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International Journal of Current Research
Vol. 7, Issue, 02, pp.12315-12320, February, 2015

INTERNATIONAL JOURNAL OF CURRENT RESEARCH

ISSN: 0975-833X

RESEARCH ARTICLE

AN ASSESSMENT STUDY ON EXTRACTION OF GLUCOSAMINE FROM POTENTIAL SOURCE AND EFFECT OF GLUCOSAMINE ACTIVITY ON ADMINISTRATION OF CONVENTIONAL DRUGS

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ARTICLE INFO

Article History:
Received 08th November, 2014
Received in revised form
29th December, 2014
Accepted 05th January, 2015
Published online 26th February, 2015

Key words:

Glucosamine,
and Cyp2C9

ABSTRACT

Glucosamine has attracted much attention owing to its therapeutic activity in osteoarthritis and widely used dietary supplement. A search for a new potential source is going on due to the allergies caused by glucosamine extracted from shell fish and to reduce the cost involved in extraction of vegetarian glucosamine which are presently used as sources. The present investigation describes the effective extraction of glucosamine from sweet potato. (Method) A maximum yield of 16.3g/kg was obtained. The study also focuses on effect of conventional drugs in interaction between cytochrome P450 receptors- Cyp2C9 and Cyp3A4 with glucosamine. In the presence of conventional drugs, the E-value of binding between glucosamine and Cyp2C9 receptor was increased by 20% and with Cyp3A4 receptor it was increased by 34% which indicates the decrease in affinity between them. Hence, the current research reports two vital findings with respect to extraction and efficacy of glucosamine in presences of conventional drugs.

INTRODUCTION

Osteoarthritis (OA) is the most common type of joint disorder characterized by the chronic condition of the synovial joint that develops over time and is the result of the thinning or loss of the cartilage, which is found in the ends of the bones. Worldwide, osteoarthritis (OA) is estimated to be the fourth leading cause of disability (**Fransen *et al.*, 2011**) and the prevalence of OA increases with age and generally affects women more frequently than men. Glucosamine is an amino monosaccharide acting as a substrate for the production of aggrecan and proteoglycans which gives hydrophilicity to the cartilage thus compounds enhancing the synthesis of aggrecan are beneficial in osteoarthritis (**Setnika *et al.*, 1991**). Glucosamine is naturally present in bone cartilage, where it forms the major cushioning ingredients of the synovial fluids of the joints and surrounding tissues (**Braham *et al.*, 2003**). Glucosamine is composed of a sugar molecule (glucose) and an amine group. While most sugars come from dietary sources and are burned for energy, amino sugars are mainly formed in the body and used primarily in manufacturing tissue components. As such, glucosamine helps form the proteoglycans that fit within the spaces in cartilage netting that is needed to restore joint structure (**Towheed and Anastassiades, 2007**). Directly or indirectly, glucosamine Plays a role in the formation of articular surfaces, tendons, ligaments, synovial fluids, skin, bone, nails, heart valves, blood vessels and mucous secretion within the digestive, respiratory and urinary systems (**De Los Reyes *et al.*, 2000**).

Most glucosamine supplements are made from the exoskeletons or outer shells, of crustaceans such as shrimp, lobster, crab and crawfish, chitin can provide trace amounts of glucosamine in their shells and tails. Commercially glucosamine is extracted by hydrolysis of crustacean exoskeletons. It has been observed that there is allergic responses related to glucosamine of shell fish origin and have been reported previously by **Anderson *et al.* (2005) and Gray HC *et al* (2004)**. In the US, supplement products containing glucosamine from this source are required carry an allergy warning statement (**US Food and Drug Administration, 2004**). Hence, glucosamine derived from plant sources would not need such warnings. Some of the plant sources like beet root, carrot, chicory, god vine, wax free pivet, mung bean were also used to produce glucosamine with different methods. Among these plant sources the highest glucosamine extract was reported by **Courtois *et al.* (2011),** 16g/kg of beetroot. According to market analysis the cost of beetroot is 25-40Rs/kg (Ministry of Agriculture, Gov. India). Hence, there is a need of potential and cheaper source for extraction of glucosamine

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The present study is based on extraction of Glucosamine from sweet potato. According to market analysis the cost of sweet potato is 10-15Rs/kg (Ministry of Agriculture, Gov. India).
The quantitative analysis was carried out using HPLC method. Further, docking studies were carried out to study the effect of conventional drugs on binding of glucosamine to cytochrome receptors.

MATERIALS AND METHODS

Extraction of Glucosamine from sweet potato

The extraction of glucosamine was carried as described previously by **Courtois *et al.* (2011).** Wash the beetroot and peel off the outer skin to avoid the impurities. Weigh 200g of and cut into fine dices. Prepare 25ml of 4M ammonium sulphate and spray it on the fine dices. Dry the dices in oven at the temperature of 91^{O} C for 48 hours. Grind the dried dices into fine powder and extract glucosamine with distilled water at room temperature. Centrifuge the sample at 10,000rpm for 10 minutes and collect the supernatant. The supernatant was placed in hot air at 100^{O}C for the evaporation. The dried Sample was analyzed for amount of glucosamine extracted by HPLC.

HPLC analysis

HPLC analysis was performed using 1260 infinity series LC system. Chromatographic separation was carried out on Zorbax Eclipse XDB-C8 Analytical column (4.6 × 250mm, 5 µ; Agilent Technologies). The mobile phase comprises of orthophosphoric acid (pH 2.5): acetonitrile (70:30) with the flow rate of 0.6 mL/min in column at ambient temperature with infusion volume of 20 µL in each experiment. 10 µL of sample was injected for each experiment and detection was carried out by measuring UV absorbance at 195 nm.

Preparation of Standard and sample

100mg of Glucosamine Hydrochloride standard was weighed in 100ml volumetric flask containing 50ml of mobile phase. The mixture was sonicated for 2 minutes and filtered using 0.22µm filter before injection into the column. The sample was also prepared in accordance with the preparation of standard. The percentage of Glucosamine from HPLC curve was estimated by using the Equation 1.

Percentage of Glucosamine=

$$\frac{1,0 \quad x6 \quad .4\ x}{4 \quad .2\ x} \qquad \text{……Equation 1}$$

Where 605.42 is the molecular weight of glucosamine sulphate KCl & 431.26 is twice the molecular weight of Glucosamine HCl, C is the concentration of Standard, W is the weight in mg of glucosamine sulfate KCl, ru is the peak response of sample and rs is the peak response of standard.

Docking studies

Retrieving of structures from PDB

Crystal structures of cytochrome P450 molecules - CYP2C9 and cyp3A4 were retrieved from PDB database with Pdb ID: 4NZ2 and 4NY4 respectively in pdb format. Conventional and Commercial drugs - Aspirin, Diclofenac, Ibuprofen and Warfarine and also Glucosamine molecular structures were retrieved from NCBI PubChem –compound database in sdf format.

Molecular Docking

Docking allows virtually screening of compounds and predicts the strongest binders based on various scoring functions. It explores ways in which two molecules, such as drugs and receptor fit together and dock to each other well. The molecules binding to a receptor, inhibit its function, and thus act as drug. The collection of drugs and receptor complexes was identified via docking and their relative stabilities were evaluated using molecular dynamics and their binding affinities, using free energy simulations.

First, Using Rasmol visualization tool, from CYP2C9 structure file (4NZ2) the bounded ligand was deleted and structure saved to be used as receptor molecule. The small molecule compounds (conventional drugs + Glucosamine) were opened in Marvin Sketch tool and saved in Pdf format. Carrying out docking with HEX, interaction efficiency of all the small compounds with the receptor molecule, CYP2C9 were first checked. Then, CYP2C9 along with each docked commercial drugs was considered as receptor and then docked with Glucosamine and the E-values were compared and analyzed. Next, the same procedure is repeated with cytochrome P450 molcule- cyp3A4

RESULTS AND DISCUSSION

HPLC analysis

The HPLC analyses were carried out for measuring the amount of glucosamine. The HPLC analysis for Standard glucosamine and Sweet Potato glucosamine are as shown in Figure 1 and Figure 2. The retention time for glucosamine peak was observed at 3.4 minutes for standard sample. The area under peak of 3.4 minutes for standard and test sample was substituted in Equation1. Thus, 3.261g of glucosamine was extracted from 200g of sweet potato. The yield of glucosamine obtained from sweet potato is 16.3g/kg and is in comparison with the reported yield of glucosamine from beet root (Courtois *et al.*, 2011).

Docking studies to check the efficiency of Glucosamine

Ligand interactions with Cyp2C9 receptor

The molecular interaction between the ligands and the cytochrome P450-Cyp2C9 are tabulated in Table 1. The E-value indicates the efficacy of receptor binding to drugs is relatively more efficient, as compared to binding efficacy of glucosamine. The docked complexes of receptor and drugs were considered to be a complete receptor and the molecular interaction with glucosamine was checked. The docking results are tabulated in Table 2. A comparison between the Table1 and Table 2 show that the docking E-value is increased by 18-20%. This comparison is shown in Figure 3.

```
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                                                  Location  :    Vial 2
   Acq Operator    : GV                           Inj. No.  :         1
                                                  Inj. Vol  :     20 µl
   Sample Name     : Glucosamine  Hydrochloride

   Sample Info     : Standard

   Method Info     : Assay

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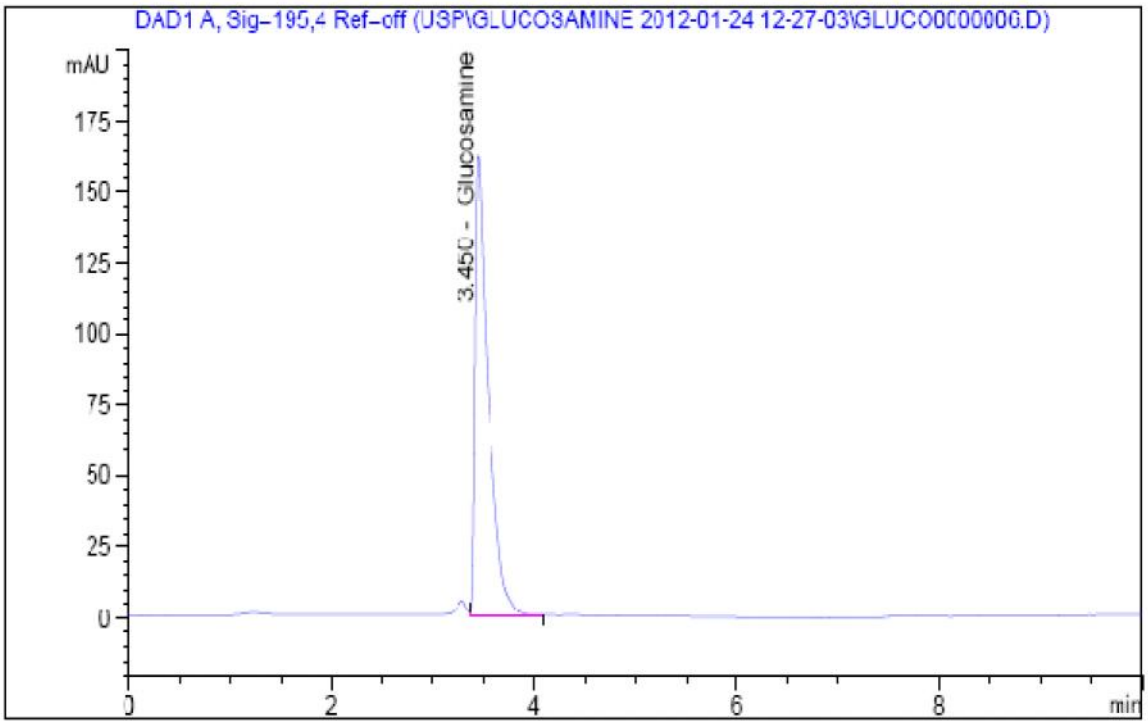


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                  Customized Report:   Robust
==========================================================================
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     |  #  |   [min]  |       |  [min] |           |       |                 |
     |----|----------|-------|-------|-----------|-------|-----------------|
     |   1|     3.450|   VB  |  0.125|  1462.155 |100.000|    Glucosamine  |
     ---------------------------------------------------------------------
```

Fig. 1. HPLC graph of standard glucosamine

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Acq Operator : GV
Inj. No. : 1
Inj. Vol : 10 µl
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Method Info : Assay
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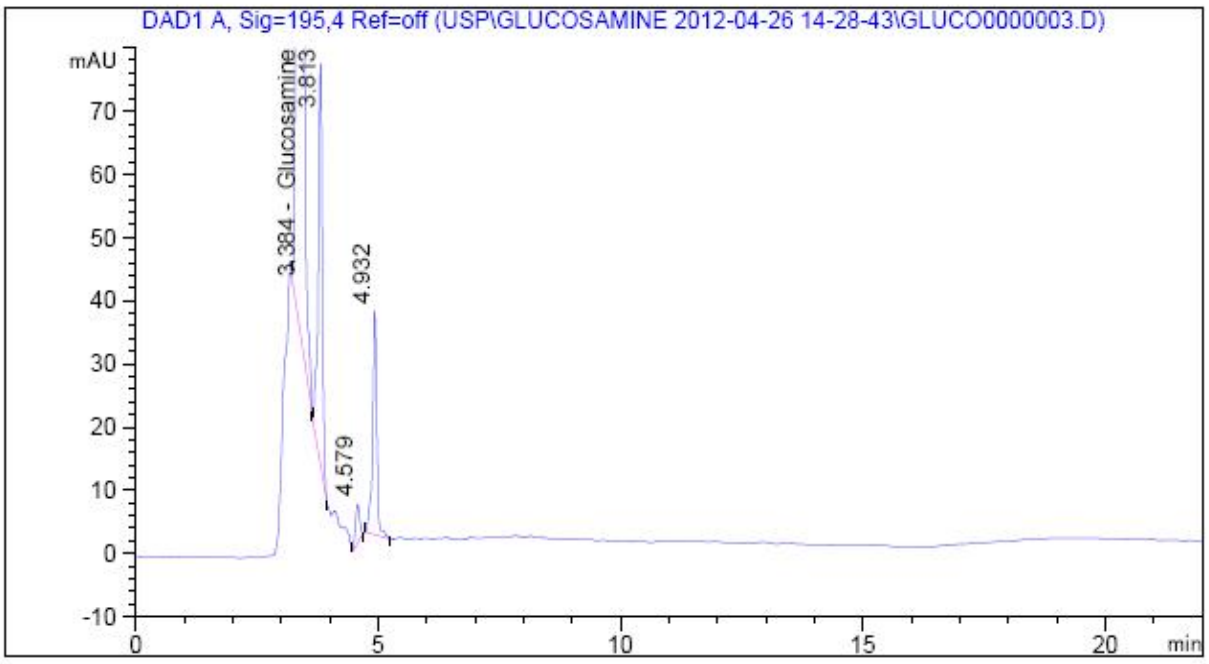

Customized Report: Robust

Signal 1: DAD1 A, Sig=195,4 Ref=off

Peak #	RT [min]	Type	Width [min]	Area	Area %	Name
1	3.384	MM	0.113	1915.754	75.428	Glucosamine
2	3.813	MM	0.098	374.077	14.728	
3	4.579	MM	0.097	36.860	1.451	
4	4.932	MM	0.100	213.168	8.393	

Fig. 2. HPLC graph of glucosamine from sweet potato

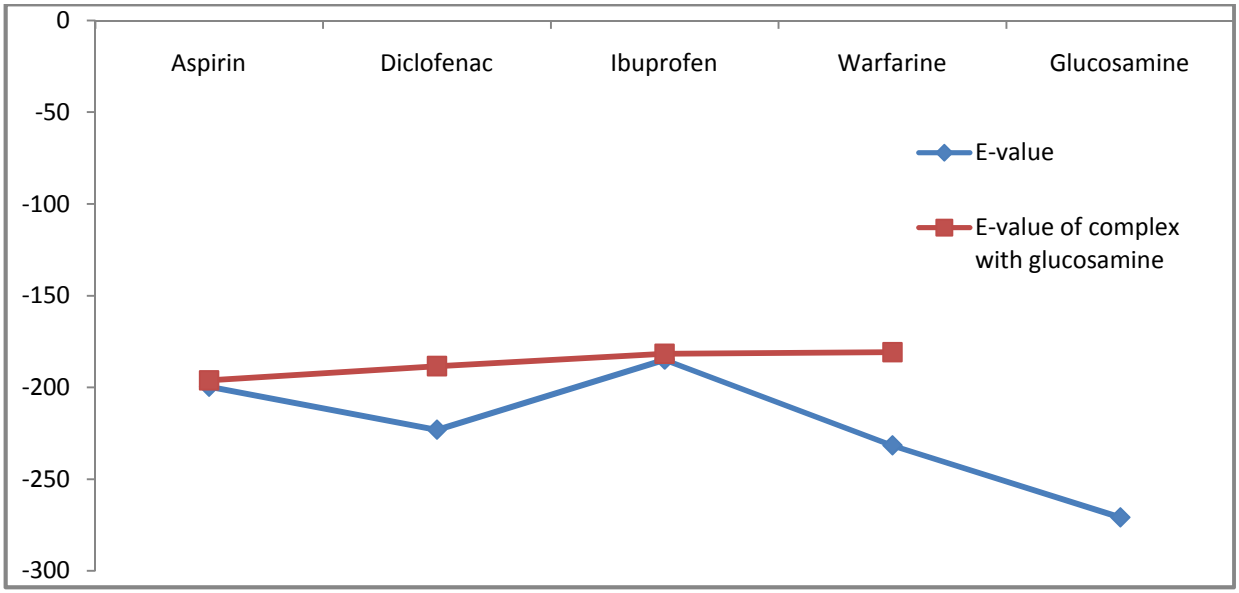

Fig. 3. Comparison of the E-values for cytochrome P450-Cyp2C9

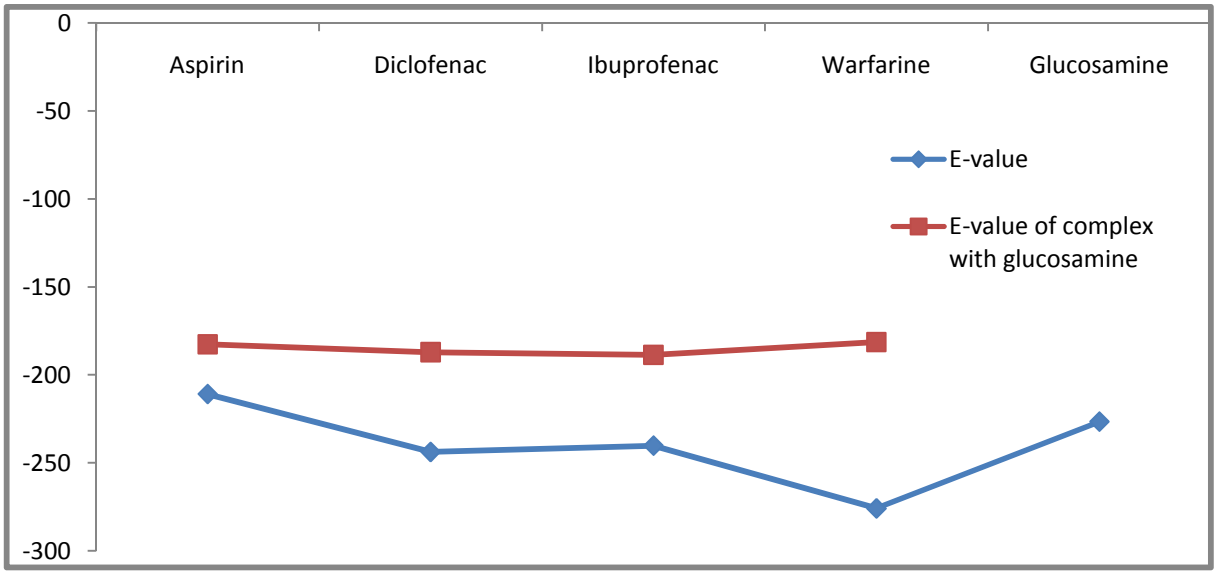

Fig. 4. Comparison of the E-values for cytochrome P450- Cyp3A4

Table 1. Molecular interaction of conventional drugs and Glucosamine with cytochrome P450 molecule - Cyp2C9

Conventional Drugs	E-value
Aspirin	-210.93
Diclofenac	-243.78
Ibuprofen	-240.20
Warfarine	-275.82
Glucosamine	-226.58

Table 2. The docked complexes of Cyp2C9 with each conventional drug were considered to be a receptor and the molecular interaction with Glucosamine was checked. The docking results are tabulated as follows

Receptors	E-value
Aspirin-cyp2C9	-181.63
Diclofenac-cyp2C9	-187.13
Ibuprofen-cyp2C9	-188.64
Warfarine-cyp2C9	-181.41

Table 3. Molecular interaction of conventional drugs and Glucosamine with cytochrome P450 molecule--Cyp3A4

Drugs	E-value
Aspirin	-199.65
Diclofenac	-223.10
Ibuprofen	-184.92
Warfarine	-231.69
Glucosamine	-270.89

Table 4. The docked complexes of Cyp3A4 with each conventional drug were considered to be a receptor and the molecular interaction with Glucosamine was checked. The docking results are tabulated as follows

Receptors	E-value
Aspirin-cyp3A4	-196.15
Diclofenac-cyp3A4	-188.41
Ibuprofen-cyp3A4	-181.64
Warfarine-cyp3A4	-180.82

Ligand interactions with Cyp3A4 receptor

The molecular interaction between the ligand and the cytochrome P450- Cyp3A4 are tabulated in Table 3. The E-value indicates the efficacy of receptor binding to glucosamine is more efficient, as compared to binding efficacy of conventionl drugs. The docked complexes of receptor and drugs were considered to be a complete receptor and the molecular interaction with glucosamine was checked. The docking results are tabulated in Table 4. A comparison between the Table 3 and Table 4 show that the docking E-value is reduced drastically by 28-34%. This comparison is shown in Figure 4.

The above data indicate the that the binding efficiency of glucosamine to cytochrome P450 receptors decreases in the presence of conventional drugs. The effect was more prominently seen for Cyp3A4 receptor. Thus, this analysis assist in postulating that glucosamine may have a decrease in its efficacy to produce the precursor for cartilage when consumed along with the above mentioned conventional drugs.

Conclusion

Glucosamine is effectively used in the treatment of osteoarthritis. It is the precursor for the formation of cartilage. The current research reports two vital findings with respect to extraction and the efficacy of glucosamine in presences of conventional drugs. Firstly, use of sweet potato as a potential source for extraction of glucosamine was carried out. The yield of glucosamine obtained per kg of sweet potato (16.3g/kg) is in comparison to that yield obtained from beetroot. The advantage of sweet potato to beet root is the cost involved per kg which is 55-60% cheaper than beet root. Hence this improves the overall economics involved in extraction of vegetarian glucosamine.

Secondly, in the docking studies, the convention drugs used during the study have shown prominent effect on the binding efficacy of glucosamine to cytochrome families receptors. The E-value of glucosamine binding to Cyp2C9 receptor was increased by 20% and to Cyp3A4 receptor was increased by 34% which indicate their decrease in binding efficiency.

The effectiveness of binding of Glucosamine to these receptors would be further hampered if the drugs would be consumed in combination. Thus, this bioinformatics analysis has laid down the base to carry out test in-vivo to test the effect of drugs on dosage of glucosamine administered during the treatment of osteoarthritis.

REFERENCES

Anderson, J. W., Nicolosi, R. J. and Borzelleca, J. F. 2005. Glucosamine effects in humans: a review of effects on glucose metabolism, side effects, safety considerations and efficacy. *Food Chem. Toxicol.*, 43, 187–201.

Braham, R., Dawson, B. and Goodman, C. 2003. The effect of glucosamine supplementation on people experiencing regular knee pain. *British Journal of Sports Medicine*, 37:45-49.

Courtois, Michaux and Goulois, 2011. Production of glucosamine from plant species, European.

De los Reyes, G., Koda, R. T. and Lien, E. J. 2000. Glucosamine and chondroitin sulfates in the treatment of osteoarthritis: a survey. *Progress in Drug Research*, 55:82-103

Fransen, M., Bridgett, L., March, L., Hoy, D., Penserga, E. and P. Brooks, 2011. The epidemiology of osteoarthritis in Asia. *Int. J. Rheumatic Diseases*, 14: 113-121.

Gray, H. C., Hutcheson, P. S. and Slavin, R. G. 2004. Is Glucosamine Safe in Patients with Seafood Allergy? *J Allergy Clin Immunol.*, 114:459-60

Ministry of Agriculture, Gov.India http://agmarknet.nic.in/

Setnika, I., Cereda, R., and Pacini, M. A., Revel, L. 1991. Antireactive properties of glucosamine sulfate. *Arzneim.-Forsch/Drug Res.*, 41: 157-161.

Towheed, T. E. and Anastassiades, T. 2007. Glucosamine therapy for osteoarthritis: An update. *The Journal of Rheumatology*, 34(9):1787-1790.

US Food and Drug Administration. Center for Food Safety and Applied Nutrition, 2004. Food Allergen Labeling and Consumer Protection Act of 2004. College Park, MD. Available from:http://www.cfsan.fda.gov/~dms/alrgact.html. Accessed June 20, 2006.

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