



EDIBLE MUSHROOMS AS FUNCTIONAL FOODS

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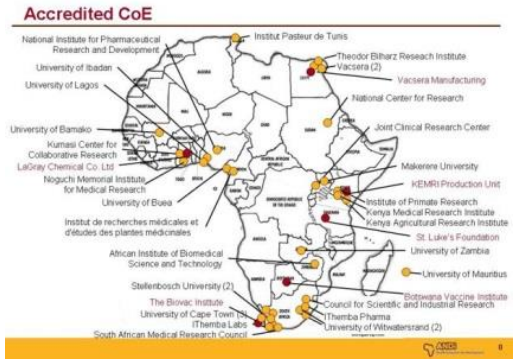

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Mushrooms as functional food

Mushrooms

High in Protein (mean proportion of 33.4%)

Low in fats (2-6% dry matter)

Calories (20.3-74.3%)

Relatively rich in riboflavin, niacin, ergocalciferol

Elevated levels of phosphorus and potassium and fairly high contents of magnesium

Main Classes of Phenolic Compounds

- PHENOLIC ACIDS
- FLAVONOIDS

ERGOTHIONEINE a potential antioxidant present in most mushroom species

Antitumor, antibacterial, and antifungal properties

Anticancer Properties of Mushrooms

Clinical trials in cancer patients

Ganoderma lucidum

"Mushroom of Immortality"

Chemopreventive and antitumour activities

Complement chemotherapy and radiation therapy

By countering the side-effects of cancer e.g. nausea, bone marrow suppression, anemia, and lowered resistance

Research in edible mushrooms is gaining popularity

Proteoglycans derived from fruiting body and mycelia of *Pleurotus ostreatus*

Possess immuno-modulatory and antitumor properties

In vitro* and *in vivo* anticancer activities of *Agaricus bisporus

Suppress aromatase activity and estrogen biosynthesis

Potential use in treatment of breast cancer

Mushroom Samples

Pleurotus sp.

Pleurotus sajor-caju

CC 201

CC 200

Pleurotus Hybrids

Mushroom Unit
(Food and Agricultural Research and Extension Unit)

Harvested at optimum maturity stage

Agaricus bisporus

Commercially available
(S.K.C Surat and Co Ltd)

Obtained at optimum maturity stage

Methanolic extracts obtained by exhaustive extraction

- **Total Phenolic content**
- **Total Flavonoid content**
- **Total Proanthocyanidin content**
- **HPLC Analysis**

Phenolic Profile of Mushrooms

- Total Phenolic content of the mushroom extracts ranged from 33.28 to 133.69 mg/g DW.

Maximum flavonoid levels (4.63 ± 0.052 mg/g DW)

Highest level of total phenolics (133.69 ± 3.204 mg/g DW)

Negligible proanthocyanidins

Negligible proanthocyanidins

Phenolic Profile of Mushrooms

HPLC Analysis of phenolic compounds and ergothioneine in the four mushroom extracts

	Pleurotus sajor-caju (CC 114)	*Pleurotus* hybrid (CC 201)	*Pleurotus* hybrid (CC 200)	*Agaricus bisporus*
	Phenolic content of mushrooms (µg/g DW)			
[I]Gallic acid	$356.90^{b} \pm 38.922$	$240.34^{c} \pm 12.186$	$224.71^{c} \pm 4.364$	$726.17^{a} \pm 4.263$
[II]Protocatechuic acid	$630.00^{a} \pm 15.161$	$590.52^{b} \pm 15.267$	$362.91^{c} \pm 10.469$	$84.85^{d} \pm 16.143$
[IV]Pyrogallol	$2831.32^{c} \pm 105.838$	$5457.03^{b} \pm 149.911$	$7791.24^{a} \pm 121.08$	$2354.68^{d} \pm 88.626$
	Ergothioneine content of mushrooms (µg/g DW)			
[III]Ergothioneine	$2518.88^{a} \pm 22.181$	$1596.867^{c} \pm 13.958$	$1351.04^{d} \pm 13.116$	$2261.18^{b} \pm 14.563$

DW: Dry weight; [I]Retention time (R.T)=5.787; [II]R.T=11.773; [III]R.T=3.829; [IV]R.T=7.339;
Data expressed as mean ± standard deviation (n=5); ANOVA and Fisher's LSD Test at 5% significance level;
Common superscripts between rows represent no significant difference between mushroom samples.

Animal Study

Balb/c mice
(7 weeks of age, 20 ± 2 g, n=120)

Group I-Control

Group II- Carcinogen treated

Treatment Period of 3 months

Phosphate Saline Buffer (PBS) only

N-methyl, N-nitrosourea (MNU) at 50 mg/kg body weight (i.p.)

Blood Sample

Haematological Biochemical Analyses

Histological Analyses

Mice Liver DNA

Laser Raman Spectroscopy

ME_1

Pleurotus sajor-caju

Group	Dose (mg/kg b.w.)
III	150 + MNU (i.p.)
IV	300 + MNU (i.p.)
V	450 + MNU (i.p.)
VI	600 + MNU (i.p.)
VII	600

ME_2

Agaricus bisporus

Group	Dose (mg/kg b.w.)
VIII	150 + MNU (i.p.)
IX	300 + MNU (i.p.)
X	450 + MNU (i.p.)
XI	600 + MNU (i.p.)
XII	600

Diets high in nitrostable foods cause cancer

- high levels of nitrates used in food preservation are carcinogenic



- Examples of foods <u>naturally</u> high in nitrates:

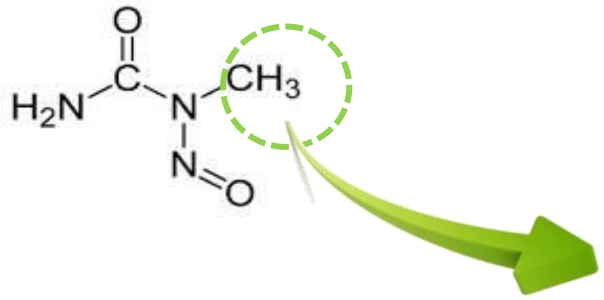
Fish, oysters, mussels, crab, lobster, Chinese cabbage, some leafy vegetables, Cigarette smoke, Beer & wine, Cheese, luncheon & sausage meats, Canned foods

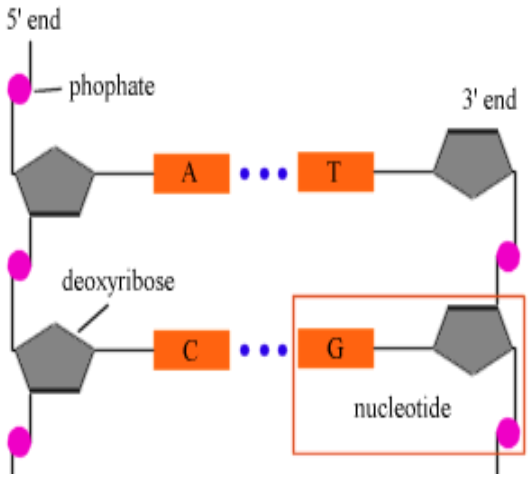
A possible reason why Asians have high rates of stomach & mouth cancers?

Example: **N-methyl-N-nitrosurea** (MNU)

MNU causes several cancers in animal models. It targets the <u>liver</u> in mice

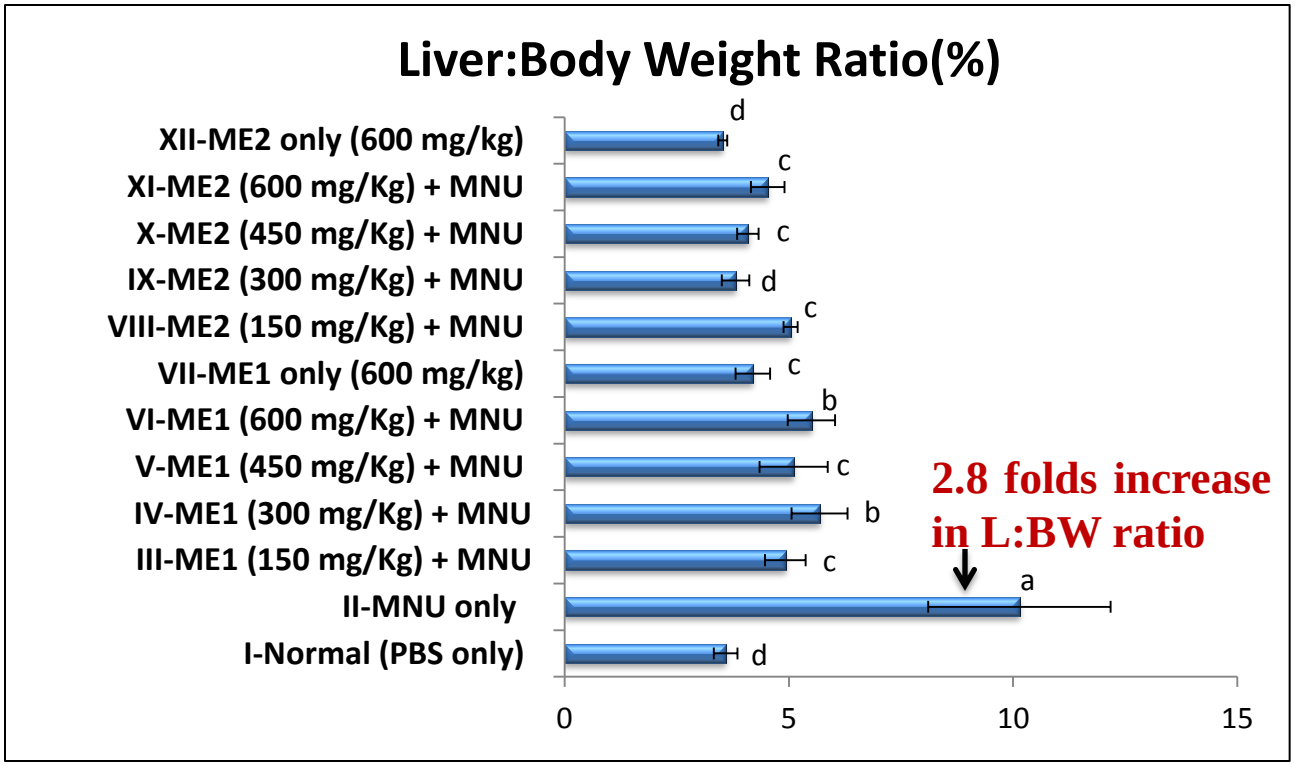
Methyl group transferred to nucleobases





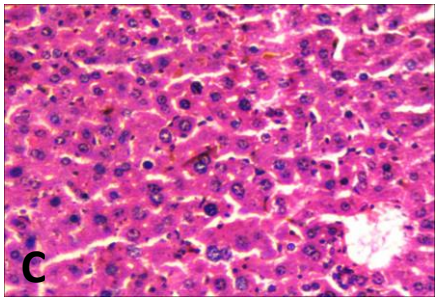
Causes **AT:GC** mutations

The liver/body weight ratio is highly indicative of tumour presence



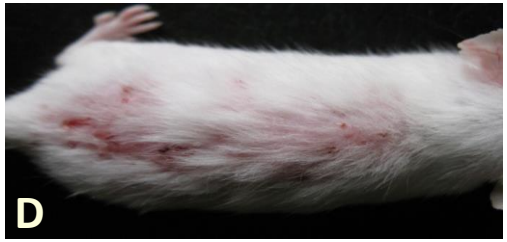
Liver:Body weight ratio for the 12 treatment groups Data expressed as mean ± standard deviation (error bars) (n=5); ANOVA and LSD at 5% significance; Similar superscripts on the mean values represent no significant differences between the treatment groups.

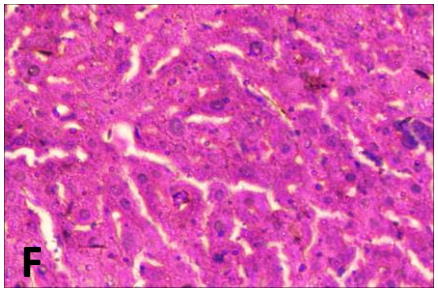
PBS-treated mice



- Smooth and even growth of hairs in normal mice
- Healthy liver

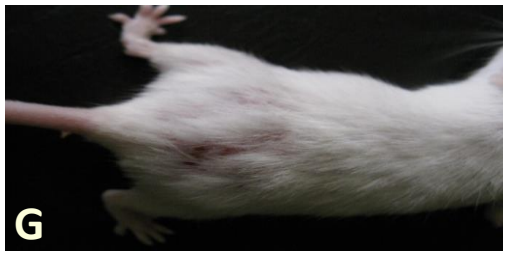
MNU-treated mice

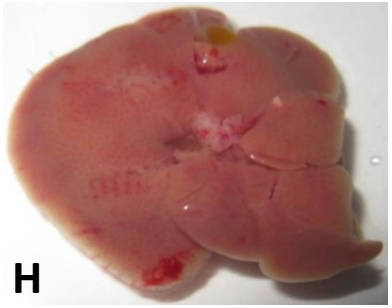


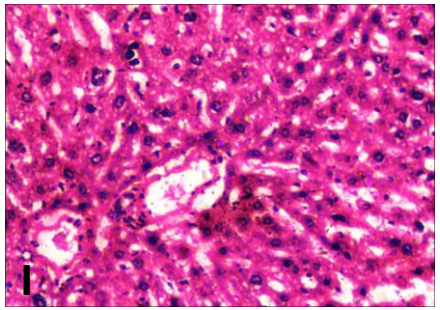


- Pachy skin and uneven shedding of hairs
- H&E stained liver section: Nucleo-cytoplasmic ratio (cells appearing leaky), enlarged vacuolated hepatocytes, altered cell structure

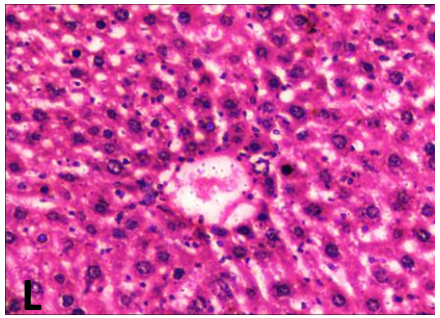
MNU + ME_1 300mg/kg







MNU + ME_2 450mg/kg



Extract Protective effect:
- Reduction in hair loss
- Reduction in lesions
- Cell architecture almost comparative to PBS

Morphological changes in mice and liver, and H&E stained liver sections from PBS, MNU, MNU+ME_1 300mg/Kg and ME_2 450mg/Kg groups after 3 months supplementation.

Reduction in lesions and mushroom protective effects (MNU + 300 mg/kg *Mushroom A* extract)

Healthy liver of Balb/c mice

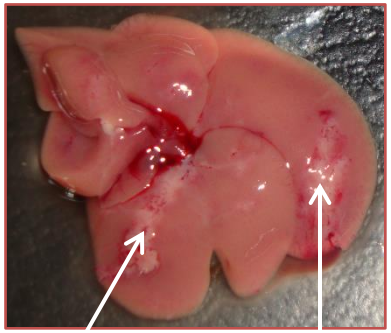
A

C

MNU

(N-Methyl-N-nitrosourea)

B

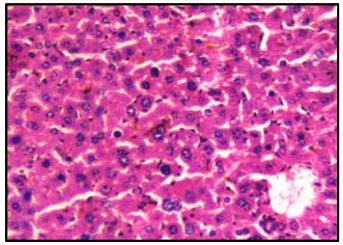


Micronodular lesions in liver of MNU-treated mice

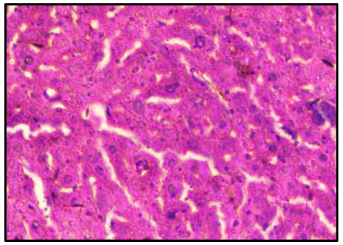
D

Histopathology of liver tissue

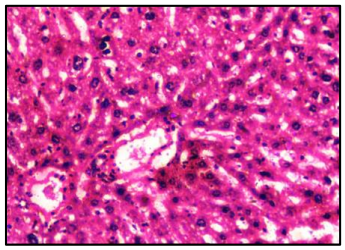
A



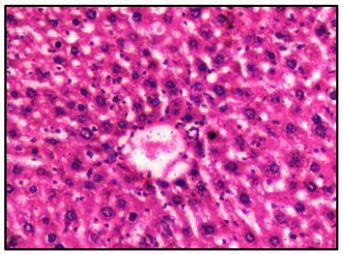
B



C



D



ONGOING

Molecular Mechanisms Works

University of Seoul, South Korea

Reduction in lesions and mushroom protective effects (MNU + 450 mg/kg *Mushroom B* extract)

MNU

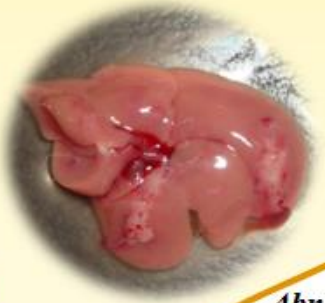
Low growth rate

T-Day 92: 57% body weight ↓

MNU

Hepatocarcinogenesis

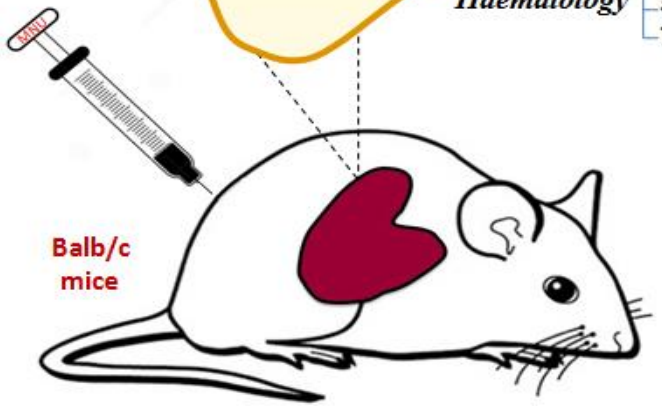
- 65 % L:B weight ratio ↑
- Liver antioxidative enzyme activity: 55% CAT, 20% SOD, 42% GR, 61 % GPx ↓
- 37 % Protein, 45% FRAP ↓
- Lipid peroxidation: 70% MDA ↑
- MNU-DNA interaction ↑
- Micronodular lesions in liver ↑



MNU

Abnormal Haemalotogy

- 31% Hb, 51% Lymphocytes ↓
- 8% polymorphs, 27% RBC ↑
- 39% platelets, 82% esonophils ↑
- 76% monocytes ↑



MNU

MNU

	ME_1	ME_2
↑ Protein	10%	10%
↑ CAT	11%	32%
↑ SOD	11%	9%
↑ GR	11%	22%
↑ GPx	21%	50%
↑ FRAP	24%	27%
↓ MDA	63%	45%

Oxidative stress

Tumorigenesis

	ME_1	ME_2
↓ Micronodular lesions in liver		
↓ L:B	44%	60%

DNA damage

ME_1 and ME_2 : ↓ raman peak intensities and MNU-DNA interations

Extract supplementation in MNU-primed mice

ME_1 (300 mg/kg b.w.)

ME_2 (450 mg/kg b.w.)

	ME_1	ME_2
↑ Hb	28%	23%
↑ Lymphocytes	5%	31%
↓ Polymorphs	10%	14%
↓ Monocytes	75%	80%
↓ Esonophils	75%	73%
↓ Platelets	30%	22%
↓ RBC	49%	33%

Immunomodulatory activity

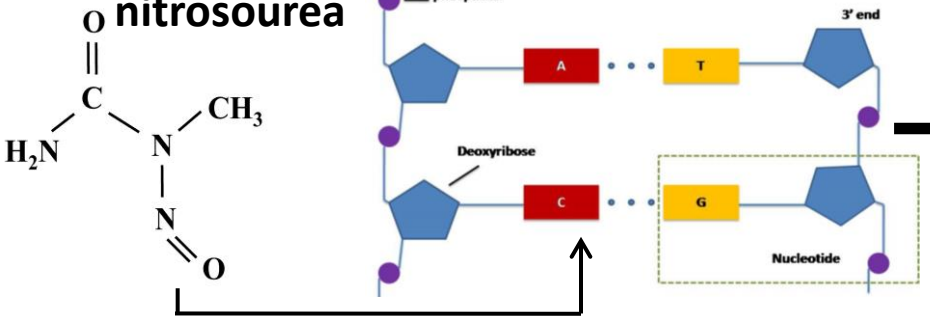
Restores normal growth

	ME_1	ME_2
↑ Body weight on T-Day 92	37%	50%

CBBR

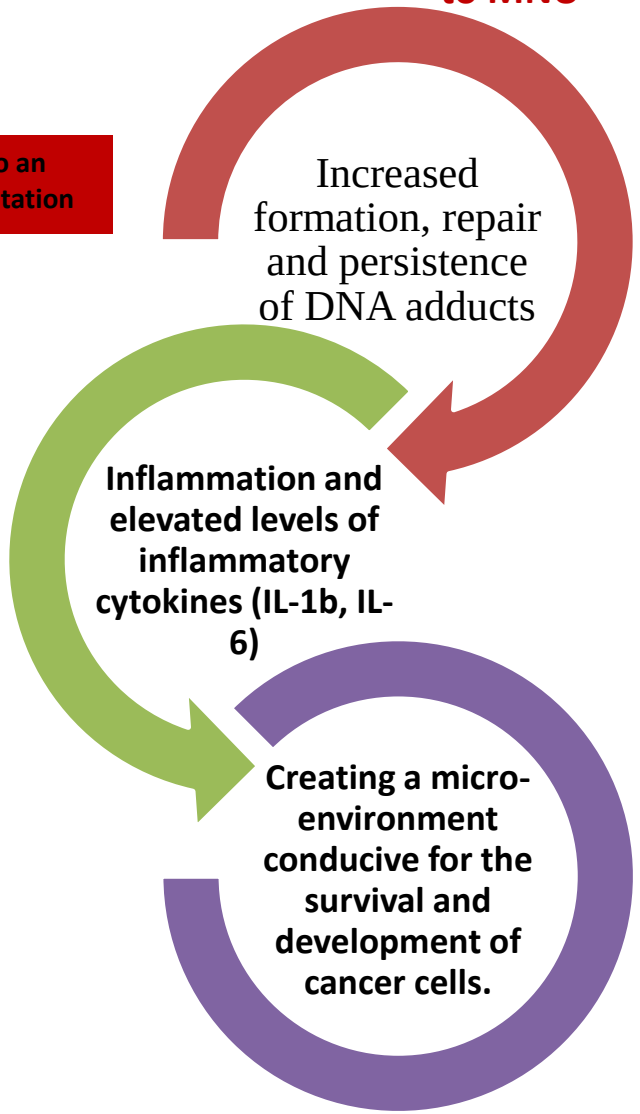
Laser Raman Spectroscopy

N-methyl-N-nitrosourea

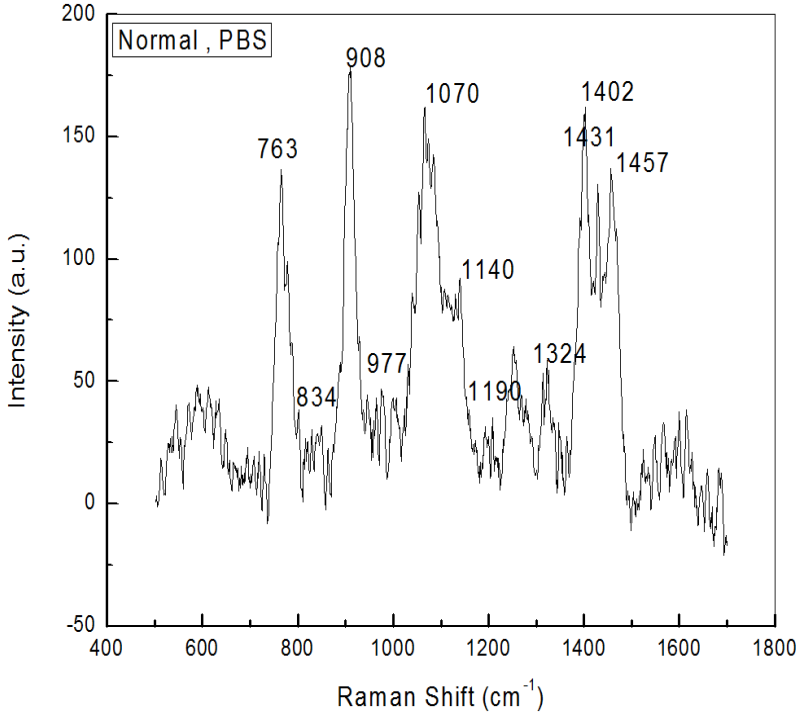


Leads to an AT:GC mutation

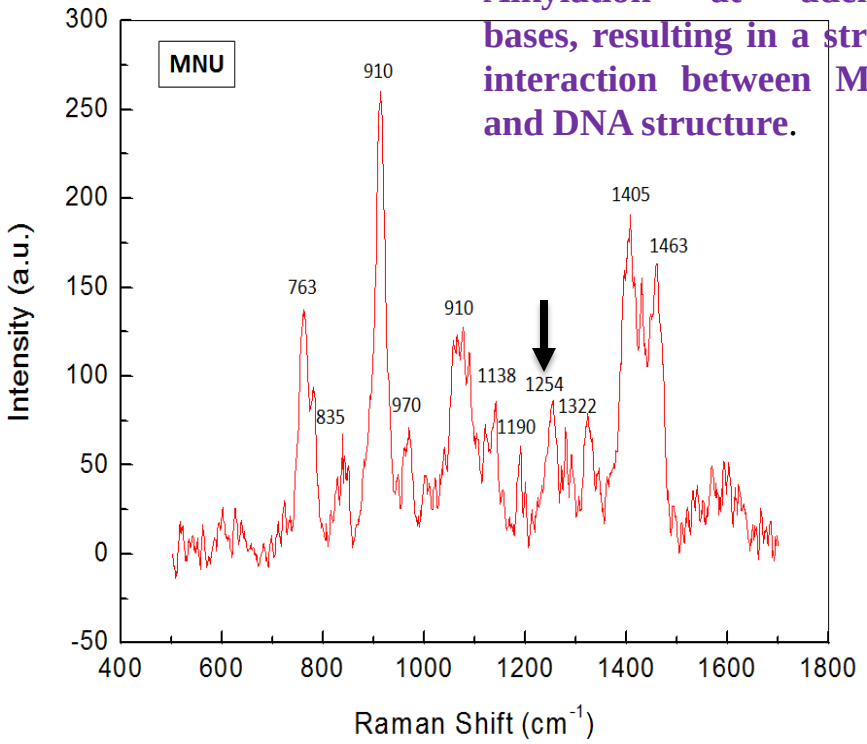
Continuous exposure to MNU

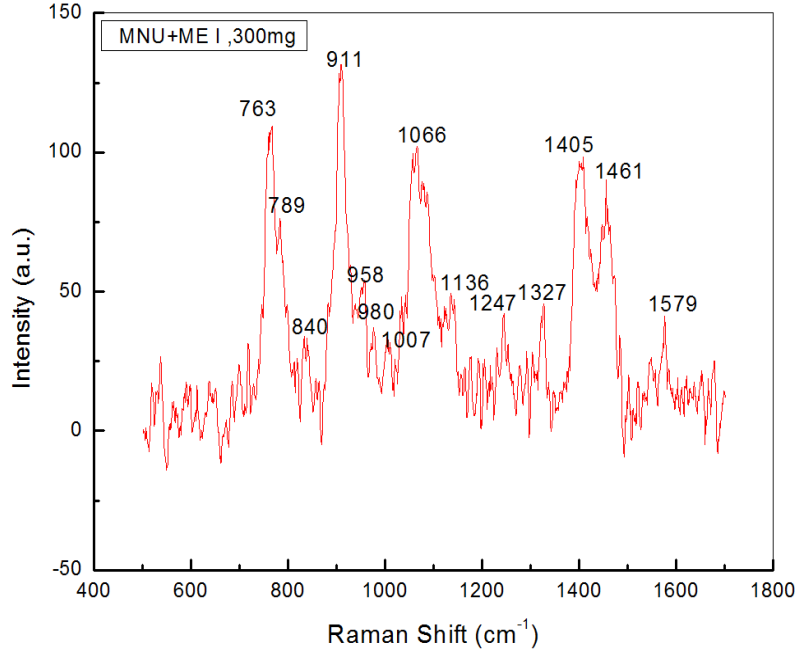


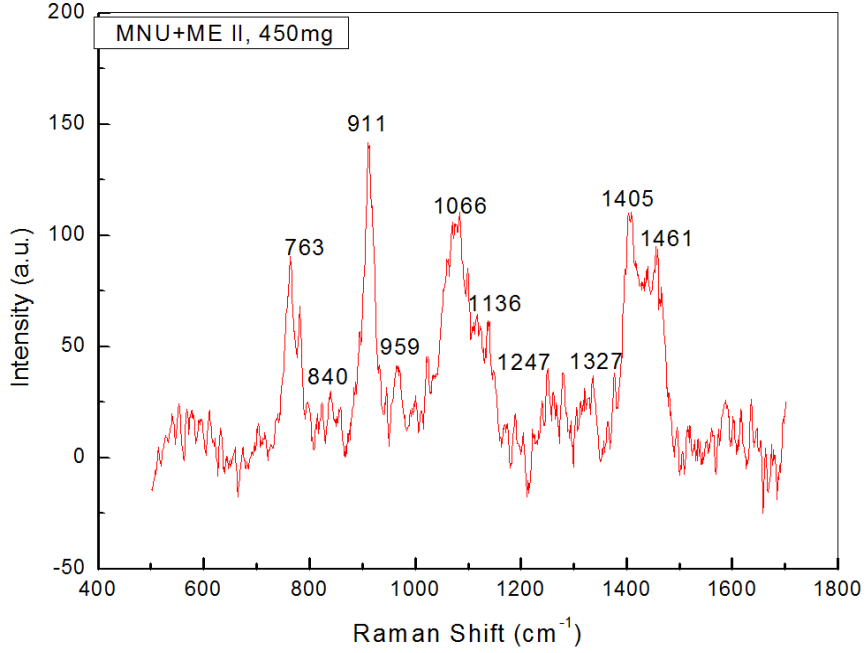
- MNU acts as an alkylating agent reacting with nucleophillic nitrogen, oxygen atoms in bases and DNA phosphate groups to create mutagenic lesions.
- The region 1200–1600 cm^{-1} (assigned to purines and pyramidines) corresponds to nucleic bases which are prone to any type of alkylation by MNU.
- Mushroom protective effect was confirmed by Raman spectroscopy where, the MNU-DNA interaction as evidenced by an intense peak at 1254 cm^{-1} was normalised. and was not apparent in any of the mushroom-treated DNA samples.



Alkylation at adenine bases, resulting in a strong interaction between MNU and DNA structure.







Food & Function

PAPER

Modulation of hepatocarcinogenesis in *N*-methyl-*N*-nitrosourea treated Balb/c mice by using mushroom extracts

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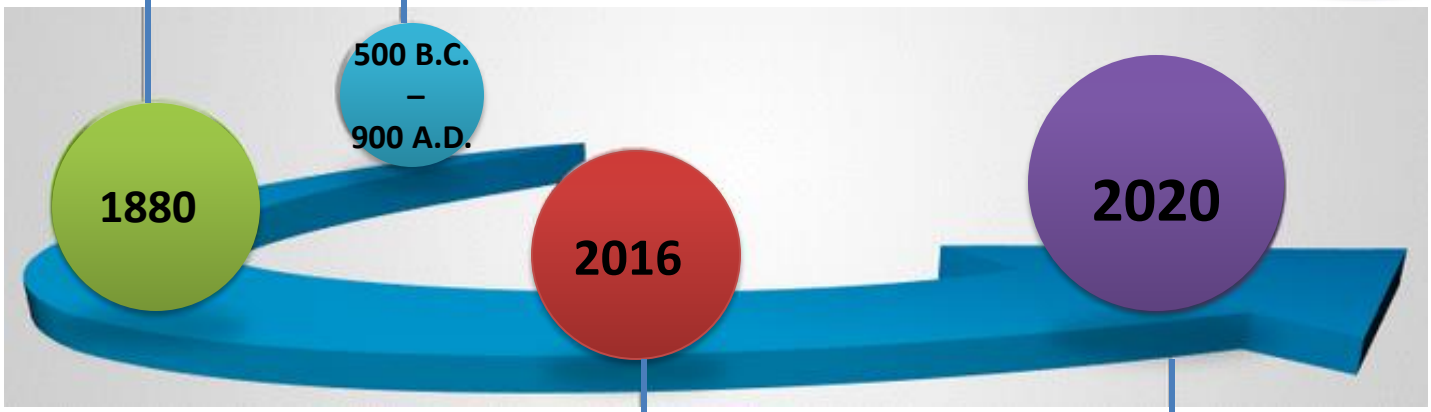
Prospects

USA: Commercial Production of *Agaricus bisporus*

Use of hallucinogenic mushrooms in Mesoamerica

Cheap source of protein with ease of home production



300 edible mushroom species identified
20 fold increase in mushroom production
Medicinal mushrooms and their derivatives-Clinical trials

Scientific Data on Mauritian mushrooms

Mauritius ?
Still not meeting local demand, high imports

Scope for increasing cultivation
Production of active extracts

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