

Ayurvedic Inheritance of India
Dr. M. S. Valiathan
National Research Professor, Manipal University
Indian Institute of Technology, Madras

Module - 9
Lecture - 19
Ayurvedic Biology: Illustrative Studies

Shortly after the beginning of the new millennium, the Indian academy of sciences brought out a decadal vision document which they called towards Ayurvedic biology; that was perhaps the first time the world heard of this term Ayurvedic biology. Now that showed a new vista in biological research very different from the drug relative research which had been going on for over a 100 years and very different from clinical trials in Ayurveda which had been going on to a much lesser extent.

Quintessentially, it is the application of modern biology which characterizes the new millennium to the study of Ayurvedic questions especially in the concepts of Ayurveda or procedures in Ayurveda. Now this Ayurvedic biology in that document itself, they had given some examples; for example the Dosha Prakriti, Vata, Pitta, Kapha, so very important in Ayurveda a concept, does it have a biological basis something that is demonstrable through experiment; that was a question they had posed. Similarly a very commonly done procedure like Panchakarma, so widely used, what are its metabolic and immunologic correlates, are there any correlates at all?

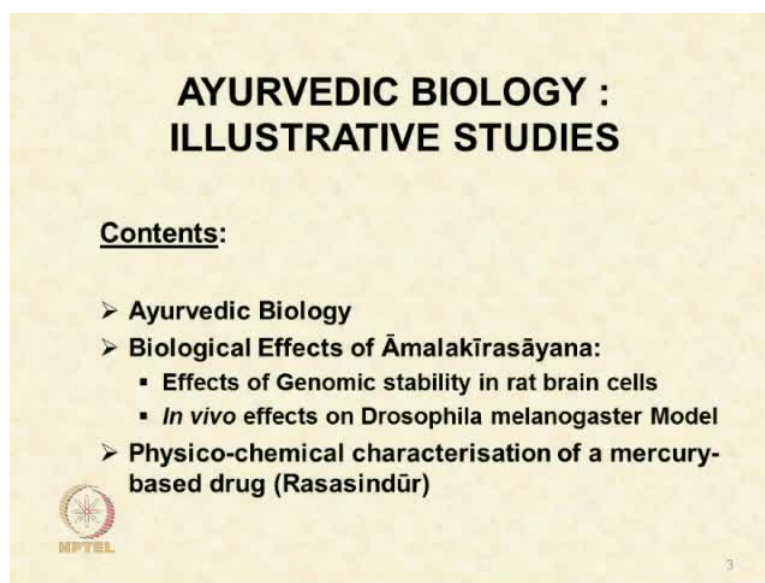
Like these they had posed several questions and this document caught the attention of the principal scientific advisor to the Government of India, Doctor Chidambaram. He noted this, and he indicated a willingness to do some probe into these questions from his office. And if that probe showed some promise, then some agency of the Government of India would be willing to take it over for regular support which was a very farsighted gesture on the part of Doctor Chidambaram. And some of these projects were approved; one of them was the Dosha Prakriti project, another was the Panchakarma project and a third project mentioned there is the Rasayanas which are again very commonly used an important branch of Ayurveda.

When you do this Rasayana therapy, what happens in the body in biological parameters which are measurable; that was the question posed. Another was Bhasmas, mercury derived Bhasmas, controversial; its toxicity is denied, is feared by a large group of people the world forbids the use of mercury whereas in Indian traditional systems they use it. So, is there something in the way it is processed that could make it not so toxic? Now these were questions; some of these were approved for funding initially by PSA's office; that is where whole project began.

Now the first 3, 4 years certainly the project showed sufficient promise for the department of science and technology to step forward and say we will take it over as a regular schemes for funding and that was called task force in Ayurvedic Biology. Now in today's lecture, I will be giving you the results of some of these projects especially two studies carried out in Rasayana and also something about the Ras-Sindoor, the mercury derived Bhasma. Now the first two Rasayana studies have been published in very good journals and the Bhasma project is under review for another very good journal, so I will be mentioning these.

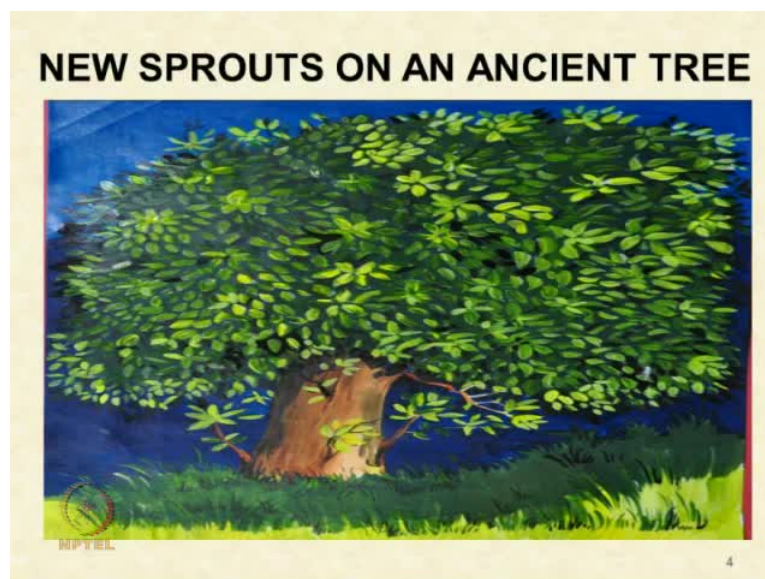
And also a fourth project which was done in CIMAP, Central for Aromatic and Medicinal plants in Lucknow a CSIR institute. The two young scientists they are Sasani and Shukla, they had investigated a question Ayurvedic plants which are anti-Vata, anti-Pitta, anti-Kapha; these are known, they are taxonomically very different. In one group anti-Vata the plants are all very different, diverse, chemically, taxonomically, then what is it that makes them therapeutically identical, they are all anti-Vata. Is there some molecular basis for this and this was a question they investigated that is the genomic basis of dosha balancing properties of medicinal plants which also has been published in a very good journal.

(Refer Slide Time: 05:09)



So, today I will be talking about this Rasayana projects, Ayurvedic biology in the Amalaki Rasayana is the main subject for today's discussion and in two models; one is in rats and, the other is in *Drosophila* and the Rasasindur project. These I will be talking about in some detail.

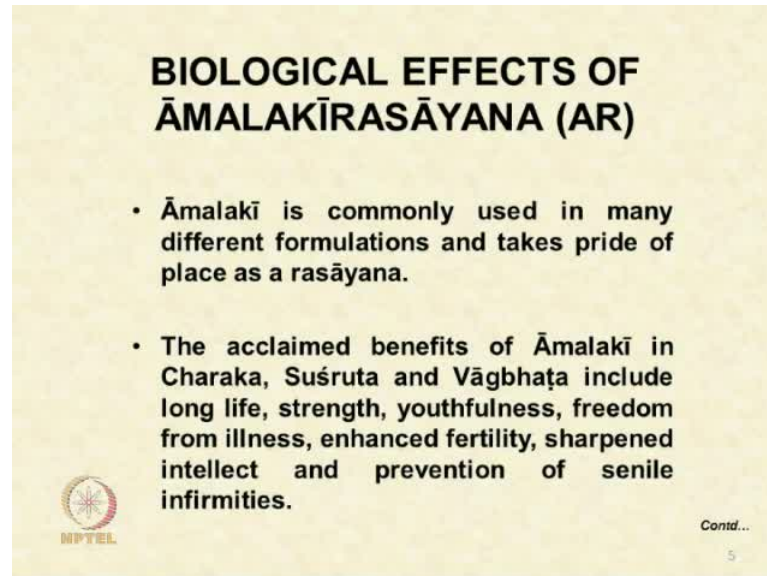
(Refer Slide Time: 05:31)



Now when I talk about these new Ayurvedic Biology projects in Ayurveda, an artist friend of mine, he got the idea that Ayurveda is like an ancient tree, suddenly it is putting


out new sprouts. So, he put it in a nice painting, which I have shown here, new sprouts on an ancient tree.

(Refer Slide Time: 05:59)



**BIOLOGICAL EFFECTS OF
ĀMALAKĪRASĀYANA (AR)**

- Āmalakī is commonly used in many different formulations and takes pride of place as a rasāyana.
- The acclaimed benefits of Āmalakī in Charaka, Suśruta and Vāgbhaṭa include long life, strength, youthfulness, freedom from illness, enhanced fertility, sharpened intellect and prevention of senile infirmities.

 Contd...
5


Now Amalaki is a very commonly used, widely used in pickles and so many different ways we use it, ordinary Indian households figured in the ancient Samhitas and it is used in many different ways, formulations and it is one of the most important Rasayanas. There are other Rasayanas like for example, Shankhpushpi. Now these are Medhya Rasayanas, they have a special property as a tonic for the brain; Medha is intellect.

So, this Amalaki is not one of those Medhya Rasayanas, it is for the whole body and Charaka Susruta, Vagbhata they have a number of Rasayana preparations based on Amalaki. And the effects include long life, strength, youthfulness, freedom from illness, enhanced fertility, sharpened, intellect and prevention of senile infirmities. These are all the general benefits which are claimed to be provided by these Rasayanas, Amalaki.

(Refer Slide Time: 07:25)

BIOLOGICAL EFFECTS OF ĀMALAKĪRASĀYANA (AR)

- Studies so far – mostly individual plant extracts of *Withania somnifera*, *Phyllanthus* (Amalaki) *emblica*, etc., - have tended to examine gross physiological parameters of the effect of rasāyanas; the present study of AR focuses on the beneficial effects on cellular DNA damage in rat brain; and several biological markers of *Drosophila melanogaster*.


6

Now most of these studies done so far, there have been many studies on this Amalaki. They have looked at the general effects that animals long life or its general health, weight; such gross measurements have been made, but here in this particular study we have been looking specifically at the DNA changes in rats, especially the brain and also the biological markers general biological markers like life span. These are measured in *Drosophila* which is certainly a very unusual model.

(Refer Slide Time: 07:43)

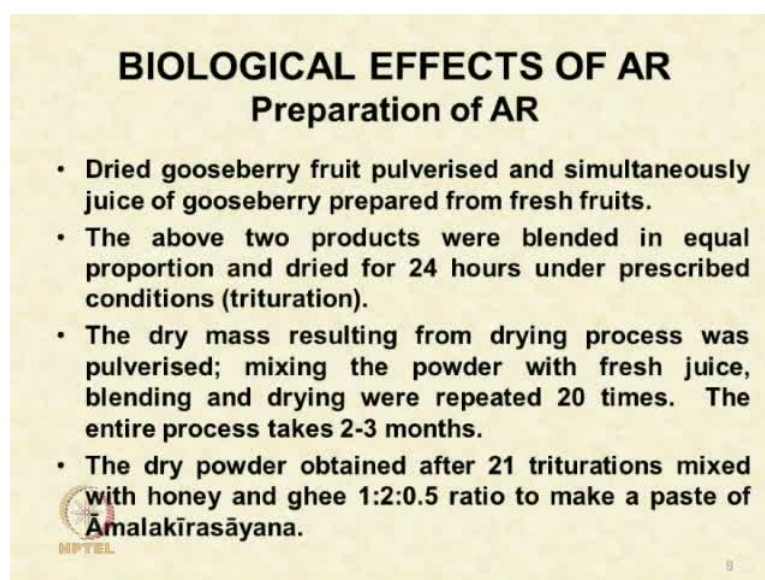
BIOLOGICAL EFFECTS OF AR

- The rasāyana for studies in rats and *Drosophila melanogaster* was prepared specially for this study at the Arya Vaidya Sala, Kottakkal; batch to batch consistency for chemical profile and quality standards assured.
- Studies on brain cellular DNA damage were done in the Jawaharlal Nehru Technological University by Professor Kalluri Subba Rao and colleagues.
- Studies on the effect of AR on *Drosophila melanogaster* were done at the Banaras Hindu University by Professor SC Lakhotia and colleagues.

7

Now Rasayana studies for rats and Drosophila, these were done in rats were done in Jawaharlal Nehru Technological University in Hyderabad and Drosophila studies were done in Banaras Hindu University and the Rasayana itself was prepared for this studies specifically by Arya Vaidya Sala, Kottakkal; it is not a commercial sample in other words. And the chief investigators in the Hyderabad study all the rat brain studies were done by Professor Kalluri Subba Rao and his colleagues and the Drosophila studies were done by professor Lakhotia and his colleagues in Banaras Hindu University. And the preparation of these Rasayanas specifically for this study was done by Doctor Burali and his colleagues in Kottakkal Arya Vaidya Sala.

(Refer Slide Time: 08:39)



BIOLOGICAL EFFECTS OF AR
Preparation of AR

- Dried gooseberry fruit pulverised and simultaneously juice of gooseberry prepared from fresh fruits.
- The above two products were blended in equal proportion and dried for 24 hours under prescribed conditions (trituration).
- The dry mass resulting from drying process was pulverised; mixing the powder with fresh juice, blending and drying were repeated 20 times. The entire process takes 2-3 months.
- The dry powder obtained after 21 triturations mixed with honey and ghee 1:2:0.5 ratio to make a paste of Amalakīrasāyana.

Preparation of the Amalaki Rasayana according to the ancient texts, dried gooseberry is pulverized and simultaneously the juice of gooseberry is prepared from fresh harvested fruits. Now these two products are blended in equal proportion and dried for 24 hours under prescribed conditions, this is called trituration. Now this dry mass resulting from the soft drying that is further pulverized and it is again mixed with same quantity of fresh juice of Amalaki. Now this process is repeated. So, trituration is repeated 21 times. Now that particular preparation which is a dry powder that is mixed with honey and ghee in the proportion of 1 is to 2 is to point 5. Now this paste is the Amalaki Rasayana which is used in this experiment.

(Refer Slide Time: 09:43)

BIOLOGICAL EFFECTS OF AR
Effects of Genomic stability in rat brain cells*

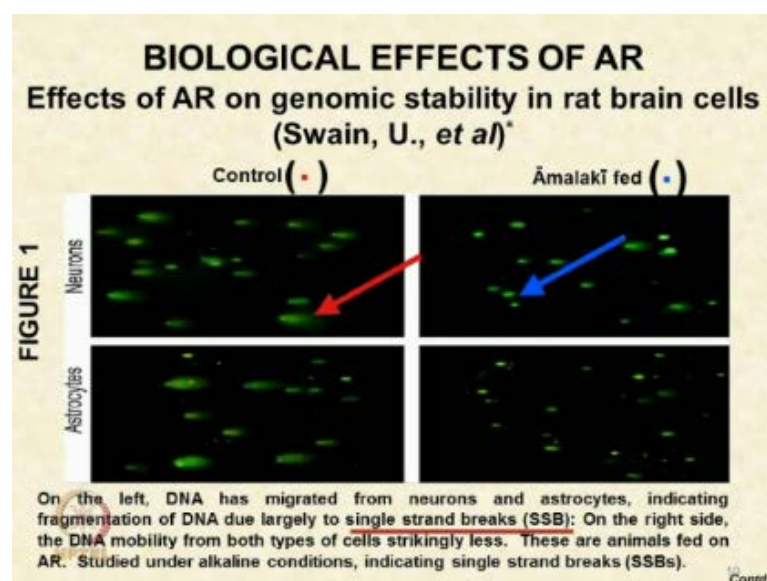
- Adult wistar rats (6 months old) fed Āmalakīrasāyana 5 days a week. (AR supplied by Arya Vaidya Sala, Kottakkal)
- At 3, 9 and 15 months, rats were killed and isolated cell suspensions of neurons and astrocytes prepared from cerebral cortex.
- DNA damage, as the prime index of genomic stability, was measured in terms of single and double strand breaks through comet assay and biochemical methods at 3, 9, and 15 months.

Swain, U., et al., Studies on the molecular correlates of genomic stability in rat brain cells following Āmalakīrasāyana therapy. Mech. Ageing Dev. (2011), doi:10.1016/j.mad.2011.10.006

Now what are the markers that we are looking for in this and that is the genomic stability which is measurable in terms of the chain breaks, single chain breaks and double chain breaks of two types of brain cells. One is neurons; the other is astrocytes in the cerebral cortex of rats. So, doctor Subba Rao's group has been using adult wistar rats, this brain preparation for DNA chain break studies for other purposes for a number of years they have a great deal of expertise in this kind of study. Now they use these adult wistar rats 6 months old, they are fed with Amalaki Rasayana 5 days a week. And this is supplied by Kottakkal Arya Vaidya Sala at 3, 9 and 15 months because these rats live around 2 years.

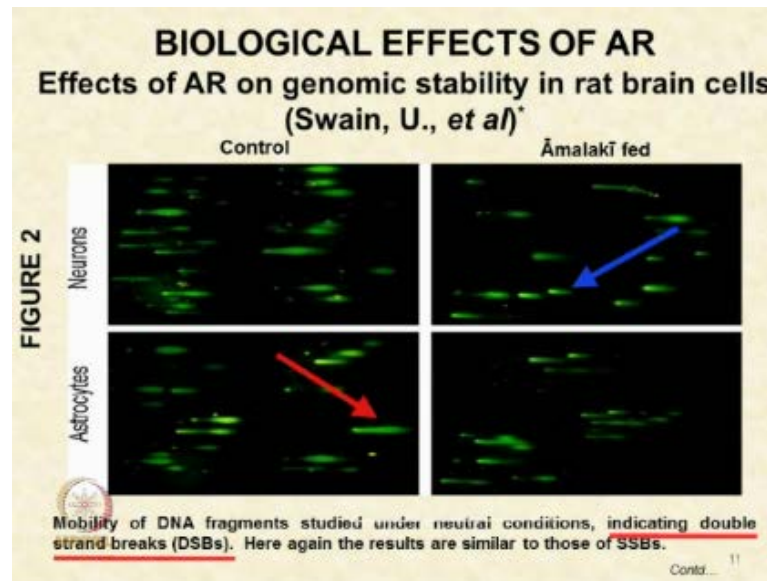
So, 3, 9 and 15 months these rats are killed and the isolated brain cell suspensions neurons and astrocytes, they are prepared from the cerebral cortex of these animals. And the DNA damage and the prime genomic index of stability is the chain break single strand and double strand and these are measured by comet assay; that is a commonly used technique for doing this. And Subba Rao has also used a biochemical method which he has an assay which he developed for double strand breaks; he has used that also for the double strand break. And this has been published in a mechanism of ageing and development in 2011 which is a high impact journal.

(Refer Slide Time: 11:25)



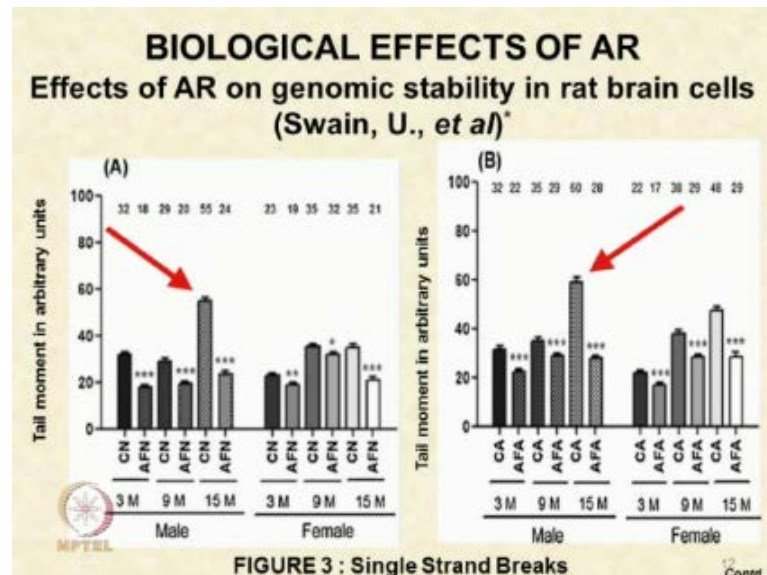
Now these are the pictures taken of the comet assay; the top two blocks those are the neurons and the bottom two are astrocytes. On the left hand side you have the controls, around the right hand side you have the Amalaki Rasayana fed animals; those are the four pictures that you see here. Now these markers which you see there, the points that you can see, these are actually pictures of DNA which has broken and then as soon as they break they begin to migrate, those are the photographs in comet assay. That is essentially what you are measuring in comet assay that is breakage of the DNA and its migration. Now if you look at it you can see not very clearly, but on the left hand side the controls both for neurons and astrocytes this breakage the tail. It is a little longer than what you see on the right hand side where the Rasayana is fed in both neurons as well as astrocytes.

(Refer Slide Time: 12:31)



But this will become much clearer because they have been and this is the double strand break, earlier you saw the single strand break. This is again a similar picture; perhaps that migration is better seen here compared to the single strand break. Now this will become much clearer when they are measured and their number is given.

(Refer Slide Time: 12:49)



Now here on the left hand side you have the neurons, right hand side you have the astrocytes. Now if you look at these bars here, one bar and the next bar has got asterisk on the top. So, here the bar without the asterisk that is the control whereas those with the


asterisk, these are the animals which have been fed Amalaki Rasayana. So, if you look at this neurons in all these you will find here the control, this is the number of chain break and this is the number of chain breaks. Now they have been measured here 32 and 18 and what is it that we are measuring? There is what is called tail movement and tail movement, in that tail which you saw in that picture, the number of DNA chain breaks a product of that and the length of the tail. Their arbitrary units, software library units they are called and those are measured; 75 measurements have been averaged and these numbers you can see here.

Now here if you look at the control at 3 months, 32 is the value whereas the Amalaki fed is 18 that is a 3 months. Now when you come to 15 months, this difference is very much magnified, in the control animals not receiving the Amalaki Rasayana is a 55 whereas those fed the Amalaki Rasayana it is only 24. So, it is significantly reduced and this is the female on the right hand side the same kind of measurements but less dramatic but same change, the trend is the same, the reading is 35 whereas those receiving the Amalaki Rasayana it is 21. So, the change is not that dramatic, but the trend is exactly the same. These are single strand brakes neurons as well as astrocytes.

(Refer Slide Time: 15:01)

BIOLOGICAL EFFECTS OF AR
Effects of AR on genomic stability in rat brain cells
(Swain, U., et al)*

- Shows cellular DNA damage expressed as “Tail moment” in control rats and those fed with AR at 3, 9 and 15 months. Panel A shows the effects in neurons and panel B shows the effects in astrocytes. (Tail moment – product of percentage of DNA in tail and tail length and expressed in software library units).
- Values above bars represent average values of tail moments derived from 75 analysis.

 Contd...


13

(Refer Slide Time: 15:11)

BIOLOGICAL EFFECTS OF AR

Effects of AR on genomic stability in rat brain cells (Swain, U., *et al*)^{*}

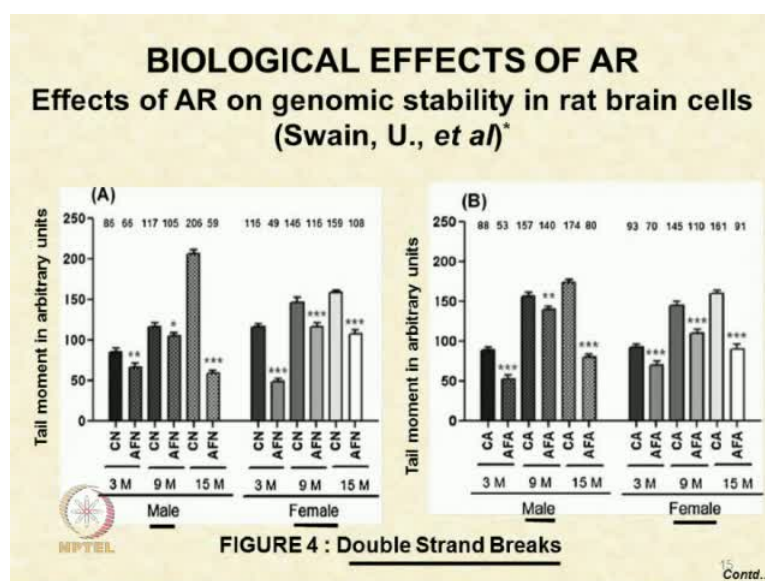
- In neurons (A) and astrocytes (B), the genomic stability in AR fed samples is better at 3 months and keeps improving to 15 months.
- The rise in cellular DNA damage seen in controls with ageing (3 to 15 months) seems to be decelerated by rasāyana therapy.


Contd...

14

Now then we move on; these are the descriptions of what I have just now told you the DNA damage expressed as ‘Tail moment’, values above bars represents 75 the number some of them I have read out to you and neurons and astrocytes, the genomic stability of the Amalaki Rasayana fed samples is better at 3 months and it keeps improving, maximal improvement is seen at 15 months. So, the rise in the cellular DNA damage in controls with ageing from 3 to 15 months seems to be decelerated, definitely slowed down by the use of Amalaki Rasayana.

(Refer Slide Time: 15:41)



Now here we look at the double strand breaks; again you see a single same kind of trend if you look at, say, 15 months the change is so much 206, these software library units whereas those which have been fed Amalaki Rasayana, it is only 59, 206 and 59. So, there is a very gray whereas in female it is 159 and 108 not so much, but again the trend is the same, so that the DNA chain breaks certainly very definite decrease in rats which have been fed Amalaki Rasayana.


(Refer Slide Time: 16:25)

BIOLOGICAL EFFECTS OF AR
Effects of AR on genomic stability in rat brain cells
(Swain, U., et al)*

- Results similar to Figure 3 and convey the same message.
- Both neuron and astrocytes in the rasāyana fed rats show greater genomic stability in AR fed rats.
- Beneficial effects of AR administration in keeping simple and double strand breaks in cellular DNA at a lower level clearly demonstrated.

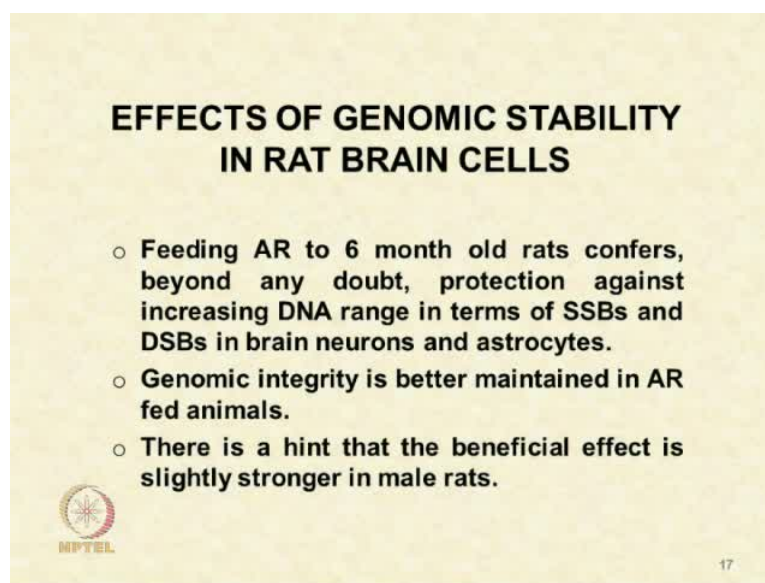
Contd...

16



Now if you look at this result again a summary of that both neuron and astrocytes in the Rasayana fed rats show great genomic stability in that Rasayana fed rats and beneficial effects of Amalaki Rasayana administration is in keeping with simple as well as double strand breaks.

(Refer Slide Time: 16:50)



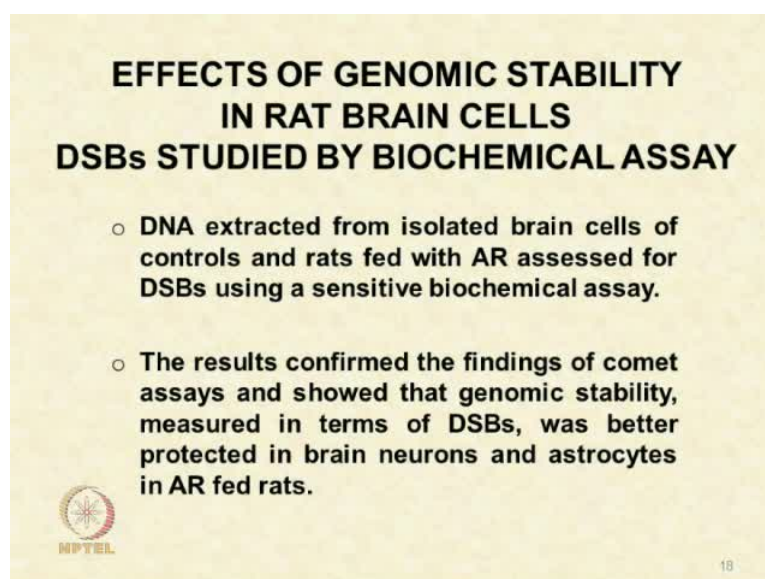
EFFECTS OF GENOMIC STABILITY IN RAT BRAIN CELLS

- Feeding AR to 6 month old rats confers, beyond any doubt, protection against increasing DNA damage in terms of SSBs and DSBs in brain neurons and astrocytes.
- Genomic integrity is better maintained in AR fed animals.
- There is a hint that the beneficial effect is slightly stronger in male rats.

MPTEL 17

Now feeding these at 6 months old rats confers beyond any doubt, protection against increasing DNA damage in terms of single strand breaks or double strand breaks, both in neurons and astrocytes, these are the two cells which have been used for this study. So, genomic integrity is much better maintained by the use of Amalaki Rasayana, even though Amalaki Rasayana is not really a Medhya Rasayana. In other words working specially on the brains, in spite of that you find this clear evidence.

(Refer Slide Time: 17:26)



EFFECTS OF GENOMIC STABILITY IN RAT BRAIN CELLS DSBs STUDIED BY BIOCHEMICAL ASSAY

- DNA extracted from isolated brain cells of controls and rats fed with AR assessed for DSBs using a sensitive biochemical assay.
- The results confirmed the findings of comet assays and showed that genomic stability, measured in terms of DSBs, was better protected in brain neurons and astrocytes in AR fed rats.

MPTEL 18

Doctor Subba Rao has developed his own biochemical assay for measuring this genomic stability. In this double strand breaks he has used that study at which collaborates the findings of the comet assay which we have looked at earlier.

(Refer Slide Time: 17:45)

BIOLOGICAL EFFECTS OF AR
In vivo* effects on *Drosophila melanogaster* model

- AR prepared from fruits of “amla” (*Phyllanthus emblica*) as per ancient protocol (*Astāngahridaya*) by Arya Vaidya Sala, Kottakkal.
- Appropriate dosage for fruit flies determined at 0.5% AR supplemented food.
- No attempt made to isolate “active principles” from AR as this approach has been unrewarding; it also contravenes the Ayurvedic principle of using formulations.

*Vibha Dwivedi, E. M. Anandan, Rajesh S. Mony, T. S. Muraleedharan, M. S. Valiathan, Mousumi Mutsuddi, Subhash C. Lakhota. In Vivo Effects of Traditional Ayurvedic Formulations in *Drosophila melanogaster* Model Relate with Therapeutic Applications. PLoS ONE Volume 7, Issue 5, e37113 May 2012

Contd...

19

Now this was the next model that was used in studying the same Amalaki Rasayana and this was not when we were discussing in a casual discussion with Professor Lakhota in Banaras who has had many years of experience working on *Drosophila* genetics. Once we were having a casual discussion I mentioned these findings of Subba Rao and he was the one who said, why not I try this on my fruit flies, so that was an interesting suggestion. So, very quickly we got this Amalaki Rasayana the same preparation from Kottakkal. And it took him time to find out the dosage, because it is not easy to determine the dosage for *Drosophila*. How much Amalaki Rasayana to give?

He took almost a year and he had his own doubts whether may be the results are produced by the ghee and honey that you are using. So, he fed them honey and ghee alone and see what happens. So, it took quite some time to determine the protocol, but anyway after a year finally it got started; they had made a personal visit to Kottakkal to see how this is being made and no attempt was made; from the beginning itself we realized the Amalaki Rasayana the preparation are you going to look for the compounds in that? Now this question always comes some referees would say, what is the compound that is doing this?

Now that question we decided in the beginning itself, we would not get involved because that is a reputation of what the Ayurveda claims in the original texts, because it is the whole preparation which has to be used not a component on that. Because often they search for a component becomes a complete diversion from what you really wanted to do and you may end up with searching for the components and not looking for the effect of Amalaki Rasayana. So, we did not want to make that mistake, so no attempt was made deliberately. But in this kind of research I remember this paper was sent to PLoS one and one of the problems which we would face in doing this kind of research the referee, one of the referee is pointed out that the procedure that you are following for making this Rasayana, it is not exactly the same as it is mentioned in the original text which we had quoted.

Now this problem comes because the original text many times it will not give you the detailed procedure such as you would see in today's description a standard operating procedure; that kind of a description you will not see in this ancient texts written 1600 hundred years ago or 2000 years ago. Some of the things are not clarified, sometimes the measurements those measures may not be well known or agreed upon today, there are many such little problems. So, what generally happens is Ayurvedic physicians with great experience, they have a consensus among themselves that basically it is the same procedure, but in the minor details there may be things that they decide not written in that original texts, because you cannot expect what happened 2000 years ago to remain exactly the same today.


So, this referee was literally correct, it is not mentioned in that original protocol. So, how can we accept this? And finally we resolved it by saying in the materials and methods part, we will describe exactly what we have done. And then in the discussion part we will say this is the method that we followed is based on this particular description in the original say Ashtanga Hrudaya. Now that was acceptable, so we have these little things we have to learn; if you want to publish this type of work in modern journals, many of these requirements you have to comply with to satisfy international standards.

Now here the *Drosophila* they are fed with this Amalaki Rasayana, Larvae as well as adult flies, they show earlier pupation; that is one of the first things that you see and also earlier adult eclosion, they become adults; they become pupae sooner compared to the flies which have not been fed Amalaki Rasayana. The salivary glands the growth in size

you will see the pictures; it is much bigger in those flies which have been fed Amalaki Rasayana and the DNA count for nucleus increases and a significant increase in the total enhatched eggs. So, the fecundity is much greater in these flies these are all shown by these.

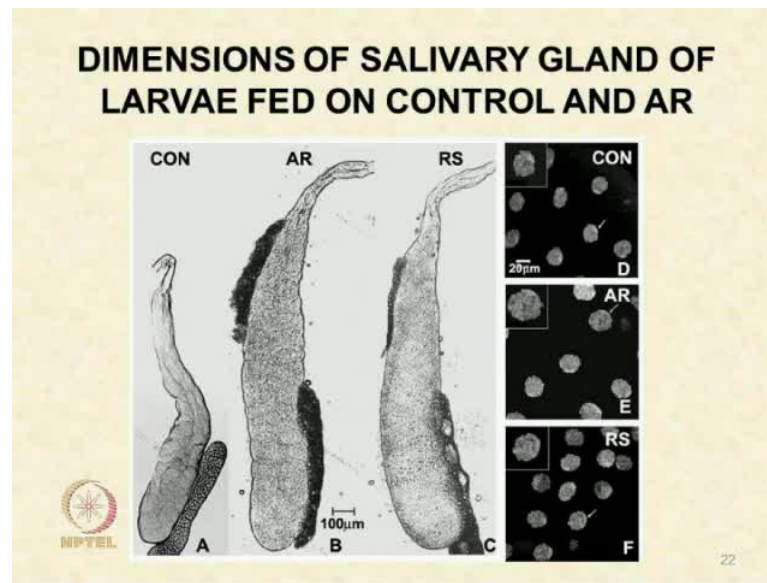
(Refer Slide Time: 22:31)

MEDIAN PUPATION AND ADULT ECLOSION TIME (HOURS)		
Food	Median Pupation	Median Adult Eclosion
Control	116.1±2.2	246.1±1.6
Honey + Ghee	116.1±2.7	245.6±1.5
AR	112.1±2.3	236.7±3.3

21

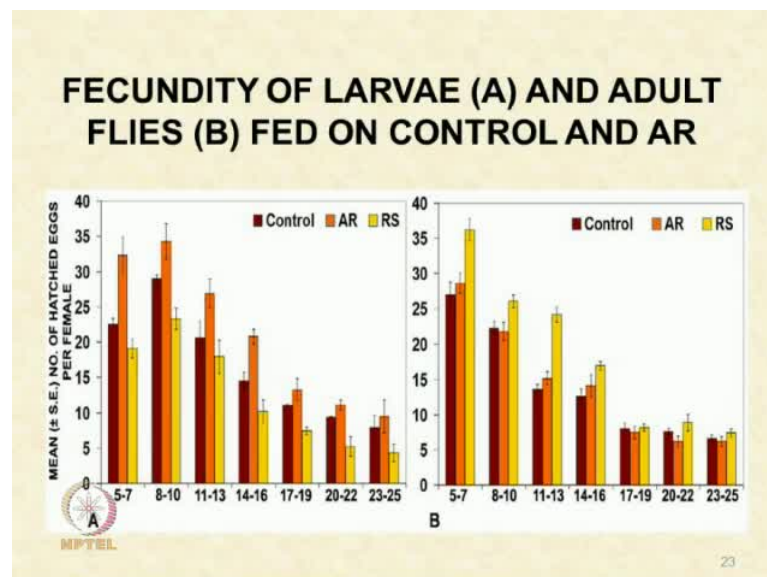
Now here if you look at them the control animals median pupation is 116 hours and hours is a long time in a fruit flies life, whereas in the median adult eclosion time is 246, those are the controls not fed Amalaki Rasayana. And as I mention Professor Lakhotia he wanted to know what happens if honey and ghee alone not the Amalaki Rasayana that was given and you can see there is hardly any change. The median pupation time remains the same, median adult eclosion time remains the same, there is no change. But Amalaki Rasayana fed you can see the median pupation time is reduced by 4 hours which is a long time in a fruit flies life. And the adult eclosion time is again reduced, so they mature faster.

(Refer Slide Time: 23:26)



And look at the salivary gland size on the left hand side is the control and the middle is Amalaki Rasayana fed; it is almost double the size. And the third one is Rasasindur, I would not get into that that is a different study but here for our purpose the control and the Amalaki Rasayana, there is no question that the size of the salivary gland very important organ of fruit flies that is very much increased.

(Refer Slide Time: 23:53)



And then you look at the fecundity or the number of eggs, and here on the left hand side that is the control without getting the Amalaki Rasayana. And the next the tall bar, that is

the animal, that is a fruit fly getting this one getting Amalaki Rasayana. You can see the hatched eggs the number is much greater. See consistently it is above the level of the controls in terms of the number of eggs produced.

(Refer Slide Time: 24:30)

BIOLOGICAL EFFECTS OF AR Life span of <i>Drosophila melanogaster</i> Medium Life Span (in days)	
Food	Medium Life Span (in days)
Control	36.0
AR supplemented 0.5%	40.4

Then we come to the life span in days, control is 36, Amalaki Rasayana supplemented feeds its 40 days statistically significant.

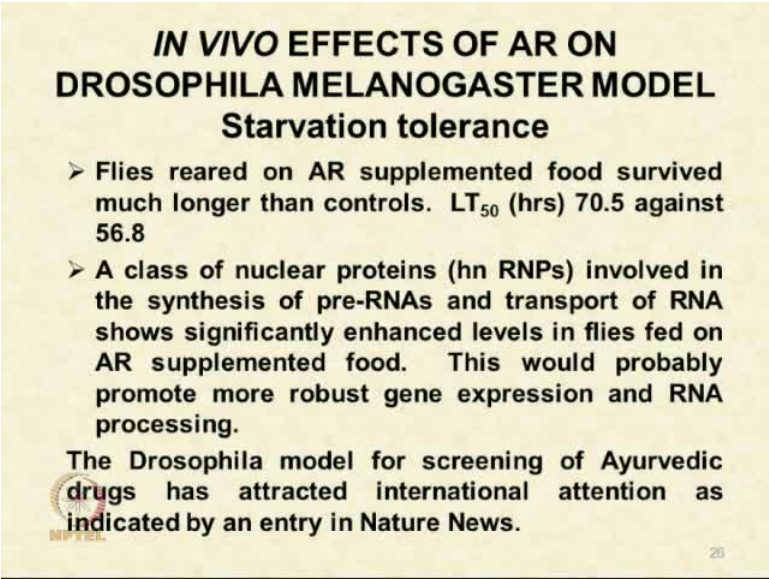
(Refer Slide Time: 24:45)

IN VIVO EFFECTS OF AR ON DROSOPHILA MELANOGASTER MODEL Thermal stress			
Heat shock (HS)	Time (mts.)	% Survival (Hrs. after HS)	
		Control	AR supplemented food
37°C	90	67.2	84.7
38°C	120	41.0	79.2
39.0	30	0	6.3

Then we have heat shock 37 degrees for 90 minutes, 38 degrees for 120 minutes and 39 degrees for 30 minutes; that is the heat shock which is being applied to these flies and

you can see the survival, the control is 67. Rasayana supplemented is 84, and 38 degrees it is 41 and 79, the difference becomes much greater. At 39 degrees there are no survivors in the controls but 6.3 surviving in those fed Amalaki Rasayana. So, here again the thermal stress the response, there is no question that the Amalaki fed flies they are doing significantly better.

(Refer Slide Time: 25:36)



**IN VIVO EFFECTS OF AR ON
DROSOPHILA MELANOGASTER MODEL**

Starvation tolerance

- Flies reared on AR supplemented food survived much longer than controls. LT_{50} (hrs) 70.5 against 56.8
- A class of nuclear proteins (hn RNPs) involved in the synthesis of pre-RNAs and transport of RNA shows significantly enhanced levels in flies fed on AR supplemented food. This would probably promote more robust gene expression and RNA processing.

The *Drosophila* model for screening of Ayurvedic drugs has attracted international attention as indicated by an entry in Nature News.

26

Then we look at starvation tolerance, these are all various types of stresses being applied very difficult to do in animals; this can only be done in the fruit flies. Now here you will find the Amalaki Rasayana fed flies they survived much longer than controls, because controls it is only 56 hours whereas the LT is the life time that is 56 in controls whereas those which are fed with Rasayana it is 70. So, it is a very significant difference in the starvation tolerance and lastly the class of nuclear proteins involved in the RNA transfer that is much greater in quantity in these Rasayana fed flies and this could possibly promote more robust gene expression that is only a kind of speculations, we have no evidence for this RNA processing. So, some of these nuclear protein functions could be improved in these Amalaki Rasayana fed flies.

Now this particular paper of Professor Lakhotia in PLoS one that attracted global attention, because it came in nature news and they picked it up because the reason why it attracted their attention is for the first time fruit flies are being used for testing two Ayurvedic preparations. Because we were primarily interested in Amalaki Rasayana and

how do the personal interest of professor Lakhotia in Rasasindur, he tried that also. So, here two entirely diverse preparation used for different purposes and Rasasindur is not really used as a Rasayana. So, he had taken these two for his own personal interest he took Rasasindur and this was a model so he tried it; he did find some interesting changes. So, this new model for testing Ayurvedic drugs; that is how nature looked at it, because there is a problem today in the regulation of Ayurvedic drugs, what kind of toxicity test do you do? This is an area which is not very clear today.

So, when you are trying to develop a protocol, if you are thinking of exporting Ayurvedic drugs for example, we have no idea what kind of requirements will come and there is all worldwide interest in developing less invasive less traumatic ways of testing. If you can get rid of animal test all together that will be the best, but that is not possible. So, if you can use lesser animal or even fruit flies for example, it would make it much easier for drug testing and certification. So, this is a subject of global interest that is how they picked it up. So, here this is a useful piece of information. So, if toxicity even at the screening preliminary, if it can be done in fruit flies; suppose, it is highly toxic in fruit flies may be there is no need to do it in rats and other animals. So, it could have implications from a regulatory angle; that is how they showed interest in this work, but it is an interesting piece of work.


Now this is what I referred to earlier because if Rasayana as you know it involves character, truthfulness, all these are involved in Rasayana which are not amenable to test in the laboratory. So, we can only take a small part. Here the chain breaks in DNA in the brain cells; that is really part of ageing, forgetfulness, deteriorating mental functions, intellectual functions that are part of old age. Now when you say that the DNA chain breaks in the neurons and astrocytes, they are slowed by taking this Rasayana; that immediately is an indication that it could have some effect in arresting the decline of intellectual functions. So, that is an important way to look at it and it is interesting that Amalaki Rasayana which is not primarily Medhya Rasayana shows these very interesting results.

(Refer Slide Time: 29:44)

**PHYSICO-CHEMICAL CHARACTERISATION OF
A MERCURY-BASED DRUG (RASASINDŪR)**

- ✓ Metal based drugs are seldom used in modern medicine as they are toxic, especially mercury-based drugs.
- ✓ The long and unhappy experience of using mercurial diuretics and other mercury based drugs which produced fatal complications such as renal failure had led to a global ban on the use of mercury-based drugs. Even the use of mercury-based amalgam is no longer permitted in dental filling.

Contd...



27


And then we come to the next project that is Rasasindur which is mercury-based Bhasma extensively used in Ayurveda and this will not be approved by any modern drug controller because of the mercury being used it is highly toxic and we have good reason to fear mercury because even when I was a medical student 1950s I remember we had no diuretic at that time oral diuretic. So, the only way to treat a patient with congestive heart failure, we have to admit him into the hospital and mercurial diuretics by injection.

And this was something greatly feared because of the danger of renal shutdown, you constantly measure the blood urea we have been watching. So, this was the situation in 1950s until a diuretic called Diamox became available in 1955 I think. So, a great deal of fear is there about using mercury; even mercury in amalgams for dental work, they do not use it anymore. So, here mercury is regularly being used if you talk to Ayurvedic physicians Rasasindur many people use it, it is a standard drug approved by the council regulatory body.

(Refer Slide Time: 31:00)

**PHYSICO-CHEMICAL CHARACTERISATION OF
A MERCURY-BASED DRUG (RASASINDŪR)**

- ✓ However, Siddha physicians in Tamil Nadu and Ayurvedic physicians everywhere had claimed that mercury-derived “bhasmas” were safe to use and had been used for centuries in their tradition. This has been a riddle.
- ✓ The observation by Siddha and Ayurvedic physicians raised the question “whether the elaborate processing of minerals in the ancient protocol brings about important physico-chemical changes including altered micro-structure in an Ayurvedic mercury-based drug? (Rasasindūr).

 Contd...

28

It is used extensively in Siddha medicine and they claim that they do not see these ill effects. So this is always been a riddle; we cannot dismiss the experience of hundreds of people over hundreds of years. So, there is a riddle here which needs to be explained and if you do chemical examination mercury is there, so immediately that objection would come. The next question is with all the new analytical techniques that we have in material science, is there some change which is taking place in the preparation of this very long preparatory step, is there some physical changes taking place which could explain a different type of behavior that is the rationale for doing this test. And this study was conducted the preparation itself of Rasasindur was made in Kottakkal specifically for this study; it is not a commercial sample, indeed in Kottakkal they do not manufacture Rasasindur.


(Refer Slide Time: 32:01)

PHYSICO-CHEMICAL CHARACTERISATION OF A MERCURY-BASED DRUG (RASASINDŪR)

✓ For this study, research-grade Rasasindūr was specially prepared by Dr Muraleedharan and colleagues at Arya Vaidya Sala, Kottakkal according to ancient protocol and the characterisation studies were done Professor Sujit Roy and colleagues at IIT, Kharagpur.

Project Title: Physico-Chemical Properties of Ayurvedic Metal-Based Drugs: A Case Study on Rasasindur*

Investigators: S. Roy, K.K. Ray, M. Bhattacharjee, Pramathesh Maji, Supriyo Samanta (IIT Kharagpur)
T.S. Muraleedharan, M. Sunil Kumar, A. Arun (Arya Vaidya Sala, Kottakal)

 *Bulletin of Materials Science (Under Review)*

29


A special lab was set up to prepare this for this study. So, he was a co-investigator Doctor Muraleedharan and colleagues from Arya Vaidya Sala, Kottakkal and the principal investigator doing these analytical studies was Professor Sujit Roy from IIT, Kharagpur. Subsequently, he has moved to the new IIT in Bhubaneswar and his colleagues. They had made several visits to Kottakkal and seen the lab, how the chemical profiling they were doing; they had satisfied themselves the quality of producing this was of a high order.

(Refer Slide Time: 32:38)

CHARACTERISATION STUDIES

✓ The following studies were done in IIT, Kharagpur on the Rasasindūr samples.

Parameters	Tests
Elemental composition (Bulk and point).	Energy-dispersion x-ray analysis (SEM-EDS)
Crystalline phase	X-ray powder diffraction.
Particle size, morphology, surface area.	SEM, FESEM, TEM, BET-isotherm.
Other spectroscopic studies.	FT-IR

 30

Now the tests which were done, this paper has been it is under review for bulletin of Material Science high impact journal publish from India. Now the referees have raised some questions asking for information, so that processing is going on. The parameters studied were elemental composition, bulk and point and using energy dispersion studies. Crystalline phase was studied using x-ray powder diffraction, particle size morphology, surface area was studied by using scanning electron microscope, transmission electron microscope, BET-isotherm, etcetera and other spectroscopic studies Fourier transform IR; these were very detailed instrumentations used for studying this.

(Refer Slide Time: 33:27)

HIGHLIGHTS OF RESULTS

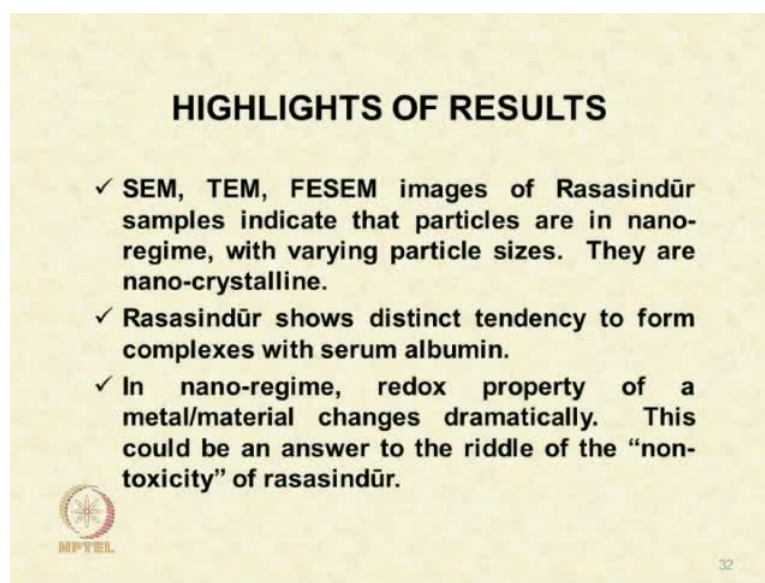
- ✓ EDS point analysis showed the absence of elements other than mercury and sulphur.
- ✓ All samples contained 4 phases of mercuric sulphide belonging to hexagonal P3121 space group. Commercial samples of Rasasindūr contain traces of HgS as impurity.
- ✓ Mercuric sulphide samples prepared in IIT, Kharagpur following modern protocol in chemistry texts do not contain any of the phases mentioned above. It shows a single phase belonging to hexagonal p6/mmm space group.

Contd...

31

And the energy disburse in studies point analysis showed the absence of elements other than mercury and sulfur. That product contains only mercury and sulfur in spite of all these processing, purification of mercury, purification of sulfur, plant extracts are used in further processing and throughout all these but the final EDS point analysis shows only mercury and sulfur and no other sub-elements in that. And all the samples contained this is the Rasasindur four phases of mercuric sulfide, it will exists in four phases whereas the mercury sulfide analyzed by chemical process in IIT, Kharagpur, that exists only in a single phase. So, there is a difference in the physical structure of this mercuric sulfide made synthetically by chemical processes that present standard method and the way Rasasindur is processed by traditional names.

(Refer Slide Time: 34:38)



HIGHLIGHTS OF RESULTS

- ✓ SEM, TEM, FESEM images of Rasasindūr samples indicate that particles are in nano-regime, with varying particle sizes. They are nano-crystalline.
- ✓ Rasasindūr shows distinct tendency to form complexes with serum albumin.
- ✓ In nano-regime, redox property of a metal/material changes dramatically. This could be an answer to the riddle of the “non-toxicity” of rasasindūr.

32

And the second all the scanning electron microscopy, transmission electron microscopy, all these studies they show that the particles in the Rasasindur, they exist in nano-regime and they are nano-crystalline. Of course particle size varies, but they are all in the nano-regime and they are nano-crystalline. So, there is a distinct tendency for these nano particles to form complexes with albumin, so there is a certain amount of activity biological activity. Now in nano-regime this is the crucial point, redox property of metal or material changes drastically from that of the bulk form. We do not know whether this is the explanation for the possible non-toxicity of Rasasindur that part this study has not addressed, but that is a possibility we cannot rule out at this stage.

Now another project which has been published I thought we may not have time to go into it. This is the study done in CIMAP that concerns Vata, Pitta, Kapha, this is as induced by perturbations of these doshas. These are counted by plants which are specific, they are classified. Plants which are used for treating Vata perturbation, plants which are used in treating Pitta perturbation, there is a classification given, but if you look at those plants there is nothing in common in the taxonomy of those plants. They are all very different chemically they are different but, therapeutically they seem to have the same action.

Now this is the puzzle and this was taken up by the two young scientists in centre of Central for Aromatic and Medicinal plants in Lucknow and Doctor Chidambaram. When

this was approved, he had indicated that the PSA's office is not a granting agency, but they can only give a start to a promising area when it is hardly born that he had done. Now that the papers already have come and some are due to come, the department of science and technology they have taken over this, there is a new scheme called task force in Ayurvedic biology with the similar objectives.

Now this task force in Ayurvedic Biology, it is on the website of DST; that does not take any drug development related programs. That it is not in their area of interest nor do they take up clinical studies trails for safety efficacy; that also is not taken. Essentially they look at basic science applied, basic science to a large extent it means modern biology and immunology. These are applied to Ayurvedic concepts, Ayurvedic procedures, and these are the projects which the task force takes. Already subsequent the first round we have mentioned all these here but in the second round new projects for example, Amalaki Rasayana. It shows positive results in Drosophila in their biomarkers, it shows interesting results in the rat brain cells.

Now scientists from Rajiv Gandhi centre in Trivandrum, Doctor Karta, he has seen this, he has a model; he is working on for heart, rat heart hypertrophy of the left ventricle. He has been studying that for various other purposes. Now Amalaki Rasayana if it is general effects, certainly it should have some effect on the heart. So, he is doing the study of Amalaki Rasayana on the rat heart hypertrophy. So, there is a study going on there. Similarly the dosha specific patterns, if it shows manifestations in these molecular markers what about autonomic functions, because many of these traits described for Vata, Pitta and Kapha. They are autonomic functions about sweating, about diarrhea; many of these are autonomic functions.

So, if you take autonomic functions and if you do a study on them or their dosha specific patterns, we are not looking at molecular markers now. We are looking at standard autonomic functions measured and do you find dosha specific patterns there. Now these are studies which are and also there is a very interesting question, when you look at these Dosha Prakritis; I was asked a question during this formulation of this project, is there heritability in this? In other words a father who is Vata, Pitta, what is the son? Does he have the same Prakriti? Now this is not discussed in the old text. It was asked by young Ayurvedic physician in SDM College. Now he has a project, he is doing that study now, so like that there are several projects which have come out of these original first round of

studies which is highly encouraging and we must also realize Ayurvedic biology is not confined to human biology.

We should also know Ayurveda itself there is a great deal of Ayurveda called Vriksha Ayurveda; Ayurveda of plants, Ayurveda of trees, there are several books on this. Similarly Ayurveda of animals, there is a very big book almost as big as Charaka Samhita on Ayurveda for elephants, Ayurveda for horses. So, there is no reason why in Ayurvedic biology, we would look for projects in these areas. For example, it is already we have a project; there is a stipulation in Ayurveda a medicinal plant not for all plants, but several times you will see it mentioned it should be collected from that particular area, it should be collected at a particular season or a particular time.

Now is there any importance attached to this, what is the significance of this? If you wish to investigate that question, one of the projects we have received is on garlic and garlic should be collected from the Himalayan areas; it should be collected in a particular season. Now if you collect garlic from that area and also collect garlic regardless of the specifications from a different area in a different season and if you compare these, what differences do you see, are they chemically different? If they are not chemically different, is there some other way that you can investigate? And here there is a new very rapidly growing area of science called microbiomes. Microbiomes are normal human beings, plants or animals, they have billions of micro organisms living within them and they are essentially providing very important service to the organisms in terms of nutrition, in terms of defense, metabolic process and so on.

So, without them it is hardly possible to live; they are beneficial, they are synergistic, they part of us. So, this microbiome now has become a huge area of interest, a new wave more or less. Now if garlic also has microbiome, so if you find that the chemical composition is different or identical, then how do you explain this particular, you have to collect it at a particular season. You may find if chemical composition is the same, why is it that you are insisting on this? You may find the differences because of the microbiome may be different. If chemical composition is the same still you insist that it should be used, then only you will have this effect that could well be due to the presence of a particular microbiome profile.

Already a scientist has put up a project; that is a plant molecular biology work. Again it comes in the Ayurvedic biology because the cue comes from Ayurveda. So, essentially all these projects because of this Ayurvedic cue it may be a project in plant molecular biology or it may be in animal science. For example, in Atharva Veda they noticed that when the pig is sick or a dog is sick, it would go and nibble at certain medicinal plants; normal times it would never do that but they do this. There is an extensive amount of work done in the western countries on Chimpanzees. When they have parasitic disease in the bowel, intestinal parasites, then they go and nibble on certain plants and it was believed to be because of their cognitive functions, they are able to identify and so on. Anyway it is an area of great importance.

Now here for example, can we do a project here because what happens is let us, say, a dog. A dog which does not normally eat a particular type of grass, but when it becomes sick then it goes and nibbles on that grass; grass is unchanged, it is the same. It is only the sickness which has made it smell and taste different, the sensitivity has changed. Now there is smell and taste institutes at the west. So, here if somebody wants to be a scientist in animal biology wants to study this particular phenomenon smell and taste in animals in relation to medicinal plants again the cue comes from Ayurveda, but it is a very up to date project in molecular biology in relation to animal science. So, like this in many of these areas the task force would be interested in receiving proposals and supporting. So, it will slowly over a period of time, may be 10 years, we would have an impressive body of knowledge, so that Ayurvedic biology becomes a reality and a promising sunlit road ahead.