered orally twice daily in the dose of 9.0 mg/kg of body weight. The entire chemical used in the experimental protocol were of analytical grade, Potash Alum was purchased from the local supplier of Dehradun (Himgiri Traders Pvt. Ltd), and cholesterol was purchased from S.D. Fine Chemicals Pvt. Ltd., Methionine from Himedia Lab. Pvt. Ltd.

Table 1: Composition of experimental high-fat diets

Ingredients	Quantity
Powdered NPD*	600 gm/kg
Coconut oil	200 gm/kg
Casein	40 gm/kg
Cholesterol	05 gm/kg
Vitamin and mineral mix	50 gm/kg
Fructose	25 gm/kg
Sucrose	25 gm/kg
dl-Methionine	03 gm/kg
Sodium chloride	02 gm/kg

*NPD – Normal Pellet Diet

Estimation of biochemical parameters and lipid content of feces

Blood samples were collected on termination of the experiment from retro-orbital plexus under light ether anesthesia without any anticoagulant and were allowed to stand for 30 minutes at room temperature, centrifuged at 2500 rpm for 10 minutes to separate the serum. The serum obtained was kept at 2 - 4 ºC for further use. Total cholesterol, Triglyceride, and HDL-cholesterol were determined

using Standard kit (Nicholas India Pvt. Ltd.) with semi-auto analyzer (Photometer 5010, Nicholas India Pvt. Ltd).

The feces excreted during the last 3 days of the experimental period were collected every day and stored at -80°C. After lyophilization, the fecal samples were pulverized and weighed before analysis. Fecal lipids were determined gravimetrically by a modification of the Saxon method. A portion of the lyophilized feces (about 1.5 g) was suspended in 1 ml of distilled water and 9 ml of conc. HCI in a sealed glass tube kept at 50°C for 30 min under nitrogen gas. The lipid fraction was then extracted with 40 ml of diethyl ether and determined gravimetrically after completely removing the solvent.

***In vitro* assay for the measurement of inhibitory effect of the potash alum on the pancreatic lipase**

Lipase Psi reagents and source of human pancreatic lipase were obtained from sigma diagnostics (Himgiri traders). Aliquots (30Al) of lipase standard and Potash Alum, (using water as blank) were added to 400 Al of reconstituted substrate solution, mixed gently and incubated for 5 min at 37-C. Activator reagent (300 Al) was added and mixed by gentle inversion, and the sample were incubated again for 3min at 37-C. The recorded rate of increase in absorbance at 550 nm due to the information of quinone diimine dye was used to determine the pancreatic lipase activity in the sample prepared.

Statistical Analysis

Data are expressed as Mean ± S.E.M. Where n=6. $^{a}P<0.05$; $^{b}P<0.01$; $^{c}P<0.001$ compared to HFD fed control (one way ANOVA followed by Tukey's post-test).

RESULT

Body weight

Consumption of HFD for 24 weeks has shown an increase in the body weight of rats as compared to normal control. Treatment with dose of Potash Alum (Alum +obese at 9.0 mg/kg) in HFD fed rats exhibited significant (p<0.001) reduction in the body weight as compared to disease control (**Table 2**).

Table 2: Effect of twenty four weeks treatment of Potash alum on body weight, hemoglobin and blood glucose of HFD fed rats

Treatment	Body weight (g)		Hemoglobin (g/100ml)	Blood glucose (mg/100ml)	
	Initial	Final		Initial	Final
Normal control	150.32±6.23	200.12±4.65c	10.45±0.11	85.33±3.87	86.45±1.324^{c}
Disease control	192.43±3.74	321.33±9.98c	11.45±0.01c	82.23±2.34	110.43±2.34^{c}
Prophylactic	188.66±5.56	290.34±8.89b	12.61±0.88c	75.76±2.31	95.52±3.34^{c}
Therapeutic	201.76±4.56	280.54±7.78c	13.22±0.32c	87.45±1.56	92.22±2.34^{c}
Alum+Obese	193.45±9.34	243.45±5.67c	12.68±1.11c	86.30±2.11	86.34±1.34^{c}
Alum control	199.47±6.67	179.98±8.57c	11.11±0.13c	82.34±1.23	87.45±3.44^{c}

Data are expressed as Mean ± S.E.M. Where n=6. $^{a}P<0.05$; $^{b}P<0.01$; $^{c}P<0.001$ compared to Disease and Normal control (one way ANOVA followed by Tukey's post-test).

Food consumption, dry weight of feces and total lipids in feces

There was significant increase in average food consumption ($^{c}P<0.001$) in disease control (20.23 ± 1.24 g/day) as compared to normal control (15.71±1.23 g/day). The average food intake in the HFD fed rats containing Potash alum 9.0 mg/kg was found to be significantly low ($^{c}P<0.001$) as compared to HFD fed control.

Rats fed with HFD for 24 weeks exhibited significant lower levels of total lipids in feces compared to normal diet fed control. Treatment with Potash Alum for 24 weeks had shown considerably higher total lipids (145.2 ± 2.31 mg/g) in feces compared to HFD fed control (129.2 ± 3.70 mg/g). Total lipid levels in feces were not changed significantly in the alum control group compared to normal diet fed control. Comparative analysis of dry weight of feces of the disease control, normal control and treated animals revealed that dry weight of the treated groups (1.85±0.25g/3day) were almost in the range of normal controls (1.52±0.13g/3day) which were lower than disease control groups (3.42±0.23g/3day). The results are summarized in **Table 3**.

Table 3: Effect of twenty four weeks treatment of Potash alum on average food intake, dry weight of feces and fecal lipid contents of HFD fed rats.

Treatment	Average food intake (g/day)	Dry weight of feces (g/rat/day)	Fecal lipids (mg/g)
Normal control	15.71±1.23	1.52±0.13	110.2±3.70
Disease control	20.23±1.24	3.42±0.23	129.3±3.20
Prophylactic	14.27±1.43^{c}	1.85±0.25^{c}	145.2±2.31^{b}
Therapeutic	15.53±2.73^{a}	2.03±0.19^{c}	152.3±3.6^{c}
Alum+Obese	15.53±1.73^{c}	2.03±0.19^{b}	165.3±2.7^{c}
Alum control	13.23±0.75^{c}	1.51±0.18^{c}	105.4±4.6^{c}

Data are expressed as Mean ± S.E.M. Where n=6. $^{a}P<0.05$; $^{b}P<0.01$; $^{c}P<0.001$ compared to Disease and Normal control (one way ANOVA followed by Tukey's post-test).

Estimation of lipid parameters

Consumption of HFD in disease control for 24 weeks had shown significant ($^{b}P<0.01$; $^{c}P<0.001$) increase in the TG, TC and decreased serum HDL-c levels as compared to Normal control. However, there was significant ($^{c}P<0.001$) reduction in TG, TC and increased serum HDL-c levels with Potash Alum treatment (group III, IV, and V) to HFD fed rats as compared to disease control groups (**Table 4**). Furthermore, the prophylactic administration of Potash Alum showed more efficacy as compare to therapeutic group in the improvement of HDLCs, TGs and TCs levels. Among all the three treated groups, better improvement of the lipid profile was observed with the group in which Potash Alum administered only after 15 days exposure to HFD (group V).

***Invitro* Assay for the pancreatic lipase activity**

Invitro assay for the measurement of inhibitory effect of the potash alum on the pancreatic lipase was done using the modified method of Moren et al and the % inhibitory effect of potash alum was 42.45% at a dose of (9.0mg/kg).

Table 4: Effect of twenty four weeks treatment of Potash alum on lipid profile of HFD fed rats.

Treatment	TC (mg/dl)	TG (mg/dl)	HDL-Cs (mg/dl)
Normal control	46.67±1.56	36.47±3.45	31.33±2.21
Disease control	160.50±3.45	175.67±2.67	15.33±0.88
Prophylactic	120.27±2.78[c]	155.54±4.68[c]	17.44±0.61[b]
Therapeutic	135.23±2.43[c]	162.22±4.12[c]	15.44±1.61[c]
Alum+Obese	105.66±4.67[c]	125.34±5.34[c]	18.55±1.71[c]
Alum control	48.23±3.61[c]	46.47±1.19[c]	28.12±1.34[c]

Data are expressed as Mean ± S.E.M. Where n=6. [a]*P*<0.05; [b]*P*<0.01; [c]*P*<0.001 compared to Disease and Normal control (one way ANOVA followed by Tukey's post-test).

DISCUSSION

Obesity is extending its tentacles with an alarming rate, both in developed and developing countries of the world and is associated with many life-style-related diseases/disorders such as hyperlipidemia, hypertension, arteriosclerosis and non-insulin-dependent diabetes mellitus [24]. Dietary fat is responsible for increased in body weight and adiposity in humans and animals when compared to dietary carbohydrate[25]. Several therapy aiming various cellular and molecular targets are available to treat obesity like affecting fat absorption by lipase inhibitor e.g. Orlistat, combined norepinephrine and serotonin re-uptake inhibitor e.g. Sibutramine, sympathomimetic amine e.g. Phentermine, selective serotonin re-uptake inhibitor e.g. Fluoxetine and inhibiting the synthesis and release of appetite stimulating factors such as hypothalamic neuropeptide Y e.g. Leptin to name a few[26].

Present study, was aimed to examine and evaluate the effect of Potash Alum on high-fat diet-induced obesity in rats. It was observed that the consumption of HFD by rats for 24 weeks increased the body weight. The increased body weight in HFD disease control might be due to the consumption of an energy-rich diet of saturated fats and its deposition in body pads coupled with decreased energy expenditure when compared to NPD fed rats (Normal control). Treatment with Potash Alum to HFD fed rats reduced the increase in body weight.

Analysis of feces from all the six groups revealed that there was an increase in fecal weight and TG in the treatment group (Alum + Obese group) compared to HFD fed rats (Disease control). The hypertriglyceridemia observed in HFD fed rats may be due to increased absorption and formation of triglycerides in the form of chylomicrons from exogenous fat-rich diet or through combination of increased endogenous production of TG-enriched hepatic VLDL and decreased TG-uptake in peripheral tissues[27].

Hypercholesterolemia may be attributed to increased absorption of dietary cholesterol from HFD[28]. We observed increased level of liver TG and serum lipids such as TG and TC in HFD fed rats. Further , hyperlipidemia might have resulted either from the inhibition of TG synthesis in liver or increased peripheral clearance of TG by stimulating LPL and/or inhibition of dietary cholesterol absorption from the intestine. The calories in excess of the requirement of the normal animal or man are known to be stored in the adipose tissue. The LPL (lipoprotein lipase) and HSL (hormone sensitive lipase) of the adipose tissue responsible for the uptake of triglycerides and mobilization in the fed and starved states respectively and skeletal muscle LPL seem to determine the level of serum triglycerides. A related aspect is the role of substrate cycle between TG and FFA between adipose tissue and liver in determining TG levels in liver, serum and adipose tissue[29]. TG secretion from liver to serum is reported to be reduced in rats fed high fat diets [30].The above effects as suggested by our study may be attributed due to inactivation of pancreatic lipase, principle enzyme responsible for the breakdown of fats and their absorption**.** The present study on potash alum decreased the activity of pancreatic lipase up to 42.45% at 14.00% aqueous solution. The administration of Potash Alum for 24 weeks resulted in a significant reduction in serum TG, TC and increased HDL level indicating their hypolipidemic activity.

CONCLUSION

This study provides clear evidence that Potash Alum is helpful in treating the HFD induced obesity. Anti-obesity action of Potash Alum in experimental animals may be partly mediated through delaying the intestinal absorption of dietary fat by inhibiting pancreatic lipase activity. The study evidently proved that Potash Alum exhibits a potent anti-obesity action and thus, supports its traditional usage. Moreover, it might help in preventing obesity complications and serve as good adjuvant in the present armamentarium of anti-obesity drugs. However, further exploration of the potash alum in terms of its toxicity, behavior as a therapeutic agent needs a detailed exploration.

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