International Journal of Pharmacology

ISSN 1811-7775

science alert

ansinet
Asian Network for Scientific Information

OPEN ACCESS International Journal of Pharmacology

ISSN 1811-7775
DOI: 10.3923/ijp.2023.25.33

Research Article
Cerebroprotective Role of Stigmasterol Against the Progression of Experimentally Induced Intracranial Aneurysms in Rats

[1,2]Haijun Mei and [1,3]Xiaoqiang Li

[1]Department of Vascular Surgery, Second Affiliated Hospital, Soochow University, 1055 Sanxiang Road, Suzhou, Jiangsu, China
[2]Department of Vascular Surgery, Affiliated Hospital of Nantong University, 20 Xisi Road, Nantong, Jiangsu, China
[3]Department of Vascular Surgery, Nanjing Drum Tower Hospital, 321 Zhongshan Road, Nanjing, Jiangsu, China

Abstract

Background and Objective: Cerebral ischemia is a medical condition caused by myocardial infarction, a coronary artery bypass graft or reversible acute hypotension that can cause serious damage or death. Intracranial aneurysms (IAs) are compromised brain artery walls that can rupture and cause cerebral haemorrhage. Stigmasterol is an important plant sterol and has good therapeutic properties. The present research reveals that stigmasterol has cerebroprotective properties against the growth of artificially produced intracranial aneurysms in rats. **Materials and Methods:** In this investigation, the intracranial cerebral aneurysm was stimulated using $CaCl_2$ in the Wister male albino rat model. After stimulating the aneurysm, smooth muscle cells were separated and treated with 30 μM stigmasterol for 48 hrs. **Results:** In comparison to the controls, the IA mice given stigmasterol performed better neurologically. Furthermore, stigmasterol therapy enhanced the biomarkers TNF-α, CCR8, IL1B, MT2A, and PIM3, which were all upregulated in IA-induced rats. Cells from animals pre-exposed to stigmasterol also exhibited oxidative stress resistance, as indicated by high mitochondrial membrane prospects and low ROS levels. Additionally, stigmasterol treatment decreased pro-inflammatory cytokines such as TNF-α, IL-13, IL-4 and IL-1. **Conclusion:** The current study's outcomes indicated that stigmasterol has a potential efficacy in enhancing acute brain function in IA and it might be a lead molecule for IA-related therapeutics.

Key words: Intracranial cerebral aneurysm, stigmasterol, necrobiosis, phytochemicals, anti-osteoarthritic, hypoglycemic, anti-mutagenicity

Citation: Mei, H. and X. Li, 2023. Cerebroprotective role of stigmasterol against the progression of experimentally induced intracranial aneurysms in rats. Int. J. Pharmacol., 19: 25-33.

Corresponding Author: Xiaoqiang Li, Department of Vascular Surgery, Second Affiliated Hospital, Soochow University, 1055 Sanxiang Road, Suzhou, Jiangsu, China Tel/Fax: +86-13678544635

Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Intracranial aneurysm (IA) is a severe cerebrovascular condition with significant morbidity and mortality that implies an outward bulging of the artery wall[1]. The IA is the characteristic of cerebral artery dilatation, producing pouch-like structures at the branching site. According to a statistical survey, 2-5% of the global population is susceptible to being impacted by IA[2]. However, in many cases, the incidence of IA is unnoticed in their lifetime. Delayed symptoms are frequently identified after aneurysm pouches rupture, leading to subarachnoid haemorrhage (SAH), which leads to a severe mortality rate annually[3]. The SAH is most prevalent in middle-aged people, influencing most of the working population[4]. The known high-risk factors include smoking, excessive consumption of alcohol, lifestyle changes and blood pressure[5]. Environmental and genetic factors also influence IA. Chronic hypertension may lead to IA formation and be regarded as a risk factor by exerting continuous stress on vascular walls[6]. Despite the recent advancement of therapeutic and diagnostic criteria, SAH remains fatal in 65% of cases observed and is remarkably disabling in 50% of surviving patients[2,7]. Effective and non-invasive pharmacological therapies to avoid rupture are still uncertain in clinical practice. Furthermore, the current lack of knowledge of the strategies and mechanisms associated with the creation, advancement and rupture of IA makes addressing its consequences difficult. Several studies using a series of animal models demonstrated the pathogenesis of IA and identified the risk factors, but the inherent pathways remain unclear.

Stigmasterol is a very common plant sterol[8]. It has demonstrated anticancer, anti-osteoarthritic, hypoglycemic, antimutagenic, antioxidant, anti-inflammatory and anti-nociceptive activities[9-14]. A study demonstrated and reported that stigmasterol could be used as a food additive and the anti-mutagenicity and lack of genotoxicity of its polyester are well-documented[15]. In a scopolamine-induced memory defacement experiment, adult animals were given stigmasterol, which improved memory by increasing the cholinergic neurotransmission system[16].

The Central Nervous System (CNS) is a cholesterol-enriched key organ. The CNS stimulates cholesterol metabolism and all forms of cholesterol are synthesized *in situ*. The inability to maintain cholesterol homeostasis throughout synthesis, transport and catabolism results in severe neurological disorders such as Alzheimer's disease, multiple sclerosis and amyotrophic lateral sclerosis. *In vivo* animal models enable the examination of hypotheses relevant to pathogenesis and to evolve of therapeutic strategies by providing a chance to identify the early stages of the diseases[17]. The use of stigmasterol in this study was stimulated by scientific evidence for its protective role. However, observations of its effect on the development of intracranial aneurysms are very limited[18]. Hence, the present investigation was conducted to evaluate its protective efficacy using *in vivo* animal models.

MATERIALS AND METHODS

Study area: The present study was carried out in the Second Affiliated Hospital of Soochow University in March to June, 2022.

Animal protocol and chemical drugs: In this study, a total of 18 Wister male albino rats were chosen and procured from the Public Animal Health Department, (Tongji Hospital, Tongji Medical College, China). During the testing period, test animals were isolated, in wide, clean cages with a constant temperature of 23±1°C and exposed to a 12 hrs dark-light sequence. Animal experiments were followed by the guidelines of the guide for the care and use of laboratory animals. The institutional ethics review board approved this study (Reg. No. 32184/2022/CPC/FTULC/17.03.2022). Stigmasterol and other reagents, including vehicle solutions, 5% Tween, 20% polyethylene glycol and 75% saline, are obtained from Sigma-Aldrich (St Louis, MO, USA).

Experimental setup: For the convenience of experimental treatments, animals were grouped into three (n = 6).

Group I: Maintained separately as a control (an intracranial aneurysm)

Group II: Maintained as an intracranial aneurysm induction group (IA)

Group III: Fed with 30 μM stigmasterol orally before IA induction

Stimulation of cerebral aneurysm: Pentobarbital sodium (35 mg kg^{-1} b.wt.) was used to sedate the test animals, which were connected to a rodent ventilator. The test animals were sedated by pentobarbital sodium (40 mg kg^{-1} b.wt.) and which is associated with a rodent ventilator. The cerebral artery was identified and treated with 0.5 mL $CaCl_2$ for 20 min using a pre-soaked gauze applicator (1.0×0.5×0.2 cm^3), treated rats were allowed with saline for 20 min. The gauze was then detached and the rats were harboured in standard laboratory conditions and allowed access to feed and water[19]. To demonstrate the protective effect of stigmasterol beyond *in vivo*, cells that were exposed to the drug *in vivo* were separated and cultured *in vitro* to show any potentiated

Table 1: List of primers used in this study

Gene	Primer	Sequence	Annealing
TNF-α	F	CGGAATGTCGATGCCTGAGT	57
	R	GGGAACAGTCTGGGAAGCTC	
CCR8	F	CCTCTACGTCGGGAGACAGA	58
	R	TAAAGAAGGGTGGCACTGGTC	
IL1β	F	CGCTTGAGTCGGCAAAGAAA	59
	R	GGCCTCCAGGTCATCTTCAG	
MT2A	F	GGCTCCTGCAAATGCAAACA	59
	R	TACACCATTGTGAGGACGCC	
PIM3	F	GCCCTGGATACTGATGACGG	57
	R	GAGCAGCGTTCAAAAAGGCA	
GAPDH	F	AATGGGCAGCCGTTAGGAAA	59
	R	GCGCCCAATACGACCAAATC	

ability due to drug pre-exposure. One of the animals was separated for a 4 weeks observation to study the *in vivo* changes related to IA and stigmasterol. Neurological functions were evaluated at the end of the trial. Standard methods were used to assess the neurological functions of performance (spontaneous movement, forelimb suspension, locomotor activity test and cliff avoidance reflex tests).

Quantitative Real-Time RT-PCR: Cerebral wall cells were examined for particular mRNA expression utilising real-time PCR analysis for the identification of specific biomarkers of IA. RNA was extracted using Qiagen commercial assay kits. An iScript cDNA synthesis kit was used to convert the same quantity of RNA to cDNA. Real-time RT-PCR was performed by SYBR green-labelled polymerase enzyme and particular primers (Table 1) were used for the genes. The CT values were used to quantify the mRNA transcript levels and the comparative CT technique (ΔΔCT), which uses GAPDH as an endogenous control. It was used to determine the fold increase in gene expression.

Separation of smooth muscle cells: Smooth muscle cells were extracted from experimental animals' aneurysmal and non-aneurysmal arteries. The cells from the aneurism section were surgically separated and maintained in PBS with antibiotics such as penicillin and streptomycin. Then smooth muscle cells split according to the procedure of Leik *et al.*[20]. The obtained cells were inoculated in DMEM (Dulbecco's Modified Eagle's medium), comprised of FBS (Fetal Bovine Serum 10%), which also contains antibiotics such as penicillin and streptomycin.

Reactive oxygen species (ROS) release and mitochondrial membrane potential (MMP) assessment: The major response mechanism in cellular dysfunction is the production of ROS with altered MMP. To measure ROS release, cells (1×10^5) cultured on coverslips were treated for 15 min at 37°C with the oxidant-sensitive fluorescent probe DCFHDA (10 μM). A fluorimeter was used to measure the quantity of fluorescence generated at excitation and emission wavelengths of 488 and 530 nm, respectively. For MMP assessment, 5 μL of JC-1 (200 μM) dye was maintained for 20 min. The reading was taken at Ex/Em: 535/595 nm for JC-1 aggregates and 485/535 nm for JC1 monomers.

Apoptosis marker assays: Caspase-3 and -9 activity, as well as cytochrome c release, were measured using commercial assay kits (Abcam Inc., USA).

ELISA measurements: The production of several cytokines was measured using commercial Test kits (Fine Biotech, China) as per the manufacturer's protocols.

The release of various cytokines was assessed using commercial ELISA kits as per the manufacturer's instruction (Fine Biotech, China). Cells were isolated and homogenised using the buffer given with the test kits and the levels of certain cytokines such as TNF-α, IL-13, IL-4 and IL-1β were measured.

Statistical analysis: The outcome data value is given as the Mean±Standard error. A One-way ANOVA was used to examine the data. The assessment medians of the gastric lesion were followed by the 25th and 75th percentiles.

RESULTS

Effects of stigmasterol on neurological functions: This study employed Wistar rats to examine the protective effects of stigmasterol against intracerebral aneurysms. In comparison to control animals, the neurological performance of IA animals was substantially reduced ($p<0.01$) in neuronal screening assessments such as spontaneous movements assay, forelimb suspension, locomotor activities and cliff avoidance. However, the animals that obtained stigmasterol pre-treatment, on the other hand, recovered from neurological performances, indicating that the treatment can protect the proper functioning of brain tissues (Fig. 1a-d).

Effects of stigmasterol on marker genes: The qRT-PCR expressions of biomarker genes before and after IA and stigmasterol administration were depicted in Fig. 2a-e. The TNF-α (2.2-fold), CCR8 (2.2-fold), IL1B (2.2-fold), MT2A (2.2-fold) and PIM3 (2.2-fold) were observed to be elevated after IA induction compared to control animals. However, these genes showed a substantial decrease ($p<0.01$) in expression in stigmasterol-treated animals, indicating that the drug-induced reversal of IA is most likely due to activity in downstream signalling (Fig. 2a-e).

Effect of stigmasterol on ROS production, mitochondrial membrane potential: The key event of the development of cellular apoptosis is increased oxidative stress and extending its influence causes an undesirable effect such as cell death. Cells isolated from animals and cultured *in vitro* showed increased ($p<0.01$) ROS levels in the IA group with decreased mitochondrial membrane potential in the current study. On the other hand, cells from the stigmasterol-treated rats had significantly reduced levels of ROS and better mitochondrial membrane potential (Fig. 3a-b).

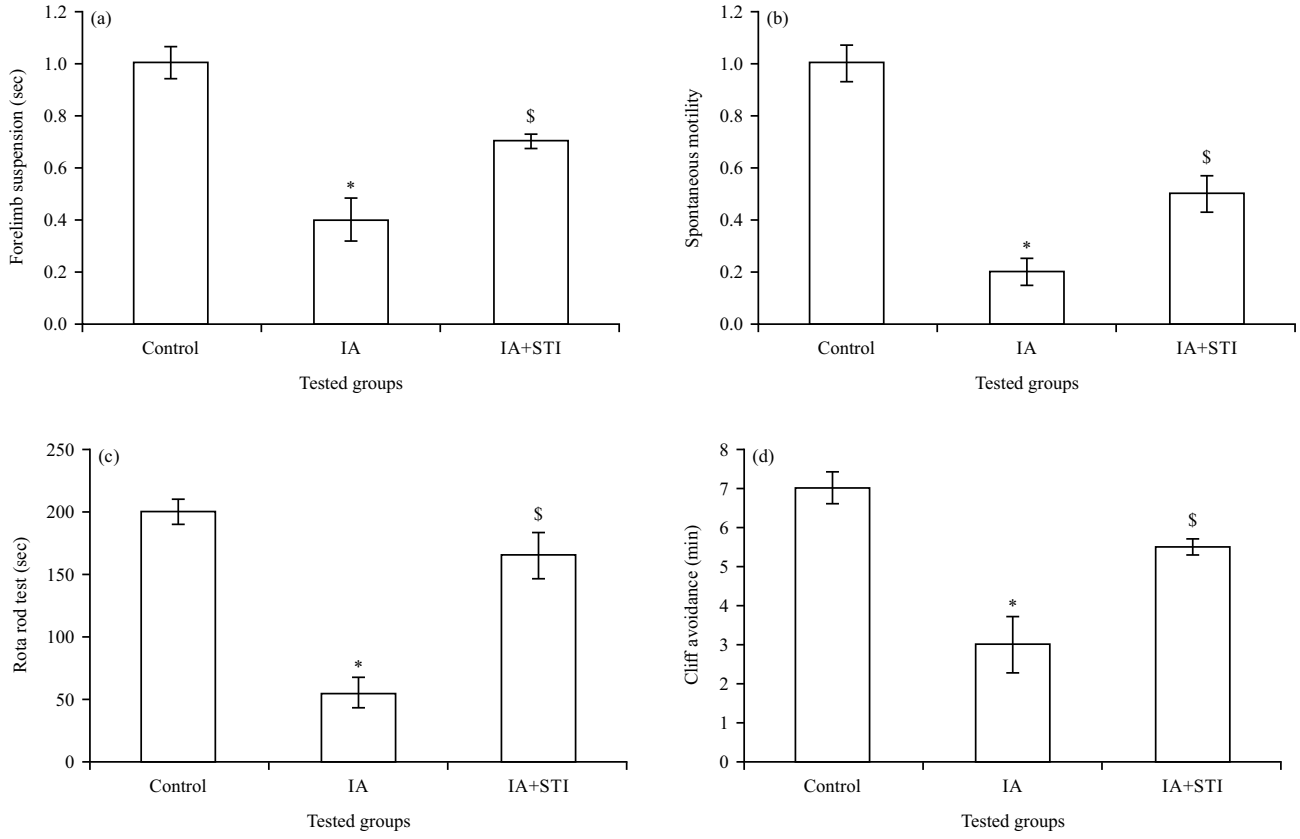

Fig. 1(a-d): Assessment of neurological performance of control and experimental group of rats

Data from the tested groups are shown as Mean±SE (n = 6). Statistical significance was expressed as *$p<0.05$ IA compared to sham-operated controls, $p<0.05 stigmasterol-treated compared to IA rats, STI: Stigmasterol and X-axis denotes the tested groups

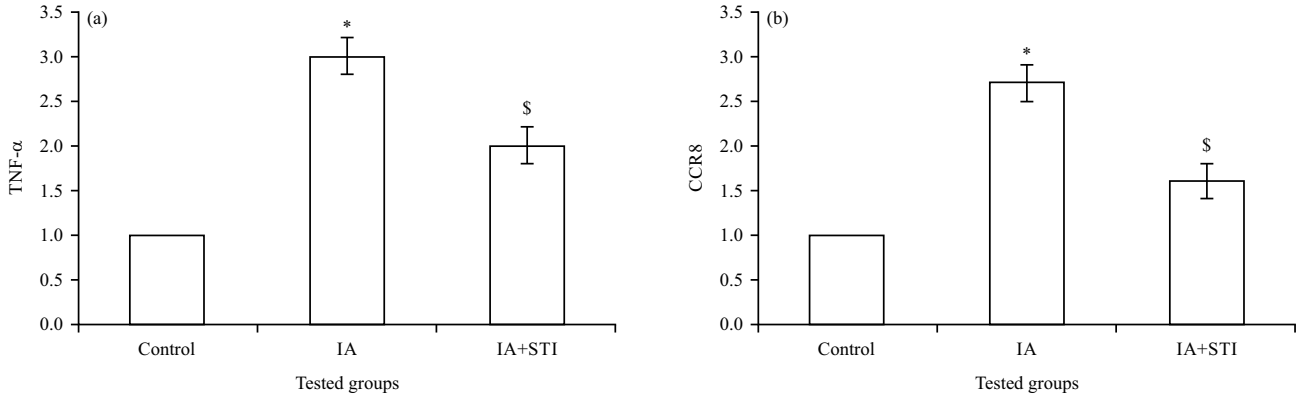

Fig. 2(a-e): Continue

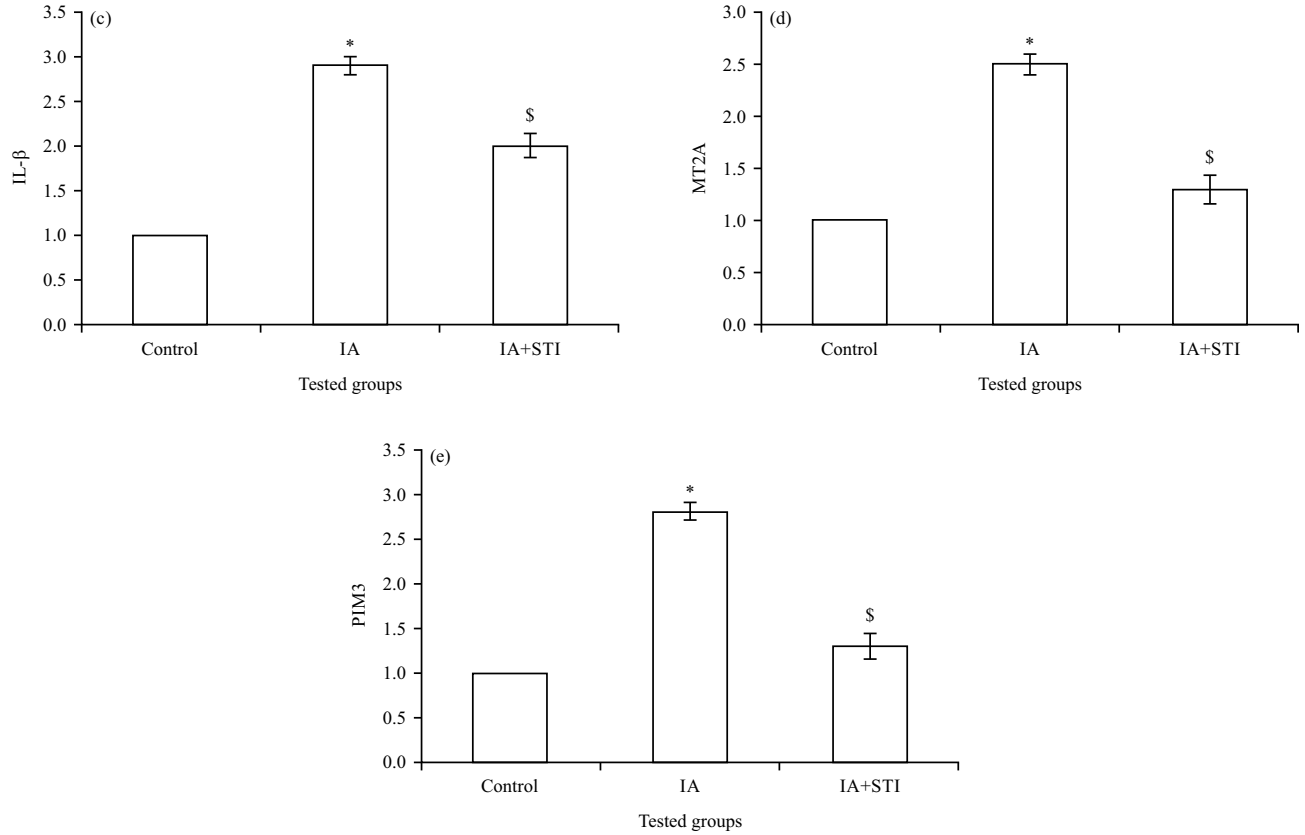

Fig. 2(a-e): qRT-PCR mRNA expression analysis of control and experimental animals

Data from the tested groups are shown as Mean±SE (n = 6). Values are expressed as Mean±SE (n = 6). Statistical significance was expressed as *p<0.05 IA compared to sham-operated controls, $p<0.05 stigmasterol-treated compared to IA rats, STI: Stigmasterol and X-axis denotes the tested groups

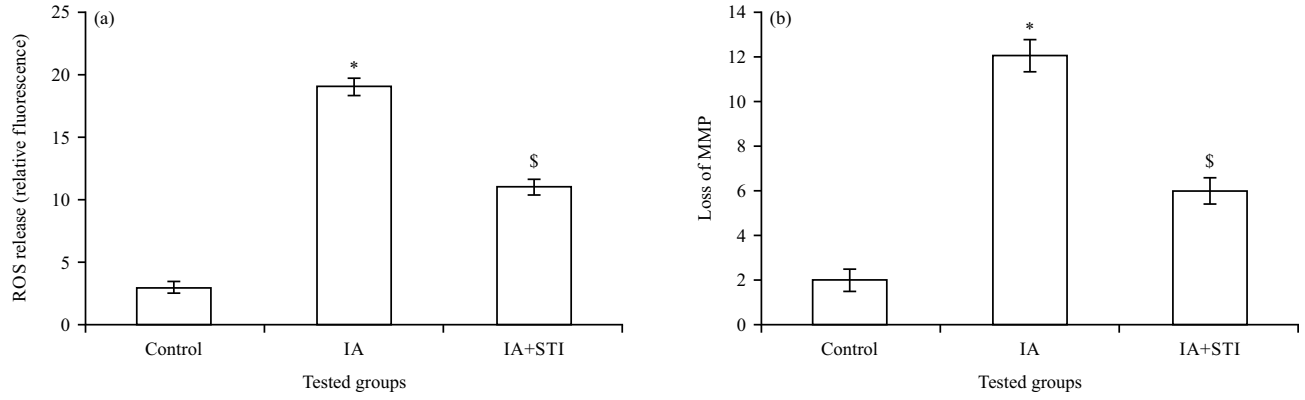

Fig. 3(a-b): ROS release and mitochondrial membrane potential of control and experimental cells

Data from the tested groups are shown as Mean±SE (n = 6). Statistical significance was expressed as *p<0.05 IA compared to sham-operated controls, $p<0.05 stigmasterol-treated compared to IA rats, STI: Stigmasterol and X-axis denotes the tested groups

Effect of stigmasterol on apoptosis markers: The IA cells demonstrated a 2-fold rise in caspase-3 (p>0.05) and -9 (p>0.05) activities in the current investigation, whereas, stigmasterol administration reduced the beginning of these apoptosis marker enzymes, demonstrating its protective effect (Fig. 4a-c).

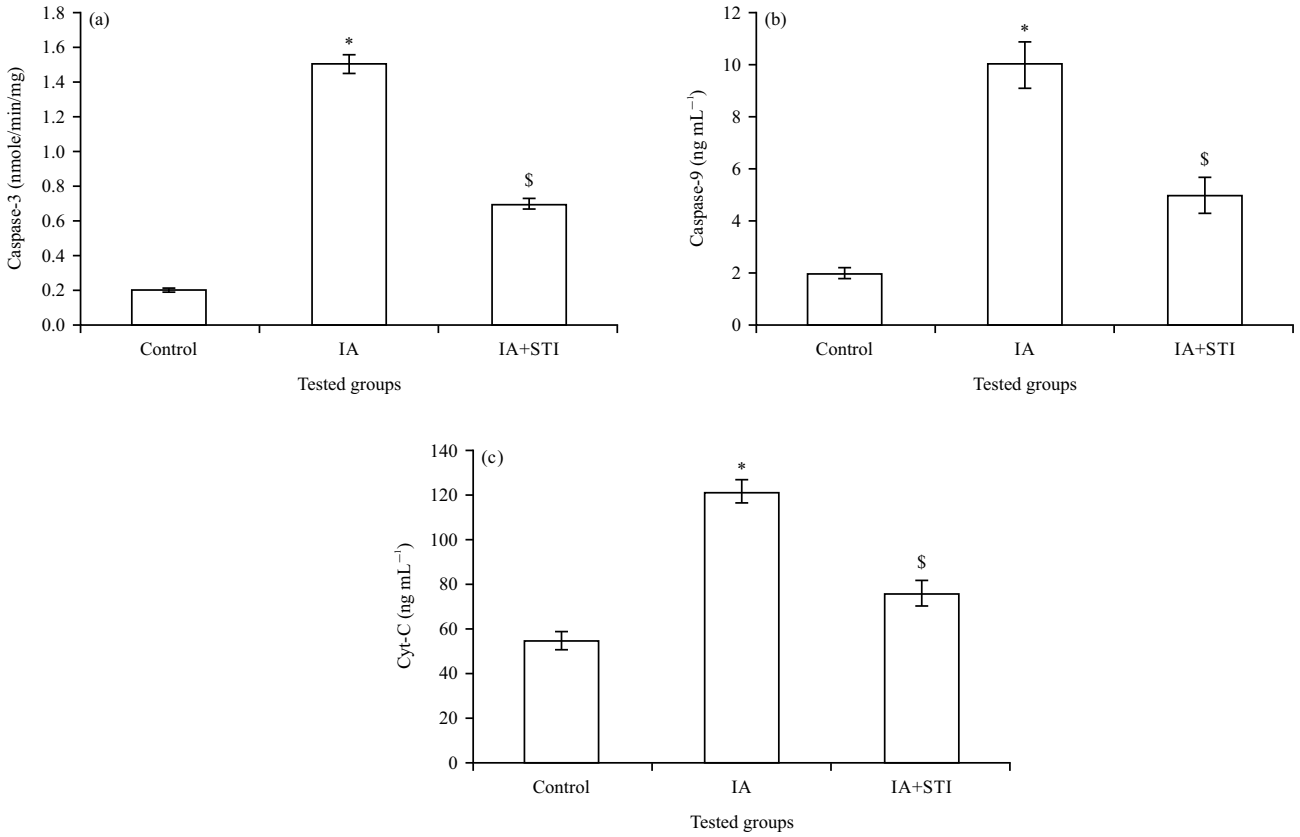

Fig. 4(a-c): Caspase-3, caspase-9 and cytochrome C activity of control and experimental cells

Data from the tested groups are shown as Mean ± SE (n = 6). Statistical significance was expressed as *p<0.05 IA compared to sham-operated controls, $^{\$}$p<0.05 stigmasterol-treated compared to IA rats, STI: Stigmasterol and X-axis denotes the tested groups

Effect of stigmasterol on cytokine level: The protective role of stigmasterol against IA was further elucidated using the levels of pro-inflammatory cytokines (Fig. 4). The TNF-α ($p<0.01$), IL-13 ($p<0.01$), IL-4 ($p<0.01$) and IL-1β ($p<0.01$) levels were significantly higher ($p<0.01$) in cells from the IA group compared to the control. While it was shown that the stigmasterol administration and the signalling of IA progression suppressed these inflammatory cytokines, this might result in the development of a novel IA therapy (Fig. 5a-d).

DISCUSSION

This study was directed at determining the use of stigmasterol for its cerebro-protective role in the intracranial aneurysm-induced rat model. Current experimental data revealed that the administration of stigmasterol could remarkably decrease cerebral degeneration evidenced by improved neurological performance. In general, people with IA often have incoordination in their neurological functions, general movement and oratory functions due to the degeneration of cells in the brain, which results in reduced bodily movements. Blood vessel pressure in the cranium along with vessel dilation is the characteristic of IA. The animals in this study were subjected to the following neurological examinations, including spontaneous locomotion, forelimb suspension, Rotarod and cliff avoidance. Animals with IA had the motor and neurological abnormalities, but animals with stigmasterol administration exhibited significant changes in their neurological functioning and motor skills. This could be possible because of activated anti-inflammatory molecules, which prevent them from entering the region of inflammation and resulting in lower oxidative stress.

It was evident that stigmasterol inhibited apoptosis, as seen in the study on apoptosis markers such as mitochondrial membrane potential and ROS release, which indicate cerebral protection. However, the increased ROS and mitochondrial damage along with the apoptotic marker enzymes revealed that the onset of apoptosis in IA rats and its inhibition by the drug demonstrated the reversal in terms of cell regeneration is very systematic through the canonical pathway evidenced by the cytokines and genes as well. Similar results were evident in the scientific literature with other phytochemicals demonstrating the use of phytocompound towards cerebral protection[21] (Fig. 4).

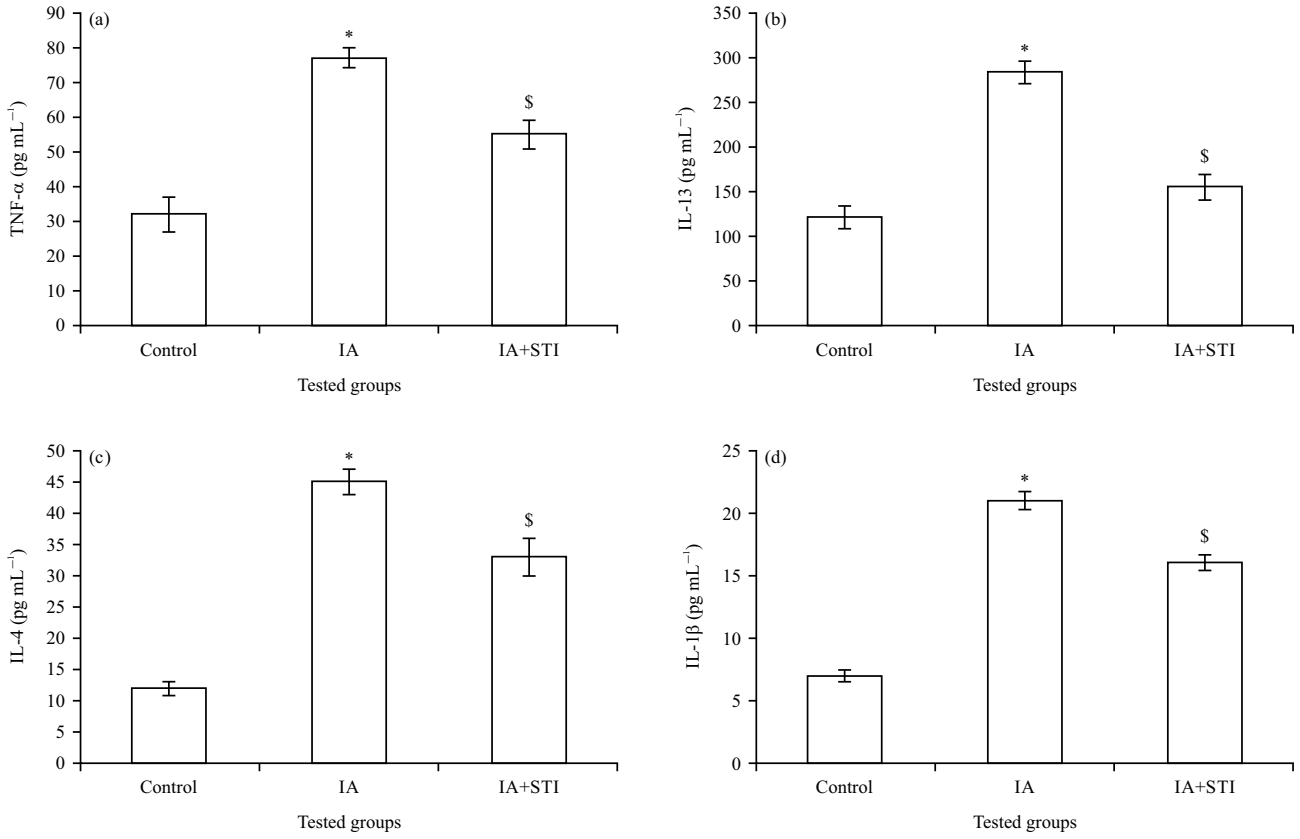

Fig. 5(a-d): Cytokine expression analysis of control and experimental cells

Data from the tested groups are shown as Mean ± SE (n = 6). Statistical significance was expressed as *p<0.05 IA compared to sham-operated controls, $p<0.05 stigmasterol-treated compared to IA rats, STI: Stigmasterol and X-axis denotes the tested groups

In aneurysm development, various pathways, such as MAPK, ERK-1/2 and JNK (nitrogen activated protein kinase), are key components and express a significant contribution to artery thickening. The JNK phosphorylation resulting causes degeneration of the aorta by inhibiting biosynthetic enzymes leading to necrosis of the extracellular matrix. For the present trial, $CaCl_2$ was used to induce cerebral aneurysms in rats. Several studies have postulated that a gradual decrease in the expression of JNK could support the inhibition of the severity of an arterial aneurysm. In another study, cerebral elastin damage and artery dilation were directly associated with necrobiosis in the cells and were considered to be crucial mechanisms which could be used the prevent and control aneurysms[22]. The degeneration of smooth muscle cells was well observed in our experiments and it was confirmed that blocking the JNK activation phosphorylation pathway in human smooth muscle cells resulted in a decrease in apoptosis[23].

An earlier study found that necrobiosis stimulation is directly related to JNK protein kinase activation[24]. Similarly, in the brain, necrobiosis in vascular wall cells has been linked to the JNK/C-JUN pathway[25]. In the present investigation, after IA induction, animal models showed significant stimulation of biomarkers such as TNF-α, CCR8, IL-1β, MT2A and PIM3 in the brain cells. While these markers were reduced by stigmasterol administration, this may be due to the effect of stigmasterol in protecting the cells from the onset of apoptosis mechanisms.

So far, scientific data suggests that inflammation in neural tissues is an important factor in IA-related problems. Local cells generate pro-inflammatory cytokines, which stimulate the production of cell adhesion molecules and the recruitment of leukocytes, which cause brain damage by activating inflammation. In our investigation, we noted that the expression of IL-1β, TNF-α and IL-6 mRNA was higher in IA animals but lower in the stigmasterol-treated group. These findings suggest that the increased inflammation in IA animals was reduced by a decrease in the inflammatory mediators directly controlled by stigmasterol therapy.

CONCLUSION

As a summary of this study, the levels of IA biomarkers and other proinflammatory cytokines were observed to rise in IA animals, but these cytokines were suppressed by stigmasterol administration, revealing an enhancement in the animals' neural connection to all organs and enhanced neuronal functioning. Thus, the current findings imply that stigmasterol has potential effectiveness in enhancing acute brain function in IA and it might be a lead molecule for IA-related therapeutics.

SIGNIFICANCE STATEMENT

Cerebral ischemia is a medical condition caused by myocardial infarction, a coronary artery bypass graft, or reversible acute hypotension that can cause serious damage or death. Intracranial aneurysms (IAs) are compromised brain artery walls that can rupture and cause cerebral haemorrhage. Stigmasterol is an important plant sterol and has good therapeutic properties. The present research reveals that stigmasterol has cerebroprotective properties against the growth of artificially produced intracranial aneurysms in rats. The current study's outcomes indicate that stigmasterol has a potential efficacy in enhancing acute brain function in Intracranial aneurysms and it might be a lead molecule for IA-related therapeutics.

ACKNOWLEDGMENT

The authors acknowledge the facilities offered by the institution to carry out this research work.

REFERENCES

1. Strange, F., B.E. Grüter, J. Fandino and S. Marbacher, 2020. Preclinical intracranial aneurysm models: A systematic review. Brain Sci., Vol. 10. 10.3390/brainsci10030134.
2. Duan, Z., Y. Li, S. Guan, C. Ma and Y. Han *et al*., 2018. Morphological parameters and anatomical locations associated with rupture status of small intracranial aneurysms. Sci. Rep., Vol. 8. 10.1038/s41598-018-24732-1.
3. Huhtakangas, J., H. Lehto, K. Seppä, R. Kivisaari and M. Niemelä *et al*., 2015. Long-term excess mortality after aneurysmal subarachnoid hemorrhage. Stroke, 46: 1813-1818.
4. Lindbohm, J.V., I. Rautalin, P. Jousilahti, V. Salomaa, J. Kaprio and M. Korja, 2019. Physical activity associates with subarachnoid hemorrhage risk-A population-based long-term cohort study. Sci. Rep., Vol. 9. 10.1038/s41598-019-45614-0.
5. Ruigrok, Y.M., G.J. Rinkel and C. Wijmenga, 2005. Genetics of intracranial aneurysms. Lancet Neurol., 4: 179-189.
6. Inci, S. and R.F. Spetzler, 2000. Intracranial aneurysms and arterial hypertension: A review and hypothesis. Surg. Neurol., 53: 530-542.
7. Wiebers, D.O., 2003. Unruptured intracranial aneurysms: Natural history, clinical outcome, and risks of surgical and endovascular treatment. Lancet, 362: 103-110.
8. Walter, G.M.O., H. Tobias, F.J. Petra, L. Ulrich and W. Oliver, 2010. Stigmasterol for the treatment of Alzheimer's disease. https://patents.google.com/patent/WO2010104394A1/en
9. Ali, H., S. Dixit, S. Alqahtani, D. Ali, S. Alkahtani and S. Alarifi, 2015. Isolation and evaluation of anticancer efficacy of stigmasterol in a mouse model of DMBA-induced skin carcinoma. Drug Des. Dev. Ther., 9: 2793-2800.
10. Gabay, O., C. Sanchez, C. Salvat, F. Chevy and M. Breton *et al*., 2010. Stigmasterol: A phytosterol with potential anti-osteoarthritic properties. Osteoarthritis Cartilage, 18: 106-116.
11. Kaur, N., J. Chaudhary, A. Jain and L. Kishore, 2016. Stigmasterol: A comprehensive review. Int. J. Pharm. Sci. Res., 2: 2259-2265.
12. Li, C., Y. Liu, Z. Xie, Q. Lu and S. Luo, 2015. Stigmasterol protects against Ang II-induced proliferation of the A7r5 aortic smooth muscle cell-line. Food Funct., 6: 2266-2272.
13. Santos, A.R.S., R. Niero, V.C. Filho, R.A. Yunes, M.G. Pizzolini, D.F. Monache and J.B. Calixtro, 1995. Antinociceptive properties of steroids isolated from *Phyllanthus corcovadensis* in mice. Planta Med., 61: 329-332.
14. Zeb, A., S. Ahmad, F. Ullah, M. Ayaz and A. Sadiq, 2016. Anti-nociceptive activity of ethnomedicinally important analgesic plant *Isodon rugosus* Wall. ex Benth: Mechanistic study and identifications of bioactive compounds. Front. Pharmacol., Vol. 7. 10.3389/fphar.2016.00200.
15. EFSA Panel on Food additives and Nutrient Sources added to Food (ANS), 2012. Scientific opinion on the safety of stigmasterol-rich plant sterols as food additive. EFSA J., Vol. 10. 10.2903/j.efsa.2012.2659.
16. Park, S.J., D.H. Kim, J.M. Jung, J.M. Kim and M. Cai *et al*., 2012. The ameliorating effects of stigmasterol on scopolamine-induced memory impairments in mice. Eur. J. Pharmacol., 676: 64-70.
17. Zaragoza, O., 2011. Multiple disguises for the same party: The concepts of morphogenesis and phenotypic variations in *Cryptococcus neoformans*. Front. Microbiol., Vol. 2. 10.3389/fmicb.2011.00181.
18. Nam, Y. and D. Lee, 2014. Ameliorating effects of constituents from *Cortex acanthopanacis radicis* on memory impairment in mice induced by scopolamine. J. Traditional Chin. Med., 34: 57-62.

19. Fan, J., X. Li, Y.W. Yan, X.H. Tian, W.J. Hou, H. Tong and S.L. Bai, 2012. Curcumin attenuates rat thoracic aortic aneurysm formation by inhibition of the c-Jun N-terminal kinase pathway and apoptosis. Nutrition, 28: 1068-1074.
20. Leik, C.E., A. Willey, M.F. Graham and S.W. Walsh, 2004. Isolation and culture of arterial smooth muscle cells from human placenta. Hypertension, 43: 837-840.
21. Cao, J., L. Jia, H.M. Zhou, Y. Liu and L.F. Zhong, 2006. Mitochondrial and nuclear DNA damage induced by curcumin in human hepatoma G2 cells. Toxicol. Sci., 91: 476-483.
22. Jones, J.A., F.G. Spinale and J.S. Ikonomidis, 2009. Transforming growth factor-β signaling in thoracic aortic aneurysm development: A paradox in pathogenesis. J. Vasc. Res., 46: 119-137.
23. Han, Y., G. Wu, J. Deng, J. Tao and L. Guo *et al*., 2010. Cellular repressor of E1A-stimulated genes inhibits human vascular smooth muscle cell apoptosis via blocking P38/JNK MAP kinase activation. J. Mol. Cell. Cardiol., 48: 1225-1235.
24. Xia, Z., M. Dickens, J. Raingeaud, R.J. Davis and M.E. Greenberg, 1995. Opposing effects of ERK and JNK-p38 MAP kinases on apoptosis. Science, 270: 1326-1331.
25. Takagi, Y., M. Ishikawa, K. Nozaki, S. Yoshimura and N. Hashimoto, 2002. Increased expression of phosphorylated c-Jun amino-terminal kinase and phosphorylated c-Jun in human cerebral aneurysms: Role of the c-Jun amino-terminal kinase/c-Jun pathway in apoptosis of vascular walls. Neurosurgery, 51: 997-1004.