PHARMACOGNOSY AND PHYTOCHEMISTRY

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Week 3

Lecture 13

Week 3: Lecture 13: Carbohydrates based drugs : Honey, Chitin and Chitosan

Thank you. Hello everyone, and welcome to the NPTEL course on pharmacognosy and phytochemistry. We are delving into the world of carbohydrates, and in this session, we will delve deeper into carbohydrates which are obtained from animal sources, and these carbohydrates are honey,

chitin, and chitosan derivatives. So, these carbohydrates are obtained from animals—that is, honey from honeybees as well as chitin and chitosan from most crustaceans. So, let's learn what these carbohydrates are and understand them. So honey, often referred to as 'mud,' is a traditional preparation which has been used for thousands of years by humankind but is gifted to us by honeybees. So, honey is a secretion that is stored in a honeycomb.

It is deposited there by bees belonging to different bee families, such as *Apis dorsata, Apis florea, Apis indica,* and *Apis mellifica*. In some cases, *Apis mellifera* and many members of the bee family, especially the Apidae family members, all belong to the order Hymenoptera. Now, if you see bees, they are abundant in forests, but they are also harvested and,

Dwelled in apiaries worldwide for the collection of honey, which is priced. And the main sources or the main places from where we get our honey of commerce are Africa, India, Jamaica, Australia, California, Chile, Great Britain, and New Zealand. Now, in some cases, you will also see that bees are dwelled in a special forest or a special group of trees to get that characteristic unique honey obtained from those plants.

So what is this, and what are the contents of honey? How is it produced? Let's understand. So honeybees are collecting nectar from the plants, as you must have seen. They have an organelle called a proboscis

which is like a straw that draws the nectar. Now, if you see the nectar from the flowers in terms of its composition, it is mostly water that is about 75% water and 25% sugars, which is predominantly sucrose. Now, once the honeybee consumes it, it goes into a special place called the honey sack, which is located in its abdomen. And there, with the help of the enzyme invertase, it is actually converted into honey.

Now, this nectar is actually the food for honeybees. So, some part of it is utilized by the bee, and the remaining part is something they deposit inside the honeycomb. So, they keep on depositing it within the honeycomb. You must have seen the cells, almost hexagonal, of it.

So, once the cell is full of the nectar, they seal it with beeswax, and beeswax is also a wax secreted from their very own bodies. So, with the help of their limbs, they seal it. Now, before sealing it, one more process happens. The first process happens in their stomach, where they convert sucrose with invertase enzyme into inverted sugars. The second process is

when they secrete it, it is still very rich in water. Now, you know sugars that are rich in water are prone to microbial contamination, fermentation, and so on. So here, nature plays an interesting role. So, the bees are said to fan the cells with their wings so much that the water content of it almost reverses. So, initially, the water content was 75%, and the sugars were 25%.

Now you have your sugars, which are 75%, and your water content goes as low as 25%. Now, what happens then? So if you have a very high sugar content, they exhibit what is called osmotic pressure, and due to their high osmotic pressure, your microbes cannot thrive in such an environment.

As a result, your honey is self-preserved and is considered to be one of the products that has absolutely endless benefits. It can stay for ages together. In fact, there have been people

who have discovered honey stored for thousands of years without deterioration. But yes, this honey, as you store it over time, tends to darken. Now, once your cells are sealed, this is where mankind intervenes.

So what is done is this honeycomb is cut off. And then it is allowed to drain. Now, in some cases, it is also centrifuged, broken, and it centrifuged so that all the liquid, all the honey within, oozes out. And then whatever the contents or the solids are, they are superficially skimmed. So this is what is called natural honey or raw honey.

Now, raw honey is rich in moisture, that is about 014 to 24%. It's rich in dextrose, levulose, which is obtained from fructose. And they are the contents or they are the ones which are highest in terms of amounts in your honey. They are called as the inward sugars.

You also have little quantity of sucrose, dextrins, gums. Now a honey has a typical pleasant odor. It has traces of essential oils because they are obtained from flour as a source. So they do carry little bit of aroma. They have beeswax because they have been obtained from your honeycomb

traces and quantities of pollen grains so when one is consuming raw honey if you see raw honey it's very translucent and turbid this translucent and turbid appearance of honey is mainly due to first pollen grains and the second reason for it is low water content because of its low water content or low moisture content many of the sugars tend to crystallize in it. You might have even seen your raw honey. If you keep it in your cupboard for a long time, you might see some crystallization happen.

So that is because the water content is really, really low. So this gives turbidity to raw honey. It contains acids, which are the result of secretion of the bees. So you have formic acid, acetic acid, and succinic acid. Then you have maltose, dextrin, some pigments obtained from flowers, as well as vitamins.

Now, in your raw honey, even the enzymes are very active. So you have diastase, invertase, inulase, and whatever those digestive enzymes which were there in the gut of the bee, which is in the honey sac, those are all secreted and are still viable within the honey, especially the raw honey. So

Now, what happens is, if you see commercial samples of honey, you would say that we never see crystallization occurring there. Now, what happens is, if you take raw honey, the raw honey contains about 14 to 24% water and has a good specific gravity of about 1.47. So it's very dense in nature and is self-sustaining, like it has an endless shelf life.

But in order to transport, in order to ensure uniformity, quality and so that the sugars don't crystallize out, many of those commercial industries dilute the honey with water. So from specific gravity about 1.47 it is diluted to bring the specific gravity to about 1.35. Now when you bring down the gravity, you increase water but at the same time you are going to decrease the sugar concentration.

Now when there is a decrease in sugar concentration and there is more fluidity, again there is a good chance of fermentation. Now this fermentation and deterioration is being brought about by microorganisms as well as enzymes. So what is done in order to take care of these enzymes, your honey is partly treated. Now how do you treat it?

It's very similar to your pasteurization. Your honey is taken. It's diluted to bring about a specific gravity of 1.35. Once that is done, it is kept at 80 degree Celsius for some time. Now, if you keep it for very long time, you know, sugars tend to caramelize.

We don't want that to happen. So, Immediately after 80 degree Celsius, it is cooled rapidly to your room temperature and stored. Now this treatment takes care of your enzymes and prevents fermentation of honey. But in the process, somewhere a compromise on the shelf life of honey is also being made.

So if you see your marketed samples, most of the marketed samples will predict the shelf life of prepared honey or what is called as industrially prepared honey. It is not artificial honey. It is just processed. So because of the processing conditions, now you have a shelf life which is generally given between one to one and a half year, mostly 18 months time.

So this honey is used as sweetening agent. Now, there is a huge dispute whether it is good for health or not. Now, if you're taking a diabetic case where you don't want to consume sugars, let us understand that because the inward sugars are already there in it and that's a good high percentage, almost 75% of the composition of honey is because of sugars.

It is a calorific component. So, it is going to give you calories and cannot be used for diabetic patients. But yes, I mean, if you compare it to sucrose, it has some little advantages in terms of other effects. Now, these other effects include being a demulcent. Especially when you have throat irritation or throat disorders.

We give honey because it has a nice soothing effect; it relieves dryness and is also antimicrobial. Despite dilution, it still contains very high sugar levels, so it exhibits high osmotic pressure. Microbes cannot withstand such high osmotic pressure, and as a result, they die off. So, it's a very good antimicrobial agent, and that's why it can almost be said to act like a preservative. Now, because it contains monosaccharides compared to all the drugs we have studied previously.

Monosaccharides are easily absorbable and highly bioavailable. So, for infants, elderly patients, or even convulsing patients, it becomes an easy food or energy source. Thus, honey is often given to young children as well as the elderly for health as a rich source of energy. But if you talk in terms of raw honey,

because it has enzymes, because it has pollens, because it has other acids, it has a very strong antimicrobial and immunomodulatory activity as compared to your processed honey. Now, because it's an animal product, it's priced and you'll see numerous adulterations happening where people create artificial syrup or add some sugars. And definitely it is contaminated, adulterated or in some cases completely substituted.

There is a simple test called as Fiehe's test, which can be done. So what you need to do is you just take your honey and then extract that honey with your Pet ether. Now take this Pet ether layer and to that Pet ether layer if you just treat it with resorcinol and HCL, you might get a reddish coloration. Now in natural honeys, this coloration is transient, that is it will fade after some time.

But if it is artificial honey, it stays for a longer period of time. Not only that, there are techniques nowadays which have been used to standardize honey. This includes a ratio of your sugars, especially sucrose, fructose, This also contains measuring your hydroxymethyl furfural which is naturally present but more abundant in your artificial honey.

It also involves measuring other percentages, specific gravity, aroma, presence of pollens, and many other tests have been devised to check the quality of honey. Now, let us move to the second animal-based product, which is chitin and chitosan. Now, these are almost similar products. We will see how.

Now, they are obtained from crustaceans. They are also obtained from spiders, such as tarantulas. Now, these are naturally occurring products. Amine, that is your amino-containing polysaccharides. So imagine glucose, and if you attach an amine group to glucose,

and more specifically, if you attach an acid amide group to glucose, that's where you get your chitin and chitosan derivatives. So these are nothing but your sugars or polysaccharide derivatives, wherein you have your chitin and chitosan coming from these animals. Now, there are numerous animals that can provide chitin and chitosan, such as tarantulas, crustaceans, insects, and worms.

You can obtain them from the hyphal or the mycelial regions of certain fungi. Now, they are huge molecular weight compounds, ranging somewhere between a few million daltons. So, if you see chitosan, chitosan is itself a high molecular weight compound, wherein the molecular weight ranges somewhere from 50 kilodaltons to about 2000 kilodaltons. Now, what specifically are these?

Now, let's discuss a little bit. To the glucose, you attach your amino group and, more specifically, an acetamino group. Now, this you attach to another acetamino-containing glucose by what are called beta linkages.

You get what is called chitin. Now, this chitin, if you selectively deacetylate, what is left back is just the amine groups. Those are called chitosan. So, it's not completely deacetylated. Some acetyl groups still remain.

But the difference between chitin and chitosan is that chitosan is a slightly deacetylated version of chitin. So, These are obtained from exoskeletons. Like I said, if you've seen insects, during their growth, they undergo what is called molting, where they shed their skin, like the external coating.

And that molting layer, you must have seen, is whitish in color, transparent to almost translucent. In crustaceans, it's not easily visible because crustaceans, even if you see crabs, may contain some pigments in them. But insect exoskeletons or fungal mycelia are very clear, transparent, translucent to white. In crustaceans, they are embedded within the calcified layer. What is done in the food industry is that the crustacean meat is taken.

So, take the crustacean shells which have been discarded, and because they contain calcium, you need to demineralize them. This demineralization is done with the help of acids. Imagine an acid like hydrochloric acid or sulfuric acid. In most cases, let's take an example of hydrochloric acid. Suppose you keep a crustacean shell in hydrochloric acid,

This hydrochloric acid will react with the calcium there to form calcium chloride. Now, this calcium chloride is water-soluble, so it dissolves. So over a period of time, when you're digesting it, what you're going to get is a clear, translucent shell which is demineralized. Now, this demineralized shell gets a pliable, soft kind of consistency but still contains

one more component which is present in animals, and that is protein. Now, generally, carbohydrates and proteins form a good association. The reason being, if you see carbohydrates, they have hydroxyl groups, whereas proteins have amine groups and they tend to form interactions, especially due to forces of attraction. That can be disentangled by treating it with an alkali, such as sodium hydroxide.

So the moment you treat it with sodium hydroxide, the associations are lost and your carbohydrates and proteins are separated. Now, your carbohydrate is taken. And it's depigmented. Like I said, one problem with crustaceans is they have orange or similar pigments present in them.

Now, these pigments are generally lipophilic pigments. So if you do take this crustacean pliable shell and extract, break it into pieces and extract it in organic solvents, most of the pigments are lost. Or what you can do is if you have a solution which contains this pigment in some cases this depigmentation can be done by a simple process such as charcoal treatment.

So the whole solution is passed through activated charcoal columns and you get a clear solution coming from it. Now this is the one which is rich in chitin. This can be concentrated evaporated to get chitin flakes or sheets which can then be crushed. But in case if you want chitosan. you have to deacetylate it.

Now, deacetylation is done by enzyme called as chitin deacetylase and in presence of more stronger concentration of sodium hydroxide, the deacetylation reaction is facilitated and you get a partially deacetylated product and that is called as your chitosan. So this is the process in which you can get your chitin as well as chitosan. But apart from this, nowadays more greener enzymatic digestions have also been utilized for this purpose.

So coming to the chemical composition, a glucose derivative containing an acetamide group. Now, this acetamide group, if you deacetylate it, becomes your chitosan. The linkages between two acetamide groups or what you call D-glucosamine groups are beta linkages. So in your chitin, it will be a homopolymer of beta-1,4 N-linked acetyl-D-glucosamine residue,

whereas chitosan, in some cases it is deacetylated, and in some cases it is not. So you will get a mixture. So it will be a randomly distributed heteropolymer containing your beta-1,4-D-glucosamine, the deacetylated unit, and alternating or sometimes spaced between N-acetyl D-glucosamine derivatives again linked by beta linkages.

Now, chitin, if you see in nature, occurs in different allomorphs. These allomorphs are your alpha forms, beta forms, as well as your gamma forms. So what happens here is, if you see your alpha forms, alpha forms are those in which the plates or the polymer sheets are antiparallel to each other, so one sheet goes in this direction, the other sheet goes in that direction.

And the alpha allomorph is the most commonly found one; the beta form is the one in which all the sheets are in a similar orientation. So gamma is actually a mixture of alpha and beta. So, you will find chitin and chitin derivatives in alpha, beta, as well as gamma forms, but mostly it will be found in the alpha form.

Now, if you see chitin and chitosan, they are yellowish to yellow-white colored powders. Again, depending upon the sources. They are odorless. The taste is slightly bland. And they are very resistant.

They don't easily dissolve in water. Even in organic solvents. So, creating a solution of chitin or chitosan is like imagining your animal exoskeleton. You are trying to dissolve it.

It is that resistant. So, how do you dissolve this? It is said that you might try to dissolve chitin and chitosan. Chitin is very difficult to dissolve because it still has an acetylated group.

Chitosan is a little simpler to dissolve, and it has amine groups which are exposed after deacetylation occurs. So these amine groups can then be converted into salts by treating them with acid. So instead of water, if you use something acidic, your chitosan will dissolve. So in your formulations or preparations, when you're trying to dissolve chitosan, generally an acidic buffer or acid such as acetic acid is used to facilitate the dissolution.

Once it is dissolved, it will form a nice, viscous, gelatinous polymer solution. It is generally preferred or solubilized at an acidic pH. You can check if your chitosan is genuine or not by a few chemical tests. You just treat it with iodine and then add 10% sulfuric acid or just a drop to it. You will see that it gives a deep violet coloration.

If you treat your chitosan with nitric acid, you know, it easily form nitrates. And this chitosan nitrates crystallize form or almost yellowish color crystals. You will see it under the microscope. Now, this crystals, if you see you under the polarized light with using nickel prisms, you might even see that cross wire like appearance on the nitrate crystals of chitosan.

It has numerous applications. Now, chitosan is something which is obtained from animal sources. It is a glucose derivative and hence very biocompatible. It is resistant, it doesn't dissolve easily in water and that's an advantage. So if you have a wound healing application being done, it is going to not be soluble easily in your exudations as well as

even if you put some water or by mystically you pour water on your wound, it's not going to get drenched out very easily. So it's used in wound healing preparations to treat cuts,

burns etc. It has a good property. It is studied extensively and has been known as a bacteriostatic, immunologic. It has antitumoral, hemostatic as well as anticoagulant activities.

It is used to prepare artificial skin or even corneal bandages. It's not much affected by water and compared to other sugars, it is more robust and biocompatible so it's used as a suture thread as well as you know if you want to do some implants or some gum cicatrization or in bone repair and dental surgery this is where your chitin and chitosan derivatives are abundantly used. It is used in textile industry for sizing rayon cotton wool

and even synthetic fibers. It has a good bioadhesivity as well as adhesivity to glass and plastics One interesting application is in the sewage water or effluent water treatment. So it is known to separate organic compounds. It is known to chelate or take care of heavy metals and even for the pesticides such as DTT or PCBs that is your polychlorobenzene derivatives.

It has been known to kind of contain in it and then precipitate or settle down so that your water gets very clear. So Here are few references if you want more reading into this topic and thank you everyone for your patient listening. Thank you.