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Antihyperlipidemic Effects of Silver Nanoparticles Synthesized from *Ventilago maderaspatana* Leaf Extract on Streptozotocin-Induced Albino Rats

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ABSTRACT

An abnormally high presence of lipid in human blood is a prelude for the emergence of cardiovascular complications. In this study, effort was made to elucidate the effect of silver nanoparticles (AgNPs) produced through the help of *Ventilago maderaspatana* on the hyperlipidemic conditions in streptozotocin (STZ)-induced Wistar rats. AgNPs were synthesized biologically using ethyl acetate leaf extract of *V. maderaspatana*. The synthesis of AgNPs was confirmed when the color of the solution turned dark brown following the addition of *V. maderaspatana* ethyl acetate leaf extract. To confirm further, UV-Vis spectroscopy analysis was conducted which gave a peak at 430 nm. The results obtained from the FT-IR studies shows that the compounds in the plant extract may have influenced the formation of AgNPs. The result obtained from further characterization showed that the synthesized nanoparticles were spherical and ranged between 10-50 nm. The XRD study indicates crystal nature of the particles with the size of 50 nm. After the injection of STZ, the lipid profiles were altered abnormally. This can be found in group II rats (hyperlipidemic control) which had the highest level of serum total cholesterol (189.1 ± 0.80 mg/dL), triglyceride (177.9 ± 0.88 mg/dL), VLDL-c (42.5 ± 1.80 mg/dL), and LDL-c (55.2 ± 3.83 mg/dL) with the exception of HDL-c which was found low (10.4 ± 1.04 mg/dL). However, hyperlipidemic groups treated with AgNPs recorded low levels of cholesterols. Based on this, it can be concluded that biosynthesized AgNPs could be helpful in lowering cholesterol level.

Keywords: Hyperlipidemia, Cardiovascular diseases, Antihyperlipidemic activity, *Ventilago maderaspatana*, Silver nanoparticles, Biosynthesis.

Introduction

In diabetic patients, hyperlipidemia is a condition that is commonly found. It is linked to the increase in vascular disease seen in diabetic patients. As a metabolic disorder, hyperlipidemia is associated with an elevated level of lipid and lipoproteins.[1,2] Very-low density lipoprotein cholesterol (VLDL-c) and chylomicrons, which transport endogenous and exogenous triglycerides, are broken down by lipoprotein lipases. In a state of insulin deficiency, there is usually a decrease in the activities of lipoprotein lipases, a common cause of hyperlipidemia in poorly controlled diabetes.

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The high presence of total cholesterol (TC) with corresponding low amount of low-density lipoprotein cholesterol (LDL-c) and triglyceride (TG) are associated with the pathogenesis of cardiovascular disease through the blockage of blood flow in heart whereas high-density lipoprotein cholesterol (HDL-c) increase prevents plaque formation in the blood vessels.[3,4]
Nanotechnology is an emerging and interdisciplinary field that is formed by the convergence of three sciences namely, physics, chemistry, and biology. In nanotechnology, the surface area to volume ratio and quantum behavior are the most important characteristics which can be seen at the nanoscale.[5] The recent advances in the search for novel nanoparticles of medical use, the noble metallic nanoparticles have gained significant interests.[6] Silver nanoparticles (AgNPs) particularly has attracted lots of interests and has been applied in many areas of medical field for their antibacterial properties amidst their good electrical conductivity, chemical stability, catalytic properties.[7,8] But the toxic effects of chemically synthesized AgNPs on rat neuronal and liver (BRL3A) cells,[9,10] murine stem cells,[11] and human lung epithelial cells,[12] has raised some questions and limited its acceptance. The *In vivo* toxicity studies of AgNPs in rats has demonstrated that the administration of AgNPs are consequently found in the blood and affects several organs including the lung, liver, kidney, intestine, and brain due to their toxic chemical effects.[13-16] However, the toxic effects of AgNPs has been found to be lower in

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AgNPs that are synthesized using plant extracts,[17] which then solves the problem of toxicity. The potentials of plants to serve useful purpose for the green synthesis of nanoparticles is hinged on the presence of plant secondary metabolites such as phenol, terpenoids, ketones, aldehyde, and flavones which has the propensity to act as reducing agents, stabilizers or both.[18-20]
Ventilago maderaspatana, family Rhamnaceae is a medicinal plant used in the traditional medical settings for the treatment of diabetes.[21,22] Thus, study was focused on evaluating the biological effects of AgNPs synthesized using *V. maderaspatana* leaf extract on lipid profile of Wistar rats.

Materials and Methods

Collection of plant materials and preparation of extract
The selected medicinal plant was collected from Kolli hills located at Latitude 11.248514 and longitude 78.338707, Namakkal District, Tamilnadu, India in the month of January 2017. It was authenticated by Dr. S. Soosairaj, (Specimen access No. SJCBT2112). The leaves of the *V. maderaspatana* were collected and brought to the laboratory, dried under shade after cleaning, and ground into powder. Extraction was done by Soxhlet method using 500 mL ethyl acetate.

Biosynthesis of AgNPs
About 10 ml of ethyl acetate leaf extract of 0.1 mg/mL *V. maderaspatana* was added drop-wise to 90 ml of 0.1M silver nitrate solution and heated up to 50ºC with continuous stirring (500 rpm) for 30 minutes and then left to stand in the dark at ambient temperature for 24hrs. The colour changes was observed and recorded.[23,24]

Characterization of biosynthesized AgNPs
The biosynthesized nanoparticles was characterized according to the method described by Zhang *et al*.[7] Ultraviolet–visible spectroscopy (UV-VIS) and fourier-transform infrared spectroscopy (FT-IR) was used to investigate the optical properties of the synthesized AgNPs whereas the shape, size, and the chemical properties of AgNPs were examined using scanning electron microscope (SEM), transmission electron microscopy (TEM) and energy dispersive X-ray analysis (EDAX).

UV-Vis Spectroscopy
Perkin Elmer spectrophotometer at St. Joseph's College, Trichy, India was used to confirm AgNPs formation during synthesis through peak analysis.[25]

FT-IR Spectroscopy
The functional groups responsible for the biological activities of *V. maderaspatana* extract used in the synthesis of the AgNPs was confirmed by FT-IR analysis.[25]

Scanning electron microscopy
The nature of the shape of the synthesized AgNPs was evaluated using SEM Viga3 Tescan scanning electron microscope.[26]

Transmission electron microscopy
The size, morphology, and chemical composition of the AgNPs biologically synthesized were analyzed by JEOL JEM 2100 High-Resolution TEM.[25]

Dynamic light scattering
Dynamic light scattering Zeta sizer Micrometric Nano plus model analyser was used to determine the particles size distribution through the dynamic fluctuations measurement of light scattering intensity.[25]

X-ray diffraction and energy dispersive X-ray analysis
The X-ray diffraction (XRD) measurement of the biosynthesized AgNPs was carried out using Rigaku Ultima III XRD for powder and thin films. EDAX was done following standard protocol.[25]

Biochemical studies
Streptozotocin (STZ) administration for Diabetes Induction
Diabetes was induced from the administration of 50 mg/kg of body weight of STZ intraperitoneally to albino rats that were fasted overnight.[27] After 48 hours of injection, the rats with blood glucose levels above 250 mg/dLwere said to be diabetic and were selected for the study. Ethical approval was obtained from the Institutional Animal ethical Committee, Bharathidasan University, Tiruchirappalli (BDU/IAEC/2017/NE/43) for conducting this research work.

Experimental set-up
Wistar albino rats used in this study were divided into 5 groups of 6 rats each:

Group I - Normal control
Group II – Hyperlipidemic control
Group III – Treated with 10 mg/kg b.w. of AgNPs
Group IV – Treated with 20 mg/kg b.w. of AgNPs
Group V – Treated with a standard 5mg/kg b.w glibenclamide (standard drug)

The drug was administered orally for 15 days in which on the 16th day. Choice of dose used was based on the LD_{50} value reported in the work of Adeyemi *et al*.[28] After administration, blood sample were collected and serum was separated for biochemical evaluation following animal sacrifice.

Antihyperlipidemic activity determination
The lipid profile (serum TC, TG, HDL, LDL, VLDL cholesterol) was evaluated using commercial kit for antihyperlipidemic activity.[29]

Statistical analysis
All the parameters studied were subjected to statistical analysis using SPSS statistical package (Version. 21). The values were expressed as mean ± SE. One-way ANOVA and SNK post hoc test was used for analyzing the impact of the treatment of *V. maderaspatana* mediated AgNPs on hyperlipidemic conditions of the diabetic rats at $p<0.005$.

Results and Discussion

Hypercholesterolemia is an abnormal condition characterized by elevated serum TG, TC, and LDL-c levels which could result in the development of cardiovascular diseases.[30,31] Animal tissues synthesize cholesterol which plays a vital role in the equilibrium of membrane structures because of its flexible planar structure and many other functions. However, the increase in the level of cholesterol is usually a cause for concern. In this study, we biosynthesized AgNPs using *V. maderaspatana*. The medicinal attributes of AgNPs have been extensively reported.[32,33] This was explored. The result obtained from the UV spectrum analysis of the biosynthesized AgNPs showed a maximum absorbance peak at 430 nm which confirmed the formation of AgNPs (Figure 1). For the FT-IR analysis, the several bands that appeared in the IR spectrum shows stretching vibration of -OH alcohols or phenols -NH amines, C=C stretch; alkenes, C-H rock; alkanes, C=O aromatic CH, aromatic CH and CH; alkene bends or C-Br or C-Cl groups that are responsible for the synthesis of AgNPs (Figure 2).
In the UV analysis at 430nm, an outstanding peak was observed which serves as a confirmation of AgNPs synthesis after the introduction of the leaf extract of *V. maderaspatana*. Previous studies suggested that a peak that occur between 410 and 450nm is the peak of AgNPs synthesis.[34] The functional groups in the leaf extract of *V. maderaspatana* play a role in stability/capping of AgNPs.[35,36] The plane of XRD from this result is in line with standard silver values. The average crystallite of AgNPs is ~25 nm.[37,38]
The DLS result revealed that the particles obtained were aggregated mixtures of sizes ranging from 10 to 50 nm, as shown in Figure 3. Twenty nanometer (20 nm) is the average particle diameter.
The SEM and HRTEM study showed that the AgNPs were spherical and the size of the particles ranges from 10 to 50 nm while the EDAX

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study revealed the presence of AgNPs in the test sample (Figure 4(A) - (D)). The XRD results were recorded from 20 to 80. Four strong reflections *viz.*, 37.86, 43.78, 64.87 and 77.61 were recorded in the planes of (1 1 1), (2 0 0), (2 2 0) and (3 1 1), respectively (Figure 4(D)).

The results obtained from the assessment of lipid levels were presented in Table 1. From the result, it was found that there was an increase in serum total cholesterol level (189.1 ± 0.80 mg/dL) in group II (hyperlipidemic control). The serum total cholesterol significantly decreased after the oral administration of AgNPs at different concentrations of 10 and 20 mg/kg b.w (Group III, 68.3 ± 3.45 mg/dLand Group IV, 51.1 ± 1.11 mg/dl) and standard drug (Group V, 71.4 ± 0.81 mg/dL) when compared to the control at $p<0.005$. Group I had a cholesterol level of 66.1 ± 2.81 mg/dLwhich is significantly different from group II at $p<0.005$. The triglyceride level was found high in group II (177.9 ± 0.88 mg/dL). After treatment with different concentrations of AgNPs (Group III, 98.5 ± 2.21 mg/dLand Group IV, 86.7 ± 6.50 mg/dL) and standard drug (Group V, 96.3 ± 1.82 mg/dL), the elevated level of TG was significantly reduced at $p<0.005$ when compared to Group II while Group I had the least triglyceride level of 80.8 ± 0.87 mg/dL. A high level of LDL was observed in Group II – hyperlipidemic control (55.2 ± 3.83 mg/dL). This value is statistically different in mean at $p<0.005$ for the groups administered AgNPs (Group III, 21.2 ± 2.31 mg/dL and Group IV, 14.0 ± 0.60 mg/dL) and standard drug (Group V, 11.9 ± 0.90 mg/dL). No significant difference was found between group I (14.4 ± 0.48 mg/dL) and group IV. A high level of VLDL was observed in the untreated diabetic rat (Group II, 42.5 ± 1.80mg/dL) which significantly reduced in groups treated with AgNPs (Group III, 24.6 ± 2.76 mg/dL and Group IV, 18.8 ± 0.94 mg/dL) and standard drug (Group V, 13.8 ± 0.85 mg/dL). Group I had a VLDL level of 14.3 ± 0.59 mg/dL. HDL level was found low in untreated diabetic rats (Group II, 10.4 ± 1.04 mg/dl) but significantly higher in AgNPs treated diabetic rats (Group III, 18.8 ± 2.01 mg/dL and Group IV, 22.7 ± 2.44 mg/dL) and standard drug-treated rats (Group V, 25.3 ± 1.25 mg/dL) at $p<0.005$). Group I had an HDL level of 21.7 ± 1.40 mg/dl. Furthermore, the SNK test revealed some similarities in the biochemical parameters of the group treated with AgNPs and gilbeclanmide (standard drug) (Table 2).

In diabetic rats induced with STZ, the TC was found to increase even when cholesterol biosynthesis has decreased. This is in line with what Gylling and Miettinen stated.[39] The elevated serum LDL is a risk factor linked to the development of coronary heart disease. In this study, after the administration of STZ, an elevated levels of TC, LDL, TG, and VLDL were observed. This can be attributed to the deficiency of insulin. This result coincided with what was previously reported by Rana *et al.*[40] and Andersson *et al.*[41]

The abnormal levels of serum lipid could be linked to abnormal insulin action probably because of the lack of regulation of lipolytic hormone actions on fat depots. In a healthy state, insulin has the ability to activate lipoprotein lipase which then hydrolyses triglycerides. However, in a diabetic state of insulin deficiency, the enzyme lipoprotein lipase is not activated which then result in hypertriglyceridemia,[42] or even hypercholesterolemia due to metabolic abnormalities that occur sequentially.[43]

The trend observed between the low levels of TG, TC and LDL levels compared to increase in the levels of HDL at $p<0.05$ may have been possible due to the increase in insulin levels which might have triggered increase in the enzymatic activity of lipoprotein lipase.[44,45] Again, the effects of AgNPs on biological system may have been possible due its size range, 10 – 50 nm which can facilitate movement across the cell membrane.[46]

In this study, we also found that *V. maderaspatana* mediated AgNPs significantly reduced high levels of serum TGs. This suggests that biologically, AgNPs could be helpful in the restoration of abnormal TG levels. The ability of AgNPs to restore abnormal TG levels is linked to the increased stimulation of the lipolytic activity of plasma lipoprotein lipase.[47,48] Again, the decrease in serum VLDL levels observed in groups administered the synthesized AgNPs may be attributed to the decrease in fatty acid re-esterification and, consequently, a decrease in triacylglycerol secretion by the liver.[49]

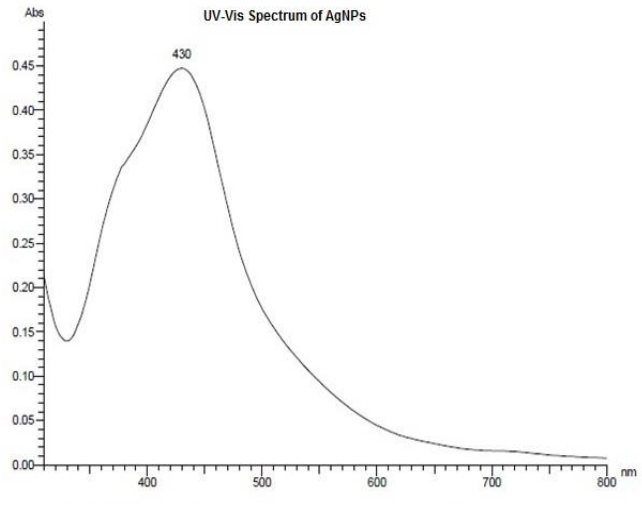

Figure 1: UV-Visible spectroscopy study

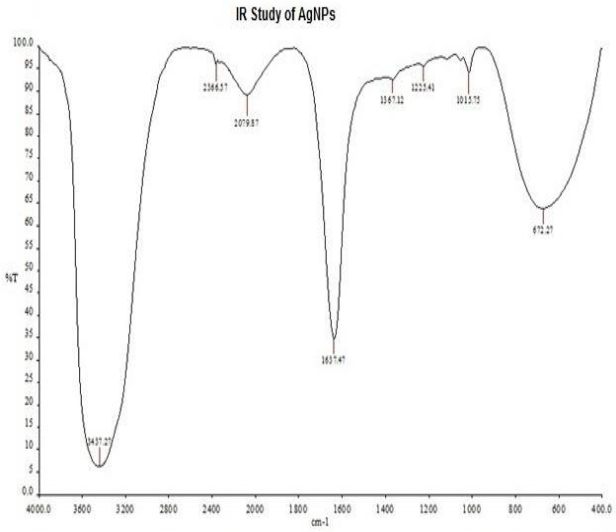

Figure 2: FT-IT spectrum of biosynthesized AgNPs

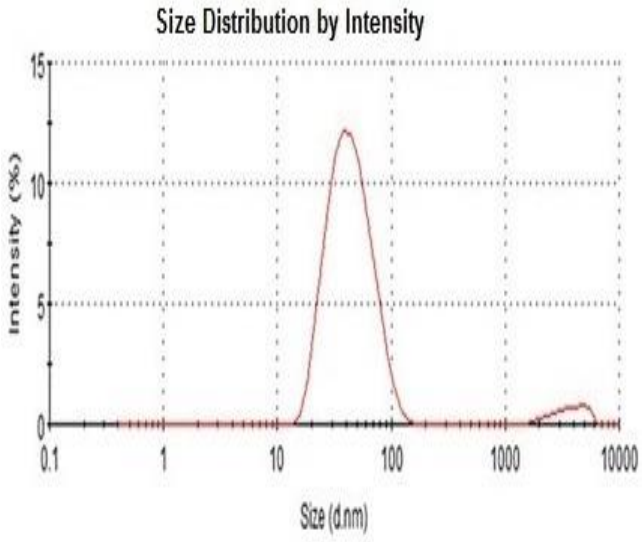

Figure 3: Dynamic light scattering study of biosynthesized AgNPs

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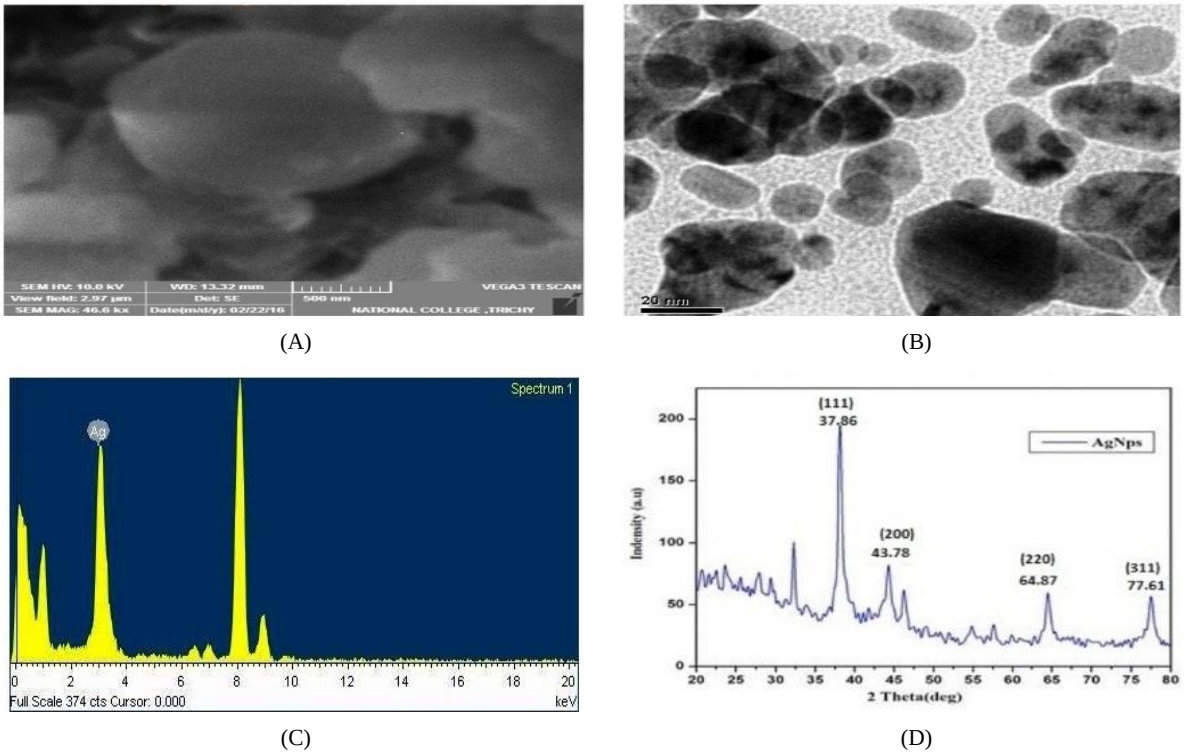

Figure 4: (A) SEM image, (B) HRTEM image, (C) EDAX spectrum, and (D) RXD spectrum study of biosynthesized AgNPs

Table 1: Effect of *V. maderaspatana* mediated AgNPs treatment on lipid profile of STZ induced albino rat model (mg/dl)

Groups	Parameters				
	Total cholesterol	**Triglycerides**	**HDL**	**LDL**	**VLDL**
Group I	66.1 ± 2.81	80.8 ± 0.87	21.7 ± 1.40	14.4 ± 0.48	14.3 ± 0.59
Group II	189.1 ± 0.80	177.9 ± 0.88	10.4 ± 1.04	55.2 ± 3.83	42.5 ± 1.80
Group III	68.3 ± 3.45	98.5 ± 2.21	18.8 ± 2.01	21.2 ± 2.31	24.6 ± 2.76
Group IV	51.1 ± 1.11	86.7 ± 6.50	22.7 ± 2.44	14.0 ± 0.60	18.8 ± 0.94
Group V	71.4 ± 0.81	96.3 ± 1.82	25.3 ± 1.25	11.9 ± 0.90	13.8 ± 0.85

Results were presented as Mean ± SEM of triplicate determination.

Table 2: Results of SNK post hoc test show the variations and similarities in lipid profile of *V. maderaspatana* mediated AgNPs treated groups

Parameter	**Student Newman- Kelus Post hoc test Groups (subset for alpha = 0.005)**				
Cholesterol	51.1 ± 1.11	66.1 ± 2.81	68.3 ± 3.45	71.1 ± 0.81	189.1 ± 0.80
	Gr IV	Gr I	Gr III	Gr V	Gr II
TG	80.8 ± 0.87	86.7 ± 6.50	96.3 ± 1.82	98.5 ± 2.21	177.9 ± 0.88
	Gr I	Gr IV	Gr V	Gr III	Gr II
HDL	10.4 ± 1.04	18.8 ± 2.01	21.7 ± 1.40	22.7 ± 2.44	25.3 ± 1.25
	Gr II	Gr III	Gr I	Gr IV	Gr V
LDL	11.9 ± 0.90	14.0 ± 0.60	14.4 ± 0.48	21.2 ± 2.31	55.2 ± 3.83
	Gr V	Gr IV	Gr I	Gr III	Gr II
VLDL	13.8 ± 0.85	14.3 ± 0.59	18.8 ± 0.94	24.6 ± 2.76	42.5 ± 1.80
	Gr V	Gr I	Gr IV	Gr III	Gr II

Horizontal lines connect similar mean.

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Conclusion

V. maderaspatana leaf ethyl acetate extract helped in the synthesis of AgNPs which are within a desired range of 10 – 50 nm. The elevated levels of serum lipids such as total cholesterol, triglycerides, LDL, and VLDL were reduced after the treatment of AgNPs whereas the level of HDL increased. On the basis of the result, it is concluded that the biosynthesized AgNPs possess antihyperlipidemic activity against STZ-induced hyperlipidemic condition in diabetic rats which might be useful for the treatment of cardiovascular diseases after advanced clinical evaluation.

Conflict of interest

The authors declare no conflict of interest.

Authors' Declaration

The authors hereby declare that the work presented in this article is original and that any liability for claims relating to the content of this article will be borne by them.

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