

# RESEARCH PAPER

# In vitro antiproliferative and hepatoprotective activity studies of *Momordica cymbalaria*

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#### ABSTRACT

Natural products are well known to exhibit the antiproliferative and hepatoprotective activities. The present study aimed at assessing the potency of hydroalcoholic extracts of fruits, aerial parts and roots of *Momordica cymbalaria*. Antiproiferative activity of the extracts assessed using *in vitro* cell lines such as MCF-7, HepG2, HeLa, PC3, A549 and Vero cell lines. The ability of extracts to exert toxic insult on cancer cells has been the basis of anticancer activity. The extracts were evaluated for hepatoprotective activity by employing primary rat hepatocytes. In the case of HeLa cells, MCR was found to be most potentially toxic with average  $\text{CTC}_{50}$  of 67  $\mu$ g/ml. MCR possesses more toxicity to PC3 cells than the others. Potential toxicity towards Vero cell lines was exhibited by MCR with average  $\text{CTC}_{50}$  value of 63  $\mu$ g/ml. The results clearly demonstrate that the extract MCR exert potential anticancer activity. *In vitro* hepatoprotective activity of the plant extracts was studied by employing primary rat hepatocytes. Our results indicate that the drug Silymarin was found to exhibit 96 per cent protection against Paracetamol induced toxicity in Hep G2 cells at the tested concentration of 250  $\mu$ g/ml. Among the parts of *Momordica cymbalaria*, MCA and MCR found to have slightly lesser activity compared to Sylimarin. In paracetamol induced toxicity of primary rat hepatocytes, the drug Silymarin was found to exhibit 85.28 per cent protection against Paracetamol induced toxicity in Primary rat hepatocytes at the tested concentration of 250  $\mu$ g/ml. MCA was found to exhibit comparatively similar protective power than silymarin.

Key Words: Momordica cymbalaria, Antiproliferative, Hepatoprotective, Hydroalcoholic extract

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of cucurbitaceae family. The synonyms are Momordica tuberose Roxb. or Luffa tuberosa Roxb. The plant is a perennial climber available only during the monsoon season and is found in thesouth Indian states of Andhra Pradesh, Karnataka, Madhya Pradesh, Maharashtra, and Tamil Nadu. The plant is a climbing annual or perennial herb with slender, scandent, branched, striate stem. The plant is traditionally used for the treatment of diabetes mellitus and also as an antiovulatory agent. Bharathi et al. (2011)

have reported the study on somatic chromosome number and detailed Karyotype analysis of six Indian Momordica species which includes *M. cymbalaria* also. The Somatic chromosome number of *M. cymbalaria* was reported to be 2n=18 against its previous reports of 2n=16, 22. The karyotype analysis reveals that it was possible to distinguish chromosomes of *M. cymbalaria* from other Momordica species and also between monoecious and dioecious taxa of the genus. A study on antiulcer activity of aqueous extracts of fruits of *Momordica cymbalaria* revealed that pretreatment

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with aqueous extract of M. cymbalaria fruits showed significant decrease in the total acidity and ulcer index and also showed improvements in all histopathological parameters in the M. cymbalaria treated group. It was also learnt that there is significant decrease in gastric lesion and NP-SH and gastric wall mucus concentration in the M. cymbalaria treated groupin ulcer induced rats (Bharathi et al., 2010). Studies on chemical composition and utilization of the wild edible vegetable Athalakkai (Momordica tuberose) was shown that it has higher amounts of minerals such as calcium, potassium and sodium. Also it is rich in vitamin C than the bitter gourd (Momordica charantia) and also it is rich in highcrude fibre (6.42g/100g) (Parvathi and Kumar, 2002). Its versatile utility as a nutritious vegetable, folk medicine and functional food ingredient provoked us to compile a comprehensive review of this multipurpose fruit on the distribution, nutritional attributes and phytochemicals composition and its medicinal properties M. cymbalaria fruits were considered as tonic, stomachic, stimulant, laxative and alterative. The fruit is useful in treating gout, rheumatism and sub-acute cases of the spleen and liver diseases. It has also been shown to havehypoglycemic properties (antidiabetic) in animal as well ashuman studies. The fruit juice and leaf tea of M. cymbalaria isemployed for diabetes, malaria, colic, sores and wounds, infections, worms and parasites, as an emmenagogue, and formeasles, hepatitis and fevers. Fruit pulp, leaf juice and seeds possess anthelmintic activity. Root is astringent, abortifacient, aphrodisiac and also used to treat constipation, indigestion, diabetes, diarrhea and rheumatism. For the lastfew decades, the medicinal value of plants has been recognized. The extracts from many plants contain not only minerals and primary metabolites but also a diverse array of secondary metabolites, mostly with antioxidant properties. Plants belonging to Momordica species have been used astherapeutic agents for the treatment of diabetes mellitus. The other species of this genus, Momordica charantia and Momordica foetida have been reported to have hypoglycemic effects (Fernandes et al., 2007 and Osinubi et al., 2008). Pramod et al. (2008) have reported the antioxidant and hepatoprotective activity of ethanolic extract of tubers of Momordica tuberosa against CCl, induced liver injury in rats. CCl<sub>4</sub> is metabolized by CYP 450 enzyme system totrichloromethyl radical (CCl<sub>2</sub>). This in turn reacts with molecular oxygen and gets converted to trichloromethyl peroxy radical. This radical forms covalent bonds with sulfhydryl group ofseveral membrane molecules like reduced glutathione leading to this depletion and causes lipid peroxidation. The lipid peroxidation initiates a cascade of reactions leading to tissue necrosis. The antioxidant property of ethanolic extract oftubers of Momordica tuberosa prevented the formation of trichloromethyl peroxy radical there by reducing tissue damage. Pramod et al. (2011) have reported the hepatoprotective activity of 70 per cent ethanol extract of tubers of Momordica tuberosa against thiacetamide (100 mg/kg of bodyweight) induced hepatic damage in albino

The current study evaluates the hepatoprotective and antiproliferative activities of fruit, aerial parts and roots of Momordica cymbalaria.

# RESEARCH METHODOLOGY

#### Chemicals:

3-(4, 5-dimethyl thiazol-2-yl)-5-diphenyltetrazolium bromide (MTT), Fetal Bovine Serum (FBS), Phosphate Buffered Saline (PBS), Dulbecco's Modified Eagle's Medium (DMEM) and Trypsin were obtained from Sigma Aldrich Co, St Louis, USA. EDTA, Glucose and antibiotics from Hi-Media Laboratories Ltd., Mumbai. Dimethyl Sulfoxide (DMSO) and Propanol from E. Merck Ltd., Mumbai, India.

3-(4, 5-dimethyl thiazol-2-yl)-5-diphenyltetrazolium bromide (MTT), Trypan blue, Fetal Bovine serum (FBS), Phosphate Buffered Saline (PBS), Dulbecco's Modified Eagle's Medium (DMEM) and Trypsin were obtained from Sigma Aldrich Co, St Louis, USA. EDTA, Glucose, Trichloroacetic acid (TCA), Acetic acid, Tris base and antibiotics from Hi-Media Laboratories Ltd., Mumbai. Dimethyl Sulfoxide (DMSO), Paracetamol and Propanol from E. Merck Ltd., Mumbai, India.

#### Cell lines and culture medium:

MCF-7, HepG2, HeLa, PC3, A549 and Vero cell lines. was cultured in DMEM supplemented with 10 per cent inactivated Fetal Bovine Serum (FBS), penicillin (100 IU/ ml), streptomycin (100 μg/ml) and amphotericin B (5 μg/ ml) in an humidified atmosphere of 5 per cent CO<sub>2</sub> at 37°C until confluent. The cells were dissociated with TPVG solution (0.2% trypsin, 0.02% EDTA, 0.05% glucose in PBS). The stockcultures were grown in 25 cm<sup>2</sup> culture flasks and all experiments were carried out in 96 microtitre plates (Tarsons India Pvt. Ltd., Kolkata, India).

HepG2 cell culture was procured from National Centre for Cell Sciences (NCCS), Pune, India. Stock cells of HEPG2 were cultured in DMEM supplemented with 10 per cent inactivated Fetal Bovine Serum (FBS), penicillin (100 IU/ ml), streptomycin (100 μg/ml) and amphotericin B (5 μg/ ml) in an humidified atmosphere of 5 per cent CO<sub>2</sub> at 37°C until confluent. The cells were dissociated with TPVG solution (0.2% trypsin, 0.02% EDTA, 0.05% glucose in PBS). The stock cultures were grown in 25 cm<sup>2</sup> culture flasks and all experiments were carried out in 96 microtitre plates (Tarsons India Pvt. Ltd., Kolkata, India).

## **Preparation of test solutions:**

For cytotoxicity studies, each weighed test drugs were

separately dissolved in distilled DMSO and volume was made up with DMEM supplemented with 2 per cent inactivated FBS to obtain a stock solution of 1 mg/ml concentration and sterilized by filtration. Serial two fold dilutions were prepared from this for carrying out cytotoxic studies.

For Hepatoprotective studies, each weighed test drugs were separately dissolved in distilled DMSO and volume was made up with DMEM supplemented with 2 per cent inactivated FBS to obtain a stock solution of 1 mg/ml concentration and sterilized by filtration. Serial two fold dilutions were prepared from this for carrying out cytotoxic studies.

#### **Determination of cell viability by MTT assay:**

The ability of the cells to survive a toxic insult has been the basis of most cytotoxicity assays. This assay is based on the assumption that dead cells or their products do not reduce tetrazolium. The assay depends both on the number of cells present and on the mitochondrial activity per cell. The principle involved is the cleavage of tetrazolium salt 3-(4, 5 dimethyl thiazole-2-yl)-2, 5-diphenyl tetrazolium bromide (MTT) into a blue coloured product (formazan) by mitochondrial enzyme succinate dehydrogenase. The number of cells was found to be proportional to the extent of formazan production by the cells used (Francis and Rita, 1986).

The monolayer cell culture was trypsinized and the cell count was adjusted to 1.0 x 10<sup>5</sup> cells/ml using DMEM containing 10 per cent FBS. To each well of the 96 microtitre plate, 0.1 ml of the diluted cell suspension (approximately 10,000 cells) was added. After 24 h, when a partial monolayer was formed, the supernatant was flicked off, wash the monolayer once with medium and 100 µl of different test concentrations of extracts were added on to the partial monolayer in microtitreplates. The plates were then incubated at 37°C for 3 days in 5 per cent CO, atmosphere and microscopic examination was carried out and observations were noted every 24 h interval. After 72 h, the drug solutions in the wells were discarded and 50 µl of MTT in PBS was added to each well. The plates were gently shaken and incubated for 3 h at 37°C in 5 per cent CO<sub>2</sub> atmosphere. The supernatant was removed and 100 µl of propanol was added and the plates were gently shaken to solubilize the formed formazan. The absorbance was measured using a microplate reader at a wavelength of 540 nm. The percentage growth inhibition was calculated using the following formula and concentration of test drug needed to inhibit cell growth by 50 per cent (CTC<sub>50</sub>) values is generated from the doseresponse curves for each cell line.

$$\% \ growth \ inhibition = 100 - \left[\frac{Mean \ OD \ of \ individual \ test \ group}{OD \ of \ control \ group}\right] \times 100$$

# Determination hepatoprotective activity in HepG2 cell line:

The monolayer cell culture was trypsinized and the cell count was adjusted to 1.0 x 10<sup>5</sup> cells/ml using DMEM medium containing 10 per cent FBS. To each well of the 96 well microtitre plate, 0.1 ml of the diluted cell suspension (approximately 10,000 cells) was added. After 24 h, when a partial monolayer was formed, the supernatant was flicked off, washed the monolayer once with medium. 50 µl of DMEM with 2 x 100mm Paracetamol and 2 x 50 µl of different non-toxic test concentrations of test drugs were added. The plates were then incubated at 37°C for 24 h in 5 per cent CO<sub>2</sub> atmosphere. After 24 h, the cell supernatants were discarded and 50 µl of MTT in PBS was added to each well. The plates were gently shaken and incubated for 3 h at 37°C in 5 per cent CO<sub>2</sub> atmosphere. The supernatant was removed and 100 µl of propanol was added and the plates were gently shaken to solubilize the formed formazan. The absorbance was measured using a microplate reader at a wavelength of 540 nm. The percentage cell viability was determined, based on which the percentage protection offered by test and standard drugs was calculated over the Paracetamol control.

## **Isolation of rat hepatocytes:**

The primary rat heptocyte culture was prepared as described earlier (Liu et al., 2001). Briefly, abdomen of an adult rat, weighing 200-220 g will be opened with a midline incision. Portal cannula will be placed and the liver will be perfused with HEPES solution fallowed by collagenase typeiv solution. The liver capsule will be disrupted and the resulting cell suspension will be filtered and washed, cell viability will be measured by trypan blue exclusion technique. The cells will be suspended in Ham's  $F_{12}$  medium with 20 per cent FBS and incubated at 37°C for further use. The handling and treatment of animals was as per Control and Supervision of Experiments on Animals (CPCSEA), Ministry of Environment and Forests, Government of India rules.

## Hepatoprotective study in isolated rat hepatocytes:

Isolated primary rat hepatocytes were suspended fresh Ham's F<sub>12</sub> medium containing 20 per cent FBS and incubated at 37°C for 16h. Medium supernatant was removed carefully and primary hepatocytes were exposed to fresh medium containing toxicant along with/without test product at different doses, along with negative and standard controls. After 24h incubation period, cell supernatant will be aspirated and cell viability was determined by MTT assay.

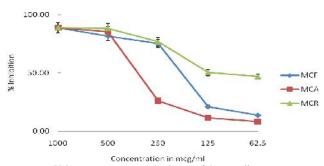
## RESULTS AND REMONSTRATION

In vitro anticancer activity of the extracts was performed by assessing cytotoxicity studies in 6 cell lines among which 5 were cancerous cell lines and one was normal cell line. The cell lines used were MCF-7, HepG2, HeLa, PC3, A549 and Vero cell lines.

Among the extracts of MCA, MCF and MCR, MCF was found to be comparatively more toxic to HEP G2 cells. Its average  $CTC_{50}$  was found to be 196  $\mu$ g/ml compared to the extract MCR which is least toxic (>1000 µg/ml) (Table 1, Fig. 1). Among AGA, AGR and AGF, AGR was found to be potentially toxic at significantly low concentration of 95 µg/ml. However, the other two extracts are comparatively

Table 1: Cytotoxicity of Momordica cymbalaria fruit, aerial parts and root extracts against HEP G2 cell line by MTT assay Sr. No.  $CTC_{50}\left(\mu G/ml\right)\pm SD$ Test sample 1. MCF 196.67±15.28 2. MCA  $346.67 \pm 15.28$ MCR >1000

Values are mean  $\pm$  standard error of three replicates



Values are mean  $\pm$  standard error of three replicates

Fig. 1: Per cent inhibition of HEP G2 cell line by Momordica cymbalaria fruit, aerial parts and root extracts

Table 2 : Cytotoxicity of <i>Momordica cymbalaria</i> fruit, aerial parts and root extracts against MCF 7 cell line by MTT assay			
Sr. No.	Test sample	$CTC_{50} (\mu g/ml) \pm SD$	
1.	MCF	$156.67 \pm 15.28$	
2.	MCA	$283.33 \pm 15.28$	
3.	MCR	>1000	

Values are mean ± standard error of three replicates

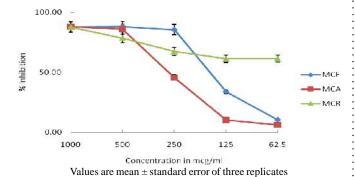


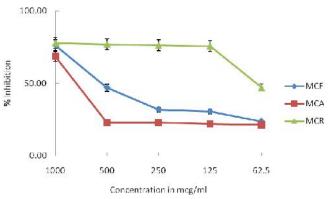
Fig. 2: Per cent inhibition of MCF 7 cell line by Momordica cymbalaria fruit, aerial parts and root extracts

not very toxic (Table 2, Fig. 2).

MCF and MCA were found to be moderately toxic at 156 and 283 µg/ml, respectively (Table 3, Fig. 3). GXA posseses the CTC<sub>50</sub> of 810 µg/ml. However, other extracts were found to be not toxic up to the concentration of 1000 µg/ml to MCF 7 cells (Table 4, Fig. 4).

Table 3: Cytotoxicity of *Momordica cymbalaria* fruit, aerial parts and root extracts against HeLa cell line by MTT assay Sr. No. Test sample AVG CTC<sub>50</sub> (µg/ml)±SD MCF 556.67±20.82 1. 2. MCA  $800.00\pm17.32$ MCR 67.50±4.33

Values are mean  $\pm$  standard error of three replicates

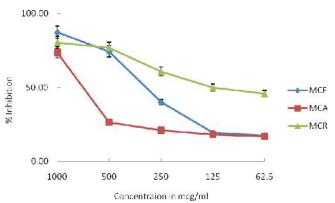


Values are mean ± standard error of three replicates

Fig. 3: Per cent inhibition of HeLa cell line by Momordica cymbalaria fruit, aerial parts and root extracts

Table 4: Cytotoxicity of <i>Momordica cymbalaria</i> fruit, aerial parts and root extracts against PC 3 cell line by MTT assay				
Sr. No.	Test sample	AVG CTC <sub>50</sub> ( $\mu$ g/ml) $\pm$ SD		
1.	MCF	406.66±11.54		
2.	MCA	$750 \pm 10.00$		
3.	MCR	123.33±15.27		

Values are mean ± standard error of three replicates



Values are mean  $\pm$  standard error of three replicates

Fig. 4: Per cent inhibition of PC 3 cell line by Momordica cymbalaria fruit, aerial parts and root extracts

In the case of toxicity to HeLa cells, MCR was found to be most potentially toxic with average CTC<sub>50</sub> of 67µg/ml (Table 5, Fig. 5). GXR was found to be toxic with average CTC<sub>50</sub> of 303 µg/ml. This was followed by GXF and GXA with average CTC<sub>50</sub> values of 303 and 456 μg/ml (Table 6, Fig. 6). MCF, AGR, AGA and MCA were found to be

Table 5: Cytotoxicity of Momordica cymbalaria fruit, aerial parts and root extracts against A 549 cell line by MTT assay Sr. No. Test sample AVG CTC50 (µg/ml)±SD MCF 1. 386.67±5.77 2. MCA 373.33±5.77 3. MCR 403.00±7.64

Values are mean  $\pm$  standard error of three replicates

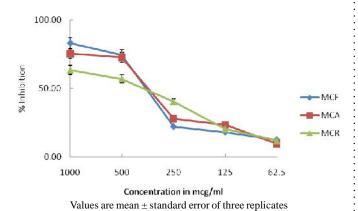


Fig. 5: Per cent inhibition of A 549 cell line by Momordica cymbalaria fruit, aerial parts and root extracts

Table 6 : Cytotoxicity of <i>Momordica cymbalaria</i> fruit, aerial parts and root extracts against vero cell line by MTT assay			
Sr. No.	Test sample	AVG CTC <sub>50</sub> (µg/ml)±SD	
1.	MCF	513.33±5.77	
2.	MCA	>1000	
3.	MCR	63.67±11.93	

Values are mean ± standard error of three replicates

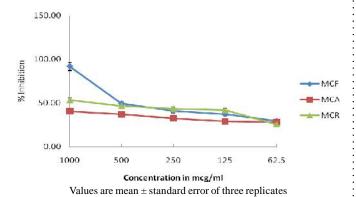


Fig. 6: Per cent inhibition of Vero cell line by Momordica cymbalaria fruit, aerial parts and root extracts

possessing average CTC<sub>50</sub> values of 556, 570, 660 and 800  $\mu g/ml$  respectively. AGF was found to be not toxic up to the concentration range of 1000 µg/ml to HeLa cells (Table 7, Fig. 7).

Table 7: Hepatoprotective activities of <i>Momordica cymbalaria</i> fruit, aerial parts and root extracts in HepG2 cell line				
Sr. No.	Plant parts	Test Concn. in µg/ml	% Protection offered over control	
1.	MCF	200	53.6±0.11	
2.	MCA	200	82.4±0.04	
3.	MCR	200	72.8±0.04	
4.	Silymarin	250	96.2±0.02	
5.	Cell control	-	100.0	
6.	Paracetamol (10mm)	-	0	

Values are mean ± standard error of three replicates

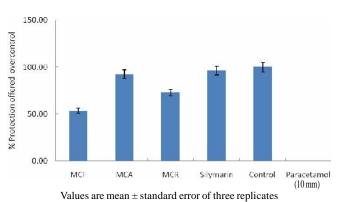


Fig. 7: Percentage protection offered by Momordica cymbalaria fruit, aerial parts and roots extracts (200 µg/ml) and standard drug Silymarin (250 µg/ml) in HepG2 cell line

Among MCA, MCF and MCR extracts, MCR possesses more toxicity to PC<sub>3</sub> cells than the others (Table 8, Fig. 8).

Table 8 : Hepatoprotective activities of <i>Momordica cymbalaria</i> fruit, aerial parts and root extracts in primary rat hepatocytes				
Sr. No.	Test drug	Test concn. in µg/ml	% Protection offered over control	
1.	MCF	200	42.61±0.04	
2.	MCA	200	78.89±0.02	
3.	MCR	200	57.66±0.03	
4.	Silymarin	250	85.28±0.03	
5.	Cell control	-	100.0	
6.	Paracetamol (10mm)	- 1	0	

Values are mean ± standard error of three replicates

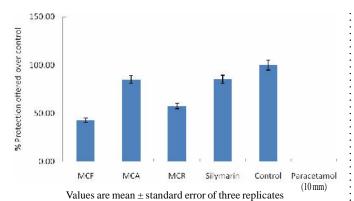


Fig. 8: Percentage protection offered by Momordica cymbalaria fruit, aerial part and root extracts (200 µg/ml) and standard drug Silymarin (250 µg/ml) in primary hepatocytes

With regard to A549 cell lines, the extracts MCA, MCR and MCF found to possess moderate toxicity and all the three extracts shown to exhibit almost same toxicity (Table 5, Fig.

Potential toxicity towards Vero cell lines was exhibited by MCR (Table 6, Fig. 6) and GXF with average CTC<sub>50</sub> values of 63 and 93µg/ml, respectively (Table 7, Fig. 7). MCA and AGF were not toxic in the tested concentrations up to 1000µg/ ml. Whereas GXR, AGR, MCF, AGA and GXA were having the  $CTC_{50}$  values of 341, 403, 513, 763 and 813µg/ml, respectively (Table 8, Fig. 8).

#### **Hepatoprotective activity:**

The drug Silymarin was found to exhibit 96.2 per cent protection against Paracetamol induced toxicity in Hep G2 cells at the tested concentration of 250mg/ml. It was found that MCA and MCR were found to have comparatively protective like Silymarin. These extract exhibited 82.4 and 72.8 per cent protection against Paracetamol induced toxicity in Hep G2 Cells at the concentration of 200µg/ml, respectively. However, other extract fruit (56.6% protection) did not exhibit much activity at the tested concentration of 200 μg/ml (Table 7, Fig. 7).

The drug Silymarin was found to exhibit 85.28 per cent protection against paracetamol induced toxicity in Primary rat hepatocytes at the tested concentration of 250 µg/ml. It was found that MCA and MCR were found to have comparatively protective like Silymarin. These extract exhibited 78.89 and 57.66 per cent protection against Paracetamol induced toxicity in primary rat hepatocytes at the concentration of 200µg/ml, respectively. However, other extract (fruit) did not exhibit much activity at the tested concentration of 200 µg/ml (Table 8, Fig. 8).

#### **Conclusion:**

In the case of HeLa cells, MCR was found to be most

potentially toxic with average  $CTC_{50}$  of 67  $\mu g/ml$ . MCR possesses more toxicity to PC3 cells than the others. Potential toxicity towards Vero cell lines was exhibited by MCR with average CTC<sub>50</sub> value of 63 µg/ml.

Our results indicate that the drug Silymarin was found to exhibit 96 per cent protection against Paracetamol induced toxicity in Hep G2 cells at the tested concentration of 250 µg/ml. Among the parts of Momordica cymbalaria, MCA and MCR found to have slightly lesser activity compared to Sylimarin. In paracetamol induced toxicity of primary rat hepatocytes, the drug Silymarin was found to exhibit 85.28 per cent protection aginstParacetamol induced toxicity in Primary rat hepatocytes at the tested concentration of 250 µg/ml. MCA was found to exhibit comparatively similar protective power than silymarin. The results clearly demonstrate that the extract MCR exert potential anticancer and hepatoprotective activities.

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