

Effect of *Hiptage madablota* Gaertn. on High Fat Diet – Induced Obese Rats

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Abstract

The present study is designed to evaluate the anti-obesity activity of the roots of *Hiptage madablota* Gaertn against high fat diet-induced obese rats. In the ethanolic extract of *Hiptage madablota* (EEHM) total saponin content is also estimated by gravimetric method. Obesity is induced in albino rats by the administration of high fat diet for 40 days. Therefore, this study is accentuated to explore the efficacy of the ethanol extract of *Hiptage madablota* root at the dose of 100, 200 and 400mg/kg by oral. The anti-obesity activity is estimated in terms of food intake, body weight, lee index, serum lipids, atherogenic index, coronary risk index and brain serotonin level in rats. Preliminary phytoconstituents analysis revealed the presence of flavonoids, terpenoids, saponins, phenolic compounds and tannins. Animal received oral EEHM (100, 200 & 400mg/kg) for 40 days, exhibited a significant reduction of food intake, body weight, lee index, serum lipids, atherogenic index, coronary risk index and inversely increased the level of brain serotonin in rats. Thus, the present study indicates that *Hiptage madablota* root extract possessed significant anti-obese efficacy due to its hypophagic and hypolipidemic effects and provoke the brain serotonin level in rats fed on high fat diet.

Keywords: *Hiptage madablota*, Anti-obesity, Lee index, Serotonin, High fat diet.

1. Introduction

Nowadays, obesity has become a global health problem because it is accompanied with various metabolic syndromes such as arteriosclerotic disease, type 2 diabetes mellitus and fatty liver disease (Després and Lemieux, 2006). In obese-diabetic patients, the pancreas loses its function of releasing insulin and insulin resistance is developed due to the excessive deposition of fats in non adipose tissue, leading to either cell death or cell dysfunction (Semenkovich, 2006; Olefsky, 2008). Despite the management or control of obesity, anti-obesity drugs are limited and they prevent the fat absorption by inhibiting lipid breakdown in intestine (orlistat) or reducing the appetite by increasing the satiety and modulating the central nervous system (sibutramine and rimonabant) (Choi *et al.*, 2007). Moreover, these drugs produce severe cardiac and psychiatric side effects. Therefore, in recent years, there has been a great increase in the use of herbal drugs for the treatment of obesity (Shi and Burn, 2004).

Hiptage madablota Gaertn. (Malpighiaceae), native from India to the Philippines, is a vine like plant that is often cultivated in the tropics for its attractive and fragrant flowers. It can be trimmed to form a small tree or shrub or can be trained as a vine (Whistler, 2000). *Hiptage madablota* (*H. madablota*) root is cultivated in

India for medicinal purposes (Bailey and Bailey, 1976). Traditionally, the root of *H. madablota* used in the treatment of obesity and reduced the weight gain. The folklore's claim of the utility of this plant in treatment of obesity has not been scientifically evaluated. For this reason, the present study attempts to explore the effect of *H. madablota* root on energy balance disorders like obesity, hyperphagia, and hyperlipidemia. Therefore, the present study is carried out to investigate the anti-obesity efficacy of the ethanol extract of root of *H. madablota* (EEHM) in high fat diet induced obesity rats.

2. Material and Methods

2.1. Collection of Plant Material and Preparation of Extract

The roots of *H. madablota* were collected from Tirumala hills, Chittoor district of Andhra Pradesh, India in March 2011. The plant was authenticated by Dr. K. Madhava Chetty, Assistant Professor, Department of Botany, Sri Venkateswara University, Tirupati. The root of *H. madablota* was dried in shade and pulverized in the grinder-mixer to obtain a coarse powder, which was passed through the 60 mesh sieve. A weighed quantity (100g) of powder was subjected to continuous hot extraction with ethanol in soxhlet apparatus for 48

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hours. Then the extract was evaporated at reduced pressure using rotary evaporator until all the solvent has been removed to give an extract sample.

2.2. Preliminary Phytochemical Investigation of EEHM

The preliminary phytochemical investigation was performed using standard phytochemical tests for the presence of various phytoconstituents in the EEHM (Harbone, 1998).

2.3. Quantification of Total Saponins in EEHM

Total saponins were quantified in EEHM by Gravimetric method. Accurately weighed 2g of the EEHM in a beaker, added 150 ml ethanol and boiled on a water bath for 10-15 minutes. The ethanol layer filtered to another beaker. Extracted the precipitate with 100 ml of ethanol and boiled on water bath for 10-15 minutes. Above process is repeated twice (with 75 ml of ethanol). The filtrate were combined and concentrated to about 20ml. To this, 150 ml of dry acetone was added to precipitate the saponins. It was filtered and dried at 100°C for constant weight (Hudson and El-Difrawi, 1979). Percentage of total saponins is calculated by (Weight of residue/ Weight of sample taken)X100

2.4. Animals

Female albino Wistar rats (150-180g) were obtained from the animal house in Sree Vidyanikethan College of Pharmacy, Tirupati, Andhra Pradesh. Female albino Wistar rats (150-180g) were obtained from the animal house in Sree Vidyanikethan College of Pharmacy, Tirupati, Andhra Pradesh. The animals were maintained in a well-ventilated room with 12:12 hour light/dark cycle in polypropylene cages and fed with standard pellet diet (Hindustan lever limited, Bangalore) and water was given ad libitum. Animals were maintained under standard housing conditions (room temperature 24–27°C and humidity (60–65%). The experiments were performed after approval (Approval no: SVCP/IAEC/I-026/2011-12) of the protocol by the Institutional Animal Ethics Committee (IAEC) and were carried out in accordance with the current guidelines on the care of laboratory animals.

2.5. Acute Oral Toxicity Studies

Acute oral toxicity studies of EEHM were done as per the OECD guideline no. 423 (Acute toxic class method). Albino wistar rats (n=6) either sex selected and kept fasting for overnight providing only water. EEHM was administered orally at the dose level of 2000mg/kg by oral needle and observed for 14 days. The animals were observed for gross behavioral, neurological, autonomic and toxic effects at short intervals of time for 24 h and then daily for 14 days (OECD, 2002).

2.6. Induction of Experimental Obesity

The rodent feed was mixed to following ingredients to prepare high fat diet (HFD): Casein-20%, D, L methionine-0.3%, corn starch-15%, sucrose-27.5%, cellulose powder-5%, mineral mixture-3.5%, vitamin mixture-1%, choline bitartrate-0.2%, corn oil -9.9%, lard oil-17.6% (Vasselli *et al.*, 2005). The high fat diet was prepared, dried, powdered and administered every day

in morning to animals with water ad libitum. Weight gain was observed in rats on third day, therefore, confirming the development of obesity in rats. This diet was continued for 40 days.

2.7. Anti-Obesity Efficacy of EEHM in HFD- Fed Rats

Thirty six female Wistar rats (150-180g) were randomly divided into six groups of six animals each. The following schedule of dose, diet administration in experimental groups was followed: Group I animals received vehicle 0.2ml of 1% CMC and were considered as normal control; Group II animals received high fat diet + Vehicle 0.2ml of 1% CMC; Group III, IV and V animals received high fat diet + EEHM of 100, 200 and 400 mg/kg body weight, per oral, respectively; Group VI animals received high fat diet + Orlistat 50 mg/kg body weight, per oral for 40 days. The body rectal temperature was measured on day 41 in order to estimate thermogenesis. The food intake of each animal was measured every day. Body weight of animal was recorded to assess the weight gain (Vasselli *et al.*, 2005).

2.8. Determination of Adiposity Level in Normal and HFD- Fed Rats

The Lee index is used for measuring the adiposity level of animals because it is highly correlated with the percentage of the total body fat (Lee, 1929; Li *et al.*, 1998). The Lee's index is expressed as cubic root of body weight in grams divided by the naso-anal length in millimeters multiplied by 10⁴.

2.9. Estimation of Lipid Profile in Normal and HFD- Fed Rats

On day 41, the animals were sacrificed by cervical dislocation and the whole brain was dissected out for estimation of serotonin. Blood samples were collected by cardiac puncture and allowed to stand for 30 min at 37°C. The clear serum was separated at 2500 rpm for 10 min and was used for the estimation of Serum total cholesterol (TC), triglycerides (TG), HDL-C were measured with the help of commercially available diagnostic kits (Span Diagnostics, India). The serum low-density lipoprotein (LDL-C), very low-density lipoprotein (VLDL-C) levels, the atherogenic index and coronary risk index were calculated by Friedewald formula: VLDL-C = TG / 5; LDL-C = TC - (HDL-C + VLDL-C) (Friedewald *et al.*, 1972). The atherogenic index and coronary risk index were calculated from LDL-C/ HDL-C and TC/ HDL-C respectively (Abbott *et al.*, 1988; Alladi and Shanmugasundaram, 1989).

2.10. Estimation of Serotonin in Normal and HFD- Fed Rats

The striatum and hippocampus were separated from the brain and a standard procedure was followed for the estimation of serotonin by Spectrofluorimetry at 360-470 nm (Schlumpf *et al.*, 1974).

2.11. Statistical Analysis

The present research observations were signified as Mean ± Standard Error Mean. Statistical significance of dissimilarities amid the groups was evaluated by one way and multiple way analysis of variance (ANOVA)

followed by Dunnett's test. *P* values less than 0.05 were deliberated as significance.

3. Results

3.1. Phytochemical analysis and Quantification of Total Saponins of EEHM

The percentage yield of EEHM was found to be 6.50%w/w. The preliminary phytochemical analysis of EEHM showed the presence of various phytochemical constituents like glycoside, flavonoids, terpenoids, saponins, phenolic compounds and tannins. By the Gravimetric method, it was found that 18.50% of total saponins were found in EEHM.

3.2. Acute oral toxicity of EEHM

Acute oral toxicity studies revealed that EEHM is safe up to 2000mg/kg. There was no lethality or toxic reactions such as tremors, convulsions, salivation, diarrhea, lethargy, sleep and coma.

3.3. Efficacy of EEHM on body temperature of normal and HFD- fed rats

Table 1 represents the body temperature of HFD-fed rats. The body temperature is significantly ($p < 0.05$) increased after 0, 30, 60, 90, 180 min administration of EEHM when compared with group II animals' body temperature. EEHM (100 & 200 mg/kg bd.wt, per oral) treated groups exhibited ($p < 0.05$) slight increases in body temperature. EEHM (400 mg/kg, b.wt, per oral) treated animals showed rise ($p < 0.05$) in body temperature than standard drug orlistat (50 mg/kg bd.wt, per oral) treated animals.

3.4. Efficacy of EEHM on body weight and food intake of normal and HFD- fed rats

The body weight and food intake in the EEHM (100, 200 and 400 mg/kg bd.wt, per oral) were treated and vehicle treated groups were monitored for a 40-day treatment period. At the end of the study, the body weight and food intake of EEHM treated rats were

significantly ($p < 0.05$) lower than Group II animals. High doses of EEHM (400 mg/kg) significantly suppressed the weight gain and food intake in rats (Table 1).

3.5. Efficacy of EEHM on Lee's index of normal and HFD- fed rats

High fat diet caused a significant rise in Lee's index in group II rats. Orlistat (50 mg/kg) and EEHM (100, 200 and 400 mg/kg) treated rats showed a significant ($p < 0.05$) dose dependent reduction in Lee's index when compared to group II animals.

3.6. Efficacy of EEHM on serum lipids of normal and HFD- fed rats

High fat diet induces significant elevations of serum total cholesterol (TC), triglycerides (TG), LDL-C, VLDL-C in untreated group II rats. Oral treatment with orlistat (50 mg/kg) and graded dose of EEHM (100, 200 and 400 mg/kg) caused a significant ($p < 0.05$) reduction in the serum total cholesterol (TC), triglycerides (TG), LDL-C and VLDL-C of HFD- fed rats. Orlistat and EEHM (200 and 400 mg/kg) caused a significant improvement in HDL-C of HFD- fed rats (Table 3).

3.7. Efficacy of EEHM on Atherogenic and coronary risk index of normal and HFD- fed rats

Additionally, orlistat (50 mg/kg) and EEHM (100, 200 & 400 mg/kg) treated rats showed a significant ($p < 0.05$) reduction in atherogenic and coronary risk index when compared to HFD induced obese rats (Table 3).

3.8. Efficacy of EEHM on serotonin level in normal and HFD- fed rats

HFD- fed rats had a reduced level of serotonin in the brain when compared to vehicle treated group rats (Group I). Treatment with EEHM (100, 200 and 400 mg/kg, per oral) and orlistat (50 mg/kg, per oral) restored ($p < 0.01$) the decreased level of serotonin in the brain in a dose dependent manner (Figure 1).

Table 1. Efficacy of EEHM on body temperature of normal and HFD- fed rats

Grops	Treatment	Body temperature (min)					
		0	30	60	90	120	180
I	Vehicle 0.2ml of 1% CMC	36.59±0.1365	36.25±0.0322	36.05±0.1543	36.39±0.0821	36.28±0.0550	36.36±0.0877
II	High Fat Diet (HFD)	36.47 ±0.1156	36.58±0.0741	36.37±0.0684	36.50±0.0379	36.82±0.0310	37.27±0.0411
III	HFD + EEHM (100mg/kg, p.o)	36.33±0.0663	36.75±0.0471	37.23±0.0398 ^{*b}	36.82±0.1828 ^{*b}	37.26±0.0330 ^{*b}	37.28±0.0358 ^{*b}
IV	HFD + EEHM (200mg/kg, p.o)	36.46±0.1137	36.92±0.0214 ^{*b}	37.49±0.0612 ^{*b}	37.39±0.0582 ^{*b}	37.54±0.0254 ^{*b}	37.47±0.0344 ^{*b}
V	HFD + EEHM (400mg/kg, p.o)	36.48 ±0.1141	37.06±0.1842 ^{*b}	37.53±0.0989 ^{*b}	37.37±0.0610 ^{*b}	37.49±0.0618 ^{*b}	37.71±0.0735 ^{*b}
VI	HFD +Orlistat (50mg/kg, p.o)	36.44±0.1108	37.61±0.0579 ^{*b}	37.60±0.1103 ^{*b}	37.55±0.0325 ^{*b}	37.62±0.0878 ^{*b}	37.56±0.0752 ^{*b}

Values are mean ± SEM of six animals; Statistical significance test for comparisons was done by ANOVA, followed by Dunnett's test. Comparisons were made between: a) Group I vs Group II; b) Group II vs Group III, IV, V & VI; **p* value < 0.05. p.o : per oral.

Table 2. Efficacy of EEHM on body weight, food intake & Lee Index of normal and HFD- fed rats

Parameters	Group I: Vehicle 0.2ml of 1% CMC	Group II: High Fat Diet control	Group III: HFD + EEHM (100mg/kg,p.o)	Group IV: HFD + EEHM (200mg/kg, p.o)	Group V: HFD + EEHM (400mg/kg,p.o)	Group VI: HFD + Orlistat (50mg/kg,p.o)
Initial body Weight (g/rat)	186.83±1.939	188.5±2.335	189.17±1.641	187.00±2.129	189.33±1.833	189.17±2.167
Body Weight on 40 th day (g/rat)	212.50±1.928 ^{*a}	255.33±4.499	241.67±1.202 ^{*b}	229.33±2.578 ^{*b}	221.50±1.708 ^{*b}	210.67±3.283 ^{*b}
Weight gain (g/rat)	25.67±1.585 ^{*a}	66.83±2.926	52.50±1.784 ^{*b}	42.33±1.585 ^{*b}	31.67±0.9545 ^{*b}	23.33±1.116 ^{*b}
Food intake (g/rat/day)	21.67±0.6667 ^{*a}	31.50±0.7638	25.83±0.6009 ^{*b}	23.67±0.4944 ^{*b}	22.33±0.7601 ^{*b}	20.50±1.118 ^{*b}
Lee Index	244.34±2.359 ^{*a}	311.12±4.632	276.97±1.577 ^{*b}	252.12±0.9481 ^{*b}	245.98±2.033 ^{*b}	240.05±1.646 ^{*b}

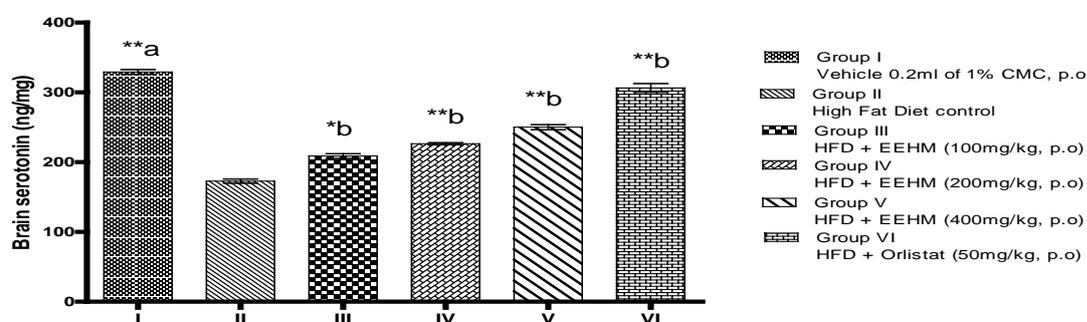
Statistical significance test for comparisons was done by ANOVA, followed by Dunnett's test; Comparisons were made between: a) Group I vs Group II; b) Group II vs Group III, IV, V & VI; **p* value < 0.05. p.o : per oral.

Table 3. Efficacy of EEHM on Serum lipids, Atherogenic Index & Coronary Risk Index of normal and HFD- fed rats

Parameters	Group I: Vehicle 0.2ml of 1% CMC	Group II: High Fat Diet control	Group III: HFD + EEHM (100mg/kg, p.o)	Group IV: HFD + EEHM (200mg/kg, p.o)	Group V: HFD + EEHM (400mg/kg, p.o)	Group VI: HFD + Orlistat (50mg/kg, p.o)
TC (mg/dl)	114.33±1.430 ^{*a}	231.50±3.085	160.33±2.376 ^{*b}	139.17±2.522 ^{*b}	121.67±3.085 ^{*b}	117.67±2.860 ^{*b}
TG (mg/dl)	113.17±1.721 ^{*a}	172.16±2.868	144.33±2.539 ^{*b}	133.67±1.944 ^{*b}	121.67±0.8819 ^{*b}	114.00±1.291 ^{*b}
LDL (mg/dl)	38.53±1.155 ^{*a}	164.73±3.838	93.80±2.309 ^{*b}	70.10±1.578 ^{*b}	53.83±2.735 ^{*b}	45.20±2.686 ^{*b}
VLDL (mg/dl)	22.63±0.3442 ^{*a}	34.43±0.5737	28.87±0.5077 ^{*b}	26.73±0.3887 ^{*b}	24.33±0.1764 ^{*b}	22.63±0.3844 ^{*b}
HDL (mg/dl)	53.17±1.352 ^{*a}	32.33±1.358	37.67±1.358 ^{*b}	42.33±1.022 ^{*b}	43.50±1.022 ^{*b}	49.67±1.282 ^{*b}
Atherogenic Index (AI)	0.73±0.0339 ^{*a}	5.15±0.2871	2.51±0.1246 ^{*b}	1.66±0.0372 ^{*b}	1.24±0.0662 ^{*b}	0.91±0.0580 ^{*b}
Coronary Risk Index (CRI)	2.15±0.040 ^{*a}	7.23±0.3298	4.28±0.1484 ^{*b}	3.29±0.0468 ^{*b}	2.80±0.0715 ^{*b}	2.37±0.0635 ^{*b}

TC - Total Cholesterol; TG - Triglycerides; VLDL - Very Low Density Lipoproteins; HDL - High Density Lipoproteins

Values are mean ± SEM of six animals; Statistical significance test for comparisons was done by ANOVA, followed by Dunnett's test. Comparisons were made between: a) Group I vs Group II; b) Group II vs Group III, IV, V & VI; **p* value < 0.05. p.o : per oral.

**Figure 1.** Efficacy of EEHM on Brain serotonin Level of normal and HFD- fed rats

Values are mean ± SEM of six animals; Statistical significance test for comparisons was done by ANOVA, followed by Dunnett's test. Comparisons were made between: a) Group I vs Group II; b) Group II vs Group III, IV, V & VI; **p* value < 0.05; ***p* value < 0.01. p.o : per oral.

4. Discussion and Conclusion

Obesity is a metabolic disorder which occurs due to an energy imbalance between an increased ratio of caloric intake and a decreased ratio of energy expenditure. Obesity is associated with disorders of hyperlipidemia, atherosclerosis and diabetes mellitus. In modern medicine, there is a great increase in the use of evidence-based complementary treatments like natural remedies in the management and prevention of obesity (Das and Maulik, 2006; Sharpe *et al.*, 2007).

In the present study, the anti-obesity efficiency of ethanolic extract of root of *H.madablota* is evaluated at 100,200 and 400mg/kg in normal and HFD-fed rats. Acute oral toxicity studies revealed that ethanol extract of *H.madablota* is found to be safe up to the dose of 2000mg/kg, p.o.

Body temperature of the HFD-fed rats was significantly ($p < 0.01$, $p < 0.05$) increased after 0, 30, 60, 90, 180 min administration of EEHM when compared with group II. EEHM has increased body temperature by overall stimulant & thermogenesis property because obesity is associated with defective thermogenesis. These mechanisms confirmed with rodents and dogs, several β 3-adrenoceptor agonists were shown to have potent thermogenic anti-obesity effects with classical adrenoceptor stimulation (Arch, 2008).

In case of body weight and adiposity level (lee index), animals which received extract suppressed the body weight gain and adiposity level (lee index) in a dose-dependent manner. Moreover, the previous studies (Haynes *et al.*, 2002; Arch, 1981) supported the body weight gain and adiposity level (lee index) was significantly decreased. In normal rats, the reference value of lee index is lower than 300. If the lee index values higher than 300, the rats classifies as obese (Bernardis and Patterson, 1968). EEHM significantly ($p < 0.01$) decreased the Lee index when compared to HFD control.

EEHM reduced food intake by hypophagic activity because obesity is associated with hyperphagia. High fat diet caused significant elevations of serum total cholesterol (TC), triglycerides (TG), LDL-C, VLDL-C in untreated group II. Oral treatment with orlistat (50 mg/kg) and graded dose of EEHM caused a significant ($p < 0.01$) reduction in the serum lipids on HFD- fed rats. EEHM may be attributed to lowering lipogenesis, enhancing lipolysis, suppressing appetite and decreasing lipid absorption (Shivaprasad *et al.*, 2014).

The atherogenic and coronary artery risk indices were potent and authentic indicator for cholesterol deposition into tissues. In human, the normal reference range of atherogenic index and coronary artery risk index should not be exceeding above 4 and 2.5, respectively (Lee *et al.*, 2013; Murray and Pizzorno, 1998). The results of the present study showed that the atherogenic and coronary artery risk indices were significantly decreased in EEHM treated HFD-fed rats. Thus strongly confirmed that EEHM exhibit hypolipidemic effects.

HFD-fed rats had a reduced level of serotonin in brain when compared to vehicle treated group rats (Group I). Treatment with EEHM and orlistat (50 mg/kg, p.o) restored ($p < 0.01$) the decreased level of the serotonin in

the brain in a dose-dependent manner. Various in-vivo studies have contributed to the understanding of the anti-obesity effects via the regulation of the serotonin level. A typical reduction of food appetite and increased serotonin availability were observed in all the weight control studies on both animal and human subjects. These were associated with reduced levels of total cholesterol, LDL, triglycerides, and increased HDL level and urinary excretion of fat metabolites (Chuah *et al.*, 2013).

Upon preliminary phytochemical studies of ethanolic extract revealed that the presence of glycoside, flavonoids, terpenoids, phenolic compounds and tannins and revealed that 18.5% saponins are present. Earlier studies reported that saponins and flavanoids mediate their hypophagic and hypolipidemic effects by reducing the food intake and inhibiting the lipid absorption or enhancing the enterohepatic excretion of cholesterol in the bile acid (Rajalakshmi *et al.*, 2004; Ruizc *et al.*, 2005; Dixit *et al.*, 2012).

In conclusion, oral administration EEHM, inhibit the food intake, weight gain, serum lipid profiles, atherogenic, coronary risk and Lee's index by hypophagic and hypolipidemic effects and elicit the brain serotonin level in HFD-fed rats. The presence of saponins is useful in the treatment of obesity and flavonoids, phenolic compounds & tannins have beneficial effects on potent hypolipidemic and anti-oxidant properties of EEHM. Further studies are needed not only to find the exact mechanism of action, but also to isolate and characterize the active phytocompounds for its effects.

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Declaration of Interest

The authors report no conflicts of interest. The authors alone are responsible for the content and writing of the paper.

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