Clinical and Experimental Health Sciences

Enhanced Enzyme Inhibitory Effects of the Nanohybrid Eggplant Extract: An Unusual Pharmaceutical Form for Medicinal Plant

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ABSTRACT

Objective: Recently, biosynthesis/synthesis of nanoflowers has become very attractive for chemical and pharmaceutical sciences, and enhanced enzyme activities. Various plant extracts and their active compounds are effectively used as organic component for novel nanoflowers synthesis. *Solanum melongena* L., commonly known as eggplant in English, a vegetable and medicinal plant belongs to Solanaceae family has several advantages in materials synthesis due to cheap and obtained easily. The aim of this study is to compare the enzyme ((alpha-glucosidase (AGase), alpha-amylase (AAase), tyrosinase (Tyr), acetylcholinesterase (AChE) and butyryl cholinesterase (BChE)) inhibitory effects of the eggplant' calyx extract and its Solanum-inorganic hybrid nanoflower (Sm-ihNFs) via *in vitro* experimental methods.

Methods: The hybrid nanoflower was formed (NF) with organic molecules, eggplant extract (Sm), and inorganic compounds, copper to enhance the catalytic activities. The inhibition capacities of the eggplant extract, and its hybrid nanoflower were evaluated on selected enzymes (AGase, AAase, Tyr, AChE and BChE) which play significant roles physiologically by *in vitro* tests in this study.

Results: According to inhibition percentages and IC_{50} values, Sm-ihNFs showed higher inhibitory activities on enzymes other than ache than the plain crude plant extract. Among all the enzymes that were studied, Sm-ihNFs demonstrated significantly higher alpha-glucosidase and alpha-amylase inhibition activities compared to acarbose. And when compared to galantamine hydrobromide Sm-ihNFs showed higher enzyme inhibition and significant IC_{50} value.

Conclusion: It was thought that Sm-ihNFs prepared from eggplant extract may have promising potential for antidiabetic drug formulations in the future. The hybrid nanoflowers will be promising and guide for the future work in terms of pharmaceutical and cosmeceutical industry.

Keywords: *Solanum melongena*; nanoflower; alpha-glucosidase amylase; tyrosinase; cholinesterases

1. INTRODUCTION

Solanum melongena L. (Solanaceae), commonly known as eggplant, is one of the most consumed, economically valuable vegetables in most of the world (1). The aerial parts of the plant are rich in alkaloids and saponins, phenolics (2,3). Dönmez et al. examined whether methanolic eggplant extract has antihemorrhoidal and antioxidant activities on rats or not. They have used eggplant calyx for preparing the extract. As a result of the study, it has been shown that the extract has high biological activity (3). Medicinal features such as analgesic, antifungal, antiasthmatic, antidiabetic, antihemorrhoidal, antiinflammatory, antipyretic, antioxidant, antiplatelet, hypocholesterolemic, hypolipidemic, hypotensive, and spasmogenic activities of the plant have been scientifically proven (4-19). Umamageswari and Maniyar demonstrated that eggplant leaf extract has antiinflammatory activity, which is related to many enzymes (20). Previously, Kwon et al. had evaluated just α-amylase (AAase), α-glucosidase (AGase) and angiotensin I-converting enzyme (ACE) inhibitory activities of eggplant extract to investigate a potential drug for type 2 diabetes and hypertension (21).

In recent years, innovative ideas on the development of biomaterials using herbal extracts have been putting forward and many plant-derived nanostructures and nanoparticles are tried to be developed with different methods (22). Hybrid nanoflower is a novel nano-bio agent formed with organic and inorganic compounds, moreover, creating hybrid nanoflowers using plant extracts and their constituents, which is green chemistry, became attractive yet convenient (23-29). It has been reported so far that nanoflowers obtained

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using plant extract exhibit improved biological activity. The organic-inorganic hybrid nanoflower (TF-Cu^{2+} hNF) was obtained using *Trigonella foenum-graecum* L. extracts with copper ions. The TF-Cu^{2+} hNF exhibited enhanced antibacterial activity against *Enterococcus faecium, E. faecalis, Staphylococcus aureus, Bacillus cereus, Salmonella typhi* and *Escherichia coli* except against *Pseudomonas aeruginosa* and *Haemophilus influenza* (30). The TF-Cu^{2+} hNFs and free TF extracts were also reported to show no antifungal activity at the same research. The synthesis of *Viburnum opulus* (European cranberry bush) extract based Cu^{2+} hybrid nano forms called "snowballs" and their effective catalytic and antimicrobial features compared to free European cranberry bush extracts were reported (31). They suggested that biologic activities may follow a fenton-like pathway. Baldemir and coworkers reported the synthesis and characterization method for hybrid nanoflower with using green tea extract and copper ion. Baldemir and coworkers reported the synthesis and characterization method for hybrid nanoflower with using *Camellia sinensis* (L.) Kuntze extract and copper ion (32). They also demonstrated enhanced antimicrobial and catalytic activities of nanoflowers. In the last nanoflower study using curcumin extract, the effect of reaction time and curcumin concentrations on nanoflower's morphology was reported (33).

Solanum melongena plant extracts with their rich alkaloid, saponin and phenolic constituents can be used in hybrid nanoflower synthesis. However, no study was recorded on enzymes inhibitory activities of calyx and the nanoflower form of the extract to this point. In addition to antidiabetic enzyme activities, it was aimed to evaluate anticholinesterase and tyrosinase enzyme inhibition activities in this article. In this study, which allows comparison of antidiabetic activity, the anticholinesterase and tyrosinase enzyme inhibition activities of calyxes were examined for the first time. For this; the hybrid nanoflowers using eggplant extract were obtained in optimum conditions, then characterized with several techniques such as Scanning Electron Microscope (SEM), Energy Dispersive X-Ray Analysis (EDX), Fourier Transform Infrared (FTIR) and X-Ray Diffraction (XRD). Then, some important enzyme inhibition activities such as alpha-glucosidase (AGase), alpha-amylase (AAase), tyrosinase (Tyr), acetylcholinesterase (AChE) and butyryl cholinesterase (BChE) of hybrid nanoflowers were investigated in comparison with the free extract. We suggested that enhanced enzymatic activities of nanoflowers may expand their uses a usual pharmaceutical form for an edible/medicinal plant.

2. METHODS

2.1. Preparation of the Extract

The cultured plant materials, which was founded in the Herbarium of Faculty of Pharmacy/Gazi University/Ankara/Turkey as authenticated voucher specimen (GUE 3500), were gathered during August-September 2016-2017 at the Central Anatolia of Turkey. Only the dark-green calyx of the mature eggplant fruit was separated and dried in shade, then ground to powder, extracted with methanol by maceration. The yield of the extract was calculated as 11.4 percent.

2.2. Synthesis of Hybrid Nanoflowers (Sm-ihnfs)

Sm-ihNFs was synthesized following modified reported method (31, 34). The calyx methanolic extract with concentrations from 0.02 to 0.2 mg mL^{-1} was separately added into the mixture containing 50 mL of 10 mM PBS (pH7.4) and 0.8 mM Cu^{2+} ion. The occurring blueish-green precipitates, washed with water, and centrifuged at 5000 rpm for 10 min. The washing process was repeated 3 times and the final product was dried at 50°C for further characterizations and enzyme assays. Sm-ihNFs was characterized by using the morphologies of the synthesized Sm-ihNFs were examined by using Scanning Electron Microscopy (ZEISS EVO-LS10). The chemical and crystal structures of the Sm-ihNFs were characterized using Fourier Transform Infrared spectroscopy (Perkin Elmer 400, Spectrometer Spotlight 400 Imaging System) and X-Ray Diffraction (Bruker, AXS D8 Advance Model) analysis, respectively. The elemental analysis of the Sm-ihNFs was performed by energy dispersive X-ray (ZEISS EVO-LS10) analysis.

2.3. Enzyme inhibition activity

Alpha-glucosidase inhibition (35): The inhibition method of α-glucosidase was followed according to Kumar et al. Acarbose was utilized as reference. The test solution (25 µL) was diluted with a PBS which added to α-glucosidase (25 µL, 0.5 U/mL). After the 10 minutes incubation at 25ºC, 25 µL of 0.5 mM PNPG was added to each well then, the mixture was further waited for 30 minutes at 37ºC. At the end of the incubation term, sodium carbonate (0.2 M, 100 µL) was joined to put an end to the reaction and the absorbances were read at 405 nm. All concentrations were carried out in triplicate to obtain an accurate statistical analysis.

Alpha-amylase inhibition (36): The inhibition method of *α*-alpha-amylase was followed by Kumar et al. While acarbose was a positive control, PBS (pH 6.9, 0.02 M, PBS) was a negative control in place of the specimen. Each specimen was conducted in triplicate with diverse concentrations. The reaction mix containing 50 µL of test solution was diluted with buffer, 25 µL of enzyme (5000 µg/mL, *α*-amylase) and incubated for about 10 minutes at 25ºC. Then 50 µL of freshly prepared 0.5 % starch solution (w/v) was annexed to each well as a substrate and incubated for a further 10 minutes at 25ºC. Incubation period was followed by addition of 1 % 3,5-dinitrosalicylic acid (DNS, 100 µL) coloring reagent and heated in a water bath for 10 minutes. The absorbances were read at 540 nm.

Tyrosinase enzyme inhibition (37): Tyrosinase inhibition was detected by the improved dopa chrome method. L-DOPA was used as substrate. A part of the plant extracts and their nanoflowers, under the optimal conditions, diffused in DMSO

(PBS, 6.8 pH, 80 µL), 40 µL of L-DOPA, and 40 µL tyrosinase enzymes were added into each well. Analysis was performed in a microplate by using ELISA plate reader and absorbances were measured at 475 nm. Results were evaluated by comparing with the DMSO and α-kojic acid.

The AChE and BChE inhibition (38,39): The acetylcholinesterase/butyryl cholinesterase inhibition assay was evaluated by Ellman colorimetric method as described by Öztürk. 150 µL of 0.1 M PBS (pH=8.0), 10 µL of test solutions in MeOH: DMSO (4k: 1k, v/v) with different concentrations and 20 µL of 0.1 U/mL enzyme solution (acetylcholinesterase-butyryl cholinesterase was obtained from equine serum) were incubate for 15 minutes at 25°C. 10 µL of a solution of 0.2 mM (acetylcholine/butyryl thiocholine) and 10 µL of 0.5 mM DTNB were mixed and the absorbances of the mix were measured at 412 nm. Galantamine hydrobromide (Sigma-Aldrich, Germany) was used as a positive control.

2.4. Statistical analysis

The differences in values between the reference and test groups were compared by using GraphPad Prism Software (8.3 Version, La Jolla/CA/USA) in statistical analysis. The results are expressed as the mean ± standard error means (S.E.M.). When one-way ANOVA with Tukey Multiple Comparison Test was used, p-values of less than 0.05 were considered statistically significant.

3. RESULTS

3.1. Synthesis of Hybrid Nanoflowers (Sm-Ihnf)

Sm-ihNFs was synthesized using calyx extract of the plant in phosphate buffered saline and Cu (II) ion for 3 days of incubation. The extract contains diverse types of phytochemicals, such as alkaloids, saponosides, flavonoids that comprise important elements such as N, O and S atoms, which can form complexes with Cu (II) ions due to their strong affinity. In the synthesis of Sm-ihNFs, the most significant interaction is the coordination chemistry between Cu (II) ions and some molecules, mostly alkaloids containing N atom, which allows the formation of the hybrid structures with flower-like shapes under definite circumstances.

Formation of hybrid nanoflower consists of three consecutive steps as nucleation, growth, and completion. Nucleation occurs formation of the key nanocrystals of copper phosphate from Cu (II) and phosphate ions interactions. The reaction with primary nanocrystals and the biomolecules leads to the formation of the petals of flowers-like shapes during growth. As a result of penetration of petal-like structures, hybrid nanoflower formation occurs.

Concentration of organic component is one of the most essential parameters that influence the morphology and the structure of organic-inorganic hybrid nanoflowers. Consequently, effect of the concentrations of the extract on the morphology of Sm-ihNFs was investigated applying SEM analysis (Fig.1). The most uniform morphology of hybrid nanoflowers was obtained as the optimum condition. The average size of nanoflowers was 5-6 µm.

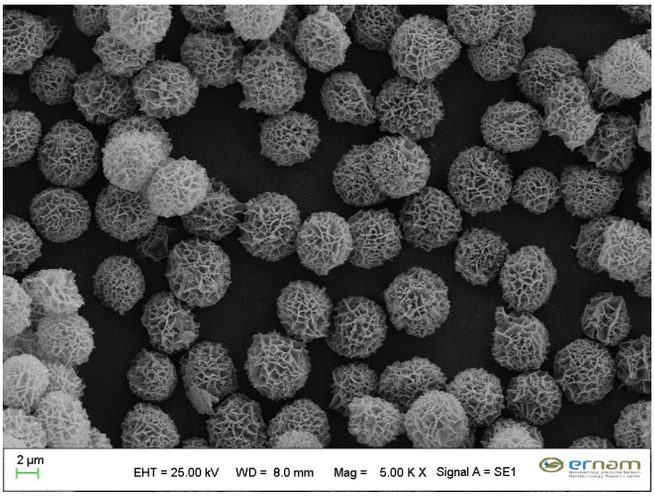

Figure 1. *Scanning electron microscopic images of hybrid nanoflowers formed with 0.1 mg mL-1 extract of S. melongena*

The elemental analysis of the synthesized Sm-ihNFs was performed by EDX (Fig.2). The EDX spectrum was revealed presence of major elements such as C, N, O, P, and Cu in the structure. The copper metal can be evaluated as a major skeleton of the hybrid nanoflower.

The functional groups in the structure of organic compounds, whether the two compounds are the same, the state of the bonds in the structure, the binding sites and whether the structure is aromatic or aliphatic were determined with the FTIR analysis, (Fig.3). The bending and stretching bonds in the free plant extract were characteristically also detected in the hybrid structure.

The measurement of particle sizes, phase equilibria and crystal structure of Sm-ihNFs were investigated by XRD (Fig.4). The all diffraction peaks of $Cu_3(PO_4)_2.3H_2O$ were matched well with the JCPDS card (022-0548).

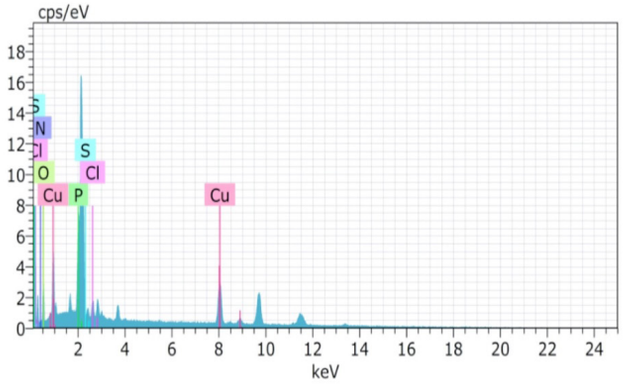

Figure 2. *EDX spectra of hybrid nanoflowers formed with 0.1 mg mL-1 extract of S. melongena*

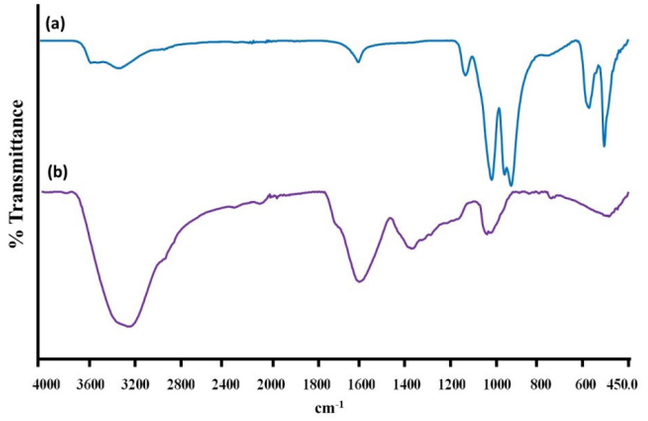

Figure 3. *FT-IR spectrums of (a) hybrid nanoflowers formed with 0.1 mg mL-1 extract of S. melongena and (b) the plant extract*

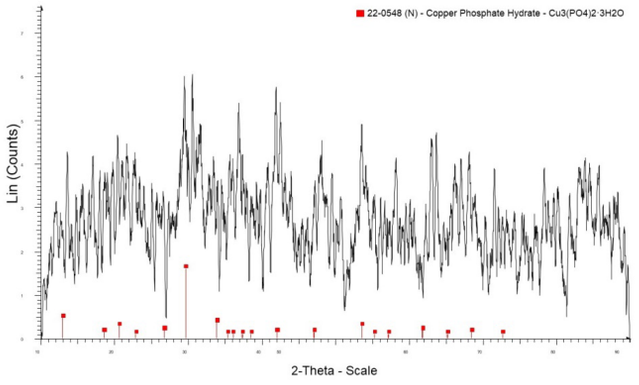

Figure 4. *XRD spectra of hybrid nanoflowers formed with 0.1 mg mL-1 extract of S. melongena*

Table 1. *Anticholinesterase activity (Inhibition % ± S.E.M.& IC_{50} value) of eggplant extract and Sm-ihNFs*

Samples	Anticholinesterase activity			
	AChE		BChE	
	Inhibition % #	IC_{50} (ppm)	Inhibition % #	IC_{50} (ppm)
Sm	70.12 ± 0.15***	17.09 ± 1.36**	75.76 ± 0.65**	28.29 ± 4.30**
Sm-ihNFs	13.83 ± 1.93***	321.46 ± 6.31***	85.12 ± 1.87*	32.76 ± 8,90**
Galantamine hydrobromide	92.23 ± 0.65***	2.31 ± 0.19**	81.82 ± 0.56*	3.01 ± 0.22*

 # Inhibition % ± S.E.M. at 0.2 mg mL^{-1} concentrations
**p<.5, **p<.05, ***p<.005, ANOVA test*

Table 2. *Antidiabetic activity (Inhibition % ± S.E.M.& IC_{50} value) of eggplant extract and Sm-ihNFs*

Samples	Antidiabetic activity			
	a-glucosidase		*a*-amylase	
	Inhibition % #	IC_{50} (ppm)	Inhibition % #	IC_{50} (ppm)
Sm	46.67 ± 4.64*	212.33 ± 17.19***	42.05 ± 5.42**	213.80 ± 21.68***
Sm-ihNFs	82.77 ± 1.31***	31.95 ± 6.72**	83.06 ± 2.92***	27.81 ± 15.21**
Acarbose	57.74 ± 0.83*	59.27 ± 15.09*	58.40 ± 0.61*	38.46 ± 11.73**

 # Inhibition % ± S.E.M. at 0.2 mg mL^{-1} concentrations
**p<.5, **p<.05, ***p<.005, ANOVA test*

Table 3. *Tyrosinase enzyme inhibition (Inhibition % ± S.E.M.& IC_{50} value) of eggplant extract and Sm-ihNFs*

Samples	Tyrosinase inhibition activity	
	Inhibition % #	IC_{50} (ppm)
Sm	4.59 ± 3.53*	704.56 ± 39.23***
Sm-ihNFs	16.43 ± 0.31**	423.80 ± 2.02**
Kojic acid	24.23 ± 5.52**	371.80 ± 36.80**

 # Inhibition % ± S.E.M. at 0.2 mg mL^{-1} concentrations
**p<.5, **p<.05, ***p<.005, ANOVA test*

3.2. Enzyme inhibitions

According to average cholinesterases inhibition ± standard error means (S.E.M.) values were given in Table 1, the BChE activity of nanoflower was higher than the galantamine hydrobromide, whereas the AChE inhibition activity was higher in the plain extract.

According to average of the *a*-glucosidase and *a*-amylase inhibition ± standard error mean (S.E.M.) inhibition values

were given in Table 2, the enzyme inhibition of the samples was elevated compared to the reference acarbose.

Average Tyr inhibition ± standard error means (S.E.M.) were demonstrated by the calculation of absorbance of the plain extract and its hybrid nanoflowers against blank sample. According to inhibition values given in Table 3, the results showed that the enzyme inhibition of the samples was low compared to kojic acid.

4. DISCUSSION

The prevalence of chronic and metabolic disorders continues to increase in humans around the world. The tendency to herbal resources has risen for the purpose of prevention or treatment of diseases (40). Today, many scientists have focused on the integration of natural resources and technological developments. In our study, nanoflower-plant hybridization studies were carried out to increase the biological activity potential of plants. One of the most important reasons for using *Solanum melongena* (eggplant) in this study was limited pharmacological studies on it despite its economically valuable. The present study demonstrated the green synthesis, characterization, and activity of the organic-inorganic hybrid Sm-ihNFs enzymes inhibitors. This easy to apply and one-step immobilization withstand the relation reactions between amine groups in the character strength of enzymes and cupric phosphate nanoflowers. Three consecutive steps were conducted for forming the nanoflower-like structure. First step was nucleation and formation of primary nano crystals. Second step was arranging crystals, and last step was formation of nanoflowers (23).

The results were promising that the hybrid nanostructures were synthesized demonstrated appealing blooming structures as 5-6 µm size. Likewise, characterization of the Sm-ihNFs was successfully completed by using different techniques (SEM, EDX, FTIR, and XRD). In a period of approximately 30-35 years, pharmacological and phytochemical studies on *Solanum species* have gained importance. At least 65 species (including *S. aculeastrum, S. aethiopicum, S. americanum S. anguivi, S. cathayanum, S. capsicoides, S. diphyllum, S. muricatum, S. nigrum, S. septemlobum, S. sessiliflorum*, *S. spirale, S. surattense, S. torvum S. tuberosum, S. violaceum,* and *S. xanthocarpum*) have been studied on scientific platform. The most antioxidants and anticancer activities of *Solanum species* have been demonstrated by scientists. The responsible compounds of pharmacological activities, such as antibacterial, anticancer, anticonvulsant, antidiabetic, anti-fungal, anti-inflammatory, antileishmanial, antioxidants, antitumor, and spasmolytic, of *Solanum* species have been attributed to steroidal alkaloids (41-43). There have been some publications related to the subject of this manuscript despite the small number of pharmacological studies on eggplant. One of these studies was conducted by Kwon and coworkers. They indicated that eggplants have radical scavenging-linked antioxidant activity and α-glucosidase inhibitory effect due to rich phenolic compounds (10). That result supported our study, moreover, the hybrid nanoflower exhibited higher effective *a*-glucosidase and *a*-amylase enzymes activities than the plain plant extract. In another *in vitro* study, calystegines, a tropane alkaloid derivative in eggplant, showed glycosidases inhibitory activity (44). Consequently, the hybrid nano-flower might contain the N including alkaloid structure, therefore.

In a poster presentation presented by Ketprayoon and Chaicharoenpong, while *S melongena*'s methanolic fruit extract showed low tyrosinase inhibitor activity, tyrosinase inhibitor activity of *S melongena*'s aqueous fruit extract could not be determined (45). Although no significant activity was observed in both the calyx extracts and the hybrid nanoflower, higher effective tyrosinase inhibitor activity was determined in the hybrid nanoflower in our study. In another study, the ethanolic eggplant peels extract have showed the skin protecting (46). There is a need for evaluation of different eggplant parts and their various extracts.

The cholinesterase inhibitors are still the most important clinical strategy to manage Alzheimer's disease. In this study, while Sm-ihNF showed BChE enzyme inhibitory effect, it was not very successful on AChE enzyme inhibition. However, this is, to the best of our knowledge, the first report of the analysis of both acetyl and butyryl choline esterase enzymes inhibition activities of eggplant. It is thought that *Solanum* glycoalkaloids may be the compounds responsible for anticholinesterase activities (47).

5. CONCLUSION

The enzyme inhibition tests have been used for getting an idea about several different illness due to their activity and specificity performance. Although there are many enzyme inhibition studies on plants, studies on nano technological-chemical studies for increasing activity based on natural sources are limited. With the studies shown in this text, a novel and elegant immobilization approach was used to form nanoflower-like structures from enzymes exhibiting highly enhanced catalytical activity and stability. The nanoflower's large surface area, limited the mass-transfer, and nanoscale-related enzyme cooperation are potential causes in enzyme inhibition tests and biological activity studies. The synthesis of protein-inorganic hybrid nanoflower containing plant extracts is an important factor considering its suitability for commercial applications in pharmacy and cosmetics.

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Author Contributions:
Research idea: UKC.

Design of the study: NÖ.
Acquisition of data for the study: CA.
Analysis of data for the study: CD.
Interpretation of data for the study: NE.
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Revising it critically for important intellectual content: UKC.
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