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3.3.1 Number of research papers published per teacher in the Journals notified on UGC CARE list during the last five years

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Title of paper	Name of the author/s	Department of the teacher	Name of journal	ISSN number	Proof
Woocommerce-The New Smart Plug in For Doing Electronic Commerce	Dr.B.Manjula	Department OF B.Com (CA)	International Organization of Scientific Research Journal of Engineering	ISSN(P):2278-8719, ISSN(e):2250-3021	Link
A Review on Security and Privacy in Manet	K.Priya	PG Department of Computer Science	International Organization of Scientific Research on Journal of Engineering	ISSN : 2250-3021,ISSN(P): 2278-8719	Link

Feasibility approach on Web Services and Security	K.Priya	PG Department of Computer Science	International Organization of Scientific Research on Journal of Engineering	ISSN : 2250-3021,ISSN(P): 2278-8719	**Link**
Architecture and Applications of Wireless Body Area Network	K.Priya	PG Department of Computer Science	International Organization of Scientific Research on Journal of Engineering	ISSN : 2250-3021,ISSN(P): 2278-8719	**Link**
S-allyl Cysteine as Potent Anti Gout Drug Insight in to The Xanthine Oxidase Inhibition and Anti-inflammatory Activity	J.Preethi	PG Department of Biochemistry	Biochimie2018	Biochimie 154(2018) 1-9	**Link**

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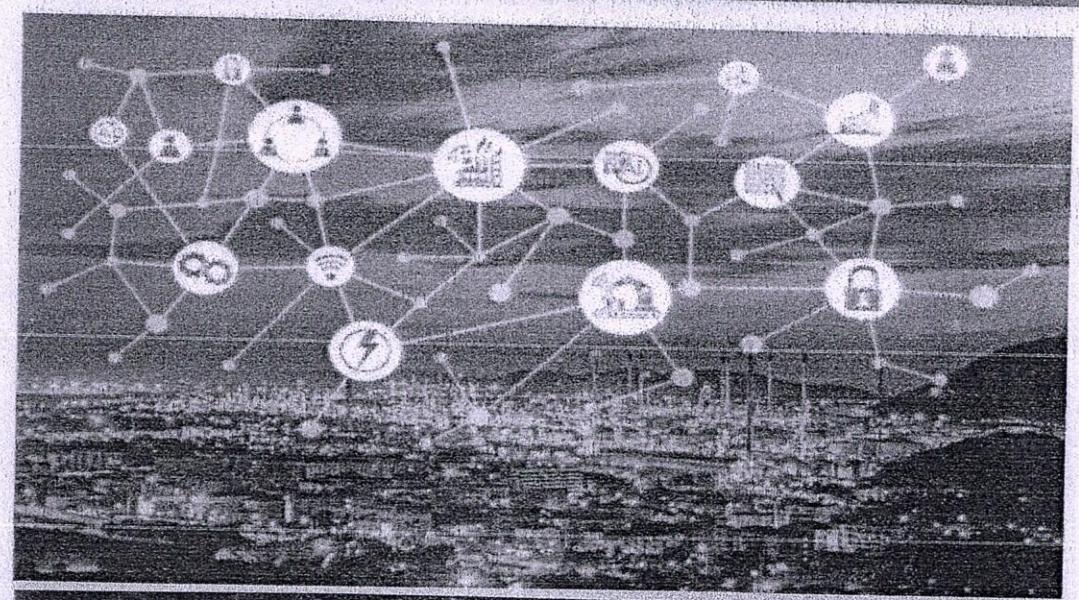

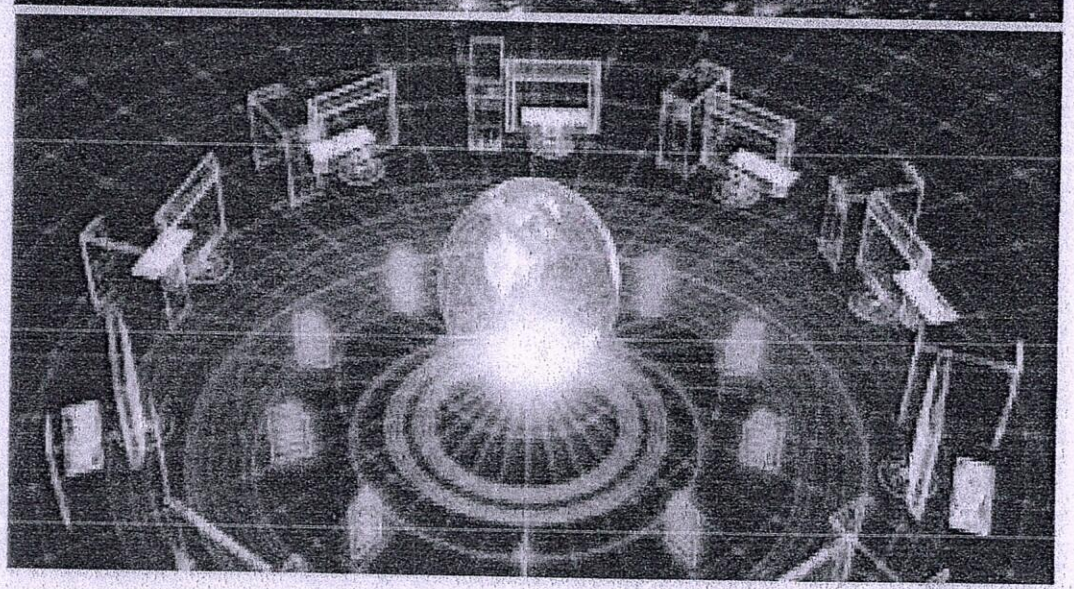

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Woocommerce- The New Smart Plug in for Doing Electronic Commerce

Dr. B.Manjula, Ms.Umrah.Daaniya Khulood
Head & Asst.Prof. Of Commerce (CA)
II B.Com (CA)

Abstract: *A few years ago, building an online store used to be an incredibly complex task. You had to install bulky software onto your own website and pay expensive developers a significant sum of money to customize even the simplest elements of your store. Luckily, nowadays, adding e-commerce functionality to your WordPress-powered website can be done by installing a single plugin and that is WOOCOMMERCE. WooCommerce preserves many of the functions core to the standard WordPress experience, but adds eCommerce capability, configuration, and customization. WORDPRESS is one of the most popular and efficient content management platforms in the world and WooCommerce was developed by it. This alone stands as a jor advantage for the uses of the plugin.*

Keywords: *Woocommerce, plugin, ecommerce, platform*

I. Introduction:

WOOCOMMERCE is an open-source e-commerce plugin for WordPress. It is designed for small to large-sized online merchants using WordPress. In simple term, WooCommerce is a WordPress plugin that can convert your WordPress website into an e-commerce store. It sells physical goods, digital products, accepts payment online, manage shipping, offer discounts and the list goes on. WooCommerce turns a regular WordPress site into a fully functioning ecommerce store.

Evolution Of Woocommerce:

WooCommerce was first developedon 27th of September 2011 by WordPress theme developer Woo Themes, who hired Mike Jolley and James Koster, developers at Jigowatt, to work on a fork of Jigoshop that became WooCommerce. In August 2014, WooCommerce powered 381,187 sites (or 17.77% of e-commerce sites online).

In November 2014, the first WooConf, a conference focusing on eCommerce using WooCommerce was held in San Francisco, California. It attracted 300 attendees.

In May 2015, Woo Themes and WooCommerce were acquired by Automatic, operator of WordPress.com and core contributor to the WordPress software.

cept Of Woocommerce:

WooCommerce is a free e-commerce plugin that allows you to sell anything beautifully. Built to integrate seamlessly with WordPress, WooCommerce is the world's favorite solution that gives both store owners and developers complete control. With endlessly flexibility and access to hundreds of free and premium WordPress extensions. WooCommerce is for selling products and services. Specifically, it makes doing these things affordable and accessible. You can sell digital and physical products, manage inventory and shipping, take secure payments, and sort taxes automatically.

Why Woocommerce

A successful sale starts long before someone clicks "buy." Create a store as unique as your brand and create a special experience for navigating your products, content and site.

- Countless themes: Pick the theme that works for you. WooCommerce is designed to work seamlessly with themes you know and love, including each year's default WordPress themes and many popular themes from around the web.
- Unrestricted customization: Edit any part of your site – from the homepage layout to the buy button – to stand out from the crowd.
- Built-in blogging: Publish great content and build a strong brand. Use WooCommerce to integrate eCommerce with the world's most popular content publishing platform.
- Embed products, checkout and more on any page: Use short codes to add your products to blog posts, or create landing pages that go straight to checkout.

Issues Of Woocommerce:

- No Frequent Updates
- Effective Knowledge To Use
- Compatibility Issues
- Seo Features Issues
- Plugin And Theme Conflicts
- Cost

No Frequent Updates

Most of the customers are dissatisfied about the updates of WooCommerce. It doesn't meet the requirement of quick updates. Sometimes your WordPress plugin is up to date but WooCommerce doesn't meet with updates.

Effective Knowledge To Use

This plugin requires effective knowledge to use. For instance, if we want to make changes in our website, it's difficult to do it by ourselves.So, you have to know about the both WordPress and WooCommerce.

Compatibility Issues: WooCommerce faces a lot of compatibility issues because of various loads of woo themes.

Plugin And Theme Conflicts: Theme and plugin conflicts are one of the biggest causes of most WordPress site issues, and this includes sites that run WooCommerce too. The more plugins you have running the higher the ances of a theme or plugin conflict arising.

Can Be Costly- WordPress and WooCommerce are free of cost and also there are various themes and plugins are available but sometimes it does not meet your requirement. If you want to customize site according to your requirement then it takes the time to do that or by expanding money you can customize your website.

Woocommerce Vs Ecommerce:

Difference Between e-commerce and WooCommerce is that E-commerce, short for electronic commerce, is a business transaction that occurs over an electronic network such as the Internet. While WooCommerce is a free WordPress e-commerce plugin developed for you to sell anything on your web.

E-commerce, short for electronic commerce, is a business transaction that occurs over an electronic network such as the Internet. Anyone with access to a computer or mobile device, an Internet connection, and a means to pay for purchased goods or services can participate in e-commerce.

WooCommerce is a free WordPress e-commerce plugin developed for you to sell anything on your web. WooCommerce is the world's favorite ecommerce plugin that install in WordPress. You can sell anything and anywhere in world. Because, WooCommerce allow you to sell anything and even features like bookings, memberships everything under your control. You can ship everywhere you like, woo-commerce allow buyers to select shipping address so you can ship products there. Woo-commerce also allows you to have different payment ons in your website to make money easily.

The Top Seven Amazing Websites Of Woocommerce:

- PORTER & YORK
- JACK RUDY COCKTAIL CO
- MINIPOP
- ROOT SCIENCE
- STRANDBERG GUITARS
- NORDIC WARE
- GOOD DYE YOUNG

PORTER & YORK

-see why we are better.

Porter and York is a meat delivery service to our door. It has been featured in Oprah magazine. The company prides itself selling on high quality meat, so that its slogan is 'see why we are better'. My favorite part of this website is the clean imagery, depicting the meat they sell.

To sum up, the development of an online store with WooCommerce is a great idea. Thousands of plugins, themes and developers are available on the market. Also, it should be necessary to help you find solutions that simply work, bring results and save your time on the one hand and on the other help you develop your software for a perfect WooCommerce store.

IV. Conclusion:

WooCommerce is a perfect solution for creating an e-commerce stores that is entirely own. With a wide variety of themes available, you can choose your favorite design and create a website that fits the unique needs of your customers.

As you can see from the examples provided, most great WooCommerce sites focus on high-quality images that clearly display the products, simple navigation and a strong brand personality reflected in color schemes and layout design. Emulating these characteristics on your own ecommerce site will make sure that it stands out. The internet has led to the birth and evolution of E-commerce just because of WooCommerce.

"If your business is not on the internet then your business will be out of business".

-Bill gates

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A Review on Security and Privacy in Manet

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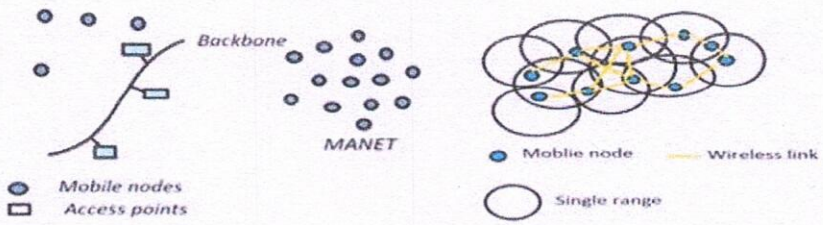
Abstract: *A Mobile Ad hoc Network (MANET) as a network that has many unrestricted or autonomous nodes, often composed of mobile devices or other mobile that can position themselves in various ways. And it is using as network simulation. That can variation locality and constitute itself on the wing because MANET or mobile communication it can be used as wireless connection to connect various network. Some MANET are regulated to local area of wireless device while other may be connected to the network so it is to be important to be attentions MANET there are archetypally are not very secure. In this paper propose an organization address to this issues security and privacy in MANET face much difficulties in this environment. So it is to be needed security production to MANET arises as future extension of a work.*

Keywords: *ad hoc network, security, privacy, mobile communication*

I. Introduction

Security and privacy is the most important concern in MANET because the nodes and the information in MANET are not secure. Privacy and security is not easy to product in this comprise identify explore and node tracking this scheme is scalable when cluster based network configuration is employed. Next generation of mobile communication will include both prestigious infrastructure wireless network and infrastructure less network mobile ad hoc (MANET) ad hoc networking concept is a new one having been around in various forms for over two decades. Service attacks of denial is the ad hoc network characteristics and also mobile devices imply higher security menaces compare with fixed operating devices. Eavesdropping spoofing and denial of services attacks are the main threads for security. Inherent characteristics of MANET (i.e.) wireless medium, broadcast transmission and lack of centralized administration of mobile ad hoc network vulnerable to security threats. This figure circumstances that the information it will not initiative unswervingly by only device to stratagem communication and in the reorganized communication at infrastructure -less network.



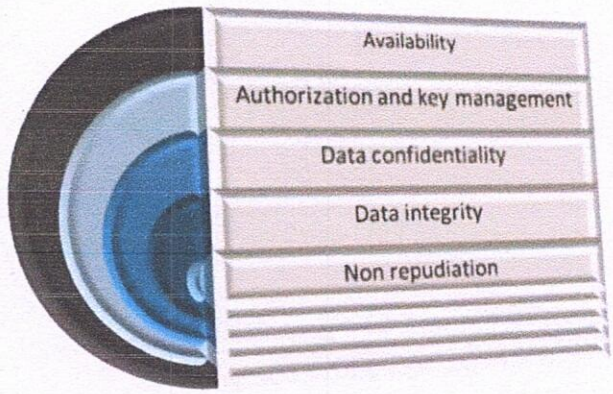
Wireless Mobile Network

II. Methodology

We here propose a MANET system that takes into contemplation the privacy and security of individuality locality and moving track of node. In this system, as usual every node still as one has unique individuality but the individuality needs to be carefully design. Each node should have multiple anonymous individualities they may be a several solution that can map a unique identity to multiple anonymous ones, and all so do a reverse mapping another concern for a MANET is that the storage capacity and processing power of the node device in MANET are very limited so we can't maintain a large mapping table on the nodes side.

- **SECURTIY THREATS IN MANETs**
  - Types of Attacks
  - Denial of service
  - Impersonation
  - Disclosure

In ad hoc network the probability of denial of an amenities attacks essentially threaten the operation the all the times of network and they are stereotypically impossible to prevent as such. The development of secure ad hoc networking. Therefore without appropriate security comestibles MANET become prone to spoofing. Hence needs to be provide security protection to MANET arises exclusively when deployed in military operation and emergency operations type of application. It is clear that the security trait related to ad hoc network form a very complex problem fields given the dynamic and unpredictable. The idiosyncrasy of ad hoc network poses both challenges and opportunities for security mechanisms.

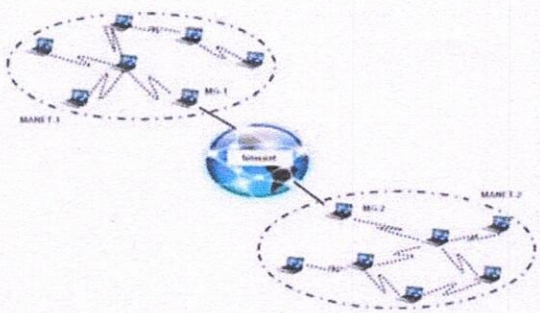


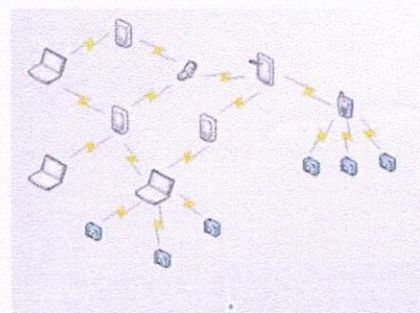
Security requirement in MANET

Mobile wireless networks generally more prone to physical security threats then are static wire network exiting link level security techniques are often applied with in wireless network to reduce this types of threats. In several mechanism has been study to detect the different attacks and conclude that the security is an essential part of adapt network is still considered to be challenging task. It shows in the manner in which the response surface methodology approach can be used to analyze and model the routing protocol performance in MANET developing models rather than conducting traditional experiential analyze important.

III. Result And Discussion

Still some of the drafts currently ignore the security issues by stating that the require security means are to be determined later in this case on can get the impression that the security mechanisms will later be retrofitted if the protection mechanisms are not designed concurrently with the basic protocol. More over some of the discussed MANET protocol have ignored the security issues completely. As mobile ad hoc network becoming widest area of research lots of modification are occur in day by day.





It is easier for hackers to eavesdrop and gain access to confidential information for them to enter or leave a wireless network because no physical connection is required. When we discussing network security general, two aspects need to be considered the services required and potential attacks. The security services

aspect includes the functionality that is require to provide a secure networking environment while the security attacks cover the methods that could be employed to break this security services. MANET proved itself a versatile network now a days but is quite unreliable due to its less attack handling capability it is less immune to attacks. Prevention mechanism require encryption techniques provide authentication, confidentiality, integrity and non-repudiation of routing information.

IV. Conclusion

The future of ad hoc network is real appealing, giving the version of anytime, anywhere cheap communication. And it is a self-configuring, infrastructure less network consists of independent mobile nodes that can communicate via wireless medium the security and privacy is essential part of ad hoc network. A tremendous advancement has been witnessed in the field of mobile communication in the past few years. MANET is a group of wireless mobile hosts which build a temporary network without the requirement of any centralized administration or backbone support services. The various criterion, upon which the safety of the network is evaluated and also realized. The area of ad hoc networking has been receiving increases attention among the researches in recent years. MANET since most of the current techniques were originally design for wire networks, many researches are engaged in improving the old techniques.

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Feasibility approach on Web Services and Security

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Abstract: *The vision of a landscape of heterogeneous web services deployed as encapsulated business software assets in the Internet is currently becoming a reality as part of the Semantic Web. When pro-active agents handle the context-aware discovery, acquisition, composition, management of applications services and data, ensuring the security if customers data become a principle task. In this paper we propose neoteric way web services and security. A methodology based on type-based Information flow to control the security of dynamically computed data and their proliferation to other web services. The business and security concern of integrated web services are separated and building them independently. Runtime modification of integrated web services. Providing compartmentalization so that one service can not affect another. We are developing flight system to demonstrate the feasibility of our approach.*

Keywords: *Web services, security, flight system, internet*

I. Introduction

As we all know that the security is must for any valuable thing and hence the topic of security is trending nowadays. In this situation clients consider security to be delivered immediately even on programs that were not developed with security in consideration. When the systems are to be developed for the web/networked environments the challenge is even competent. A web service [1] is a standards-based, language-agnostic software entity that accepts specially formatted request from other software entities on remote machines via vendor transport neutral communication protocols, producing application specific responses.
The simplest web service system has two participants:
(i) A service producer (Provider).
(ii) A service consumer (Requester).

Instances of these security features can be the enforcement of user authentication, access control and data to validate the feasibility of our proposition, we developed a Flight System (FS) that is composed of several Web services. A RBACK (Role Based Access Control) model for the flight system, which we called RBAC-FS, is elaborated. Confidentiality in web services. Varietal standard languages have been determined to apply Web services security. The devised aspect realize the elaborated RBAC-FS model and provide authentication and access control features to the flight system. Case studies and experimental result are also presented to defend our propositions.

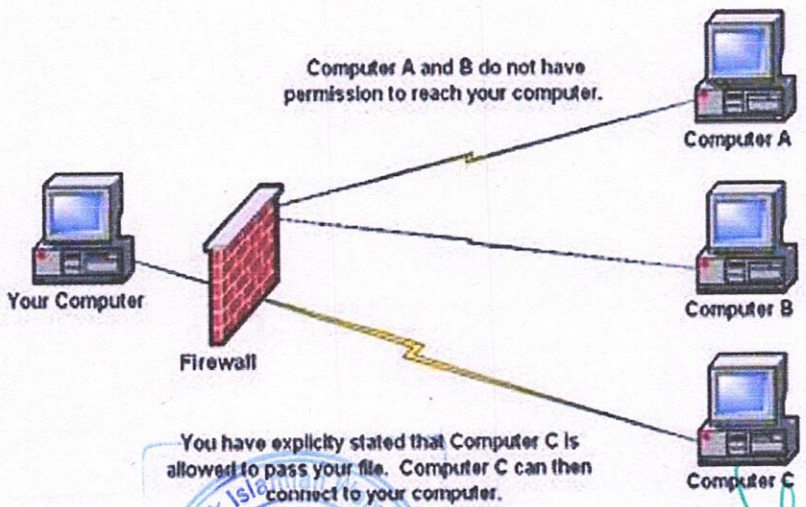

Fig 1. web service system.

II. Related Work

In this context, several standards such as Security Assertion Markup Language (SAML), WS-Security and WS-XACML were considered. The Security Assertion Markup Language (SAML), developed by the Security Services Technical Committee of OASIS, is an XML-based framework for communicating user authentication, entitlement, and attribute information.

As its name suggests, SAML allows business entities to make assertion regarding the identity, attributes, and entitlements of a subject (an entity that is often a human user) to other entities, such as a partner company or another enterprise application. WS-Security protocol was originally developed by IBM, Microsoft, and VeriSign.

Their original specification was published on 5 April 2002, and was followed up by an addendum on 18 August 2002. WS-Security addresses security by leveraging existing standards and specification. XML Encryption and XML Signature describe ways of encrypting and signing the contents of XML messages. XML canonicalization describes ways of making the XML ready to be signed and encrypted. What WS-Security adds to existing specifications is a framework to embed these mechanisms into a SOAP message.

This is done in a transport-neutral fashion. OASIS proposed "The Web Services extensible Access Control Language (WS XACML)" as XML based language to specify and exchange access control policies.

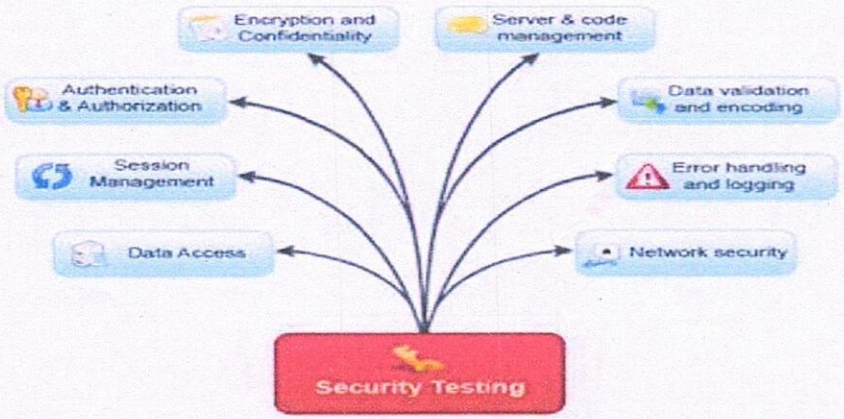

Fig 2. WS-Security assertion matching

Many security features require run-time verification of the security policies, which may often be modified and updated. This means that when the security policies and/or the verification strategy change, the developer has to go back to the design/code of the web services and update them accordingly. This mechanism is bulky, error-prone and tedious. Our approach relies on the dynamic injection of AOP aspects into BEPL processes.

Digital labs proposed an AOP language called CSAW, which is a small superset of Ph.D. thesis, discussed an aspect-oriented approach that allowed the integration of security aspect within applications. The approaches in the AOP are useful to

Explore the feasibility of using AOP in software security. Hence, we can benefit from their achievements in building our security model.

The interaction between the user, the BPEL process and the web services of the flight system. As depicted in the fig, the security features are deployed on the web services side (i.e. not in the BPEL process). This clearly shows that any changes in these security features need a modification in the corresponding web service.

The system available services are shown in the system main page. First, the financial data service allows the user to request the revenues and expenses of the flight agency for a given month, second, the flight inquiry service returns a list of the airline, and the available seats and tickets price. The employee information service allows the user to view information about the flight system staff by entering their ID number.

This information includes the employee's full name, phone number, email, address, post and his office number. In other words, each user has an ID and a password stored in the database, in addition to his/her personal information. Each time a user wishes to access one of

the flight system services, both the authentication and access control services are invoked to ensure that he/she is not only a valid user, but he/she also has the permission to view the requested information.

III. Approach description

Aspect Oriented Programming (AOP) is one of the most prominent paradigms that have been devised for integrating Non-functional requirements (e.g. security) into software. A point cut identifies one or more join points. A join point identifies one or many flow points in a program (in our case a program is a BPEL process). At these points, some advices will be executed. An advice contains some code that can alter the process behavior at a certain flow point.

This means that any change in the security strategy has to be done on the application code, which can have impact on the business logic. AOP solves this issue by embedding security in aspects. Aspects allow to application, which make them interesting solutions for many security issues. Many contributions in addition to our experiments, have proven the usefulness of AOP for integrating security features into software. we present in this section an aspect-oriented approach for the dynamic enforcement of web services security. Our proposition is based on the use of AOP in the BPEL process of the composed Web services. It allows to specify the security concerns into separate components called aspects. These aspects are then weaved in the BPEL process at runtime.

IV. Conclusion

We presented an approach to use language based information flow control to ensure the confidentially, integrity of user's data provided to dynamically composed web services. Our hypothesis is based on the coactions between AOP and formation of web services. It permits the partition of business and security concerns of web services, and hence building them independently. It also permits the alteration of the web services at run time and provides distinction for designing cross-cutting concerns between web services.

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Architecture and Applications of Wireless Body Area Network

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2Student PG Department of Computer Science
3Student PG Department of Computer Science

Abstract: *Over the course of years a booming Internet is comprehended in the field of wireless communication for the evolution of a monitoring system to notice the human vital organs activities remotely. Wireless Body Area Network (WBAN) can be wearable or implantable in the human body. Due to its applications in the field of health, medical, entertainment services and many more, WBAN have received great attention. In this paper a concise survey consisting of the existing approaches of WBAN and its challenges has been discussed. Its abundance applications in the field of medical and non-medical sectors have been outlined. And finally this paper describes the future scope for further research in the field.*

Keywords: *Wireless Body Area Network, Auto medication, easily accessible, affordable.*

I. Introduction

Recently, the research community has directed its interest towards the development of substantial and adaptable networks, which is made up of small devices having the ability to collect information regarding the enclosed environment. This approach is commonly called "sensing" and the corresponding devices are called "sensors" [1]. As a result of the exceptional advances in the field of wireless communications, computer networking and hardware design, the recognition of sensors has become recently possible and cost efficient.

The recent technological improvement in integrated circuits, physiological sensing and wireless communications qualify for the production of miniature, light-weight, ultra-low power intelligent monitoring devices. Many of these devices can be integrated into a Wireless Body Area Network (WBAN), a new enabling technology for healthcare monitoring.

Due to the increasing poverty and the number of elder people existing in the world, the need for personal home health care is growing. To end this, wireless sensor technologies have validated new types of applications for monitoring and regulating people's physiological parameters.

The first generation of e-healthcare results were to some degree replacement of a wire with a wireless communication channel, i.e., another set of protocols on top of a new physical communication media. In the second generation, with a local system host, the devices communicated wirelessly, whichtransmit alarms. In the third generation the mobile body area network are wirelessly attached to healthcare sensors and actuators.

The Wireless Body Area Network (WBAN) varies from other Wireless Sensor Networks (WSN) with some eloquent points. The first difference between a WBAN and WSN is energy consumption. WBAN consumes less energy than other WSNs. The second difference is mobility. The user can move with sensor nodes with same mobility patterns in WBAN whereas WSNs are usually immobile. Based on geographical coverage, WBAN operates close to the human body (1m-2m). WPAN (Wireless Personal Area Network) surrounds the person (up to 10m). WLAN (Wireless Local Area Network) surrounds the person (up to 100m). WWAN (Wireless Wide Area Network) covers the largest geographical area. WBANs are subset of WSN or WSAN (Wireless Sensor and Actuator Network) [2]. Furthermore, WBAN sensor devices are found cheaper than WSNs.

II. Wban Architecture:

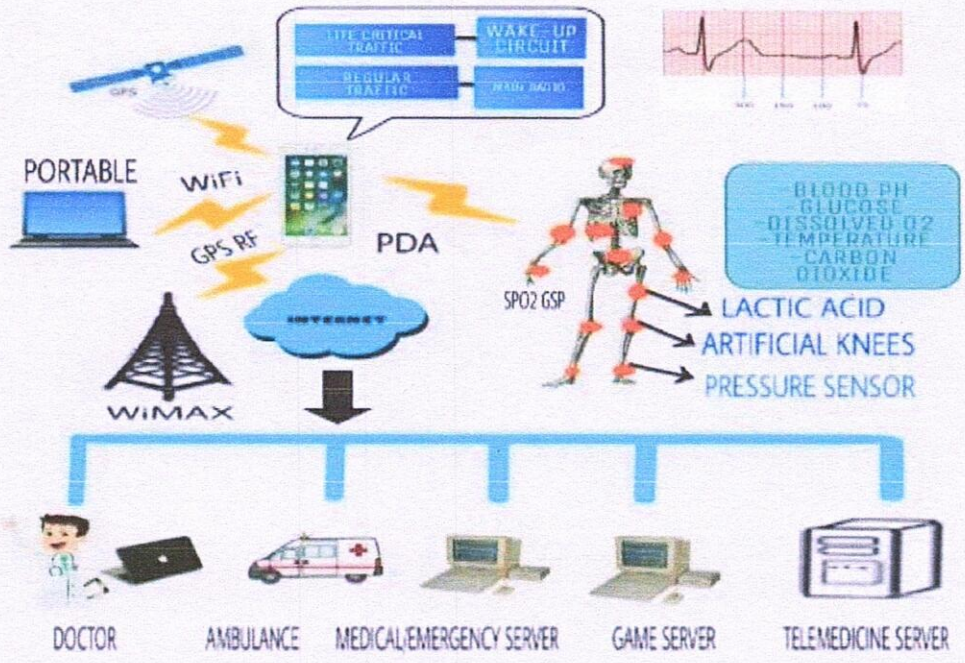

Figure 1: Architecture of WBAN

A structure for the wireless real-time monitoring of physiological data from a body can be systematically arranged in a wireless BAN as illustrated in the Figure 1. Tier 1 takes in or contains different sorts of wireless medical sensor nodes. The physiological signs from these little sensor nodes are been displayed by WBAN which have remote transmission capacity set either inside or around a man's body [3]. They are deployed to collect important wellbeing information of a person which occurs during medicinal or game or training activities. Every single sensor has the capability to inspect and recognize as well as to take action on at least one of the physiological signs. For example, Electrocardiogram sensor (ECG) have the ability to check on cardiovascular activity [4], the level of oxygen in our body can be measured by Oxygen saturation sensor (SpO2), and many others. Tier 2 takes in or contains the personal server(PS) application running on a client Laptop , Personal Computer, iPod or some other suitable gadgets which have the information collected of the remote devices and at a particular time the whole data is transferred to a appropriate PC when a compatible interface is accessible. Tier 3 incorporates several remote based-stations that have kept recorded all the personal/therapeutic/non-medical information and based on those reports recommendation are given.

Examples:

There are many examples available for the wireless technology that are already in existence and are been used. They can be wearable also. Some of the examples that can be provided are Smart shirt which, when worn by a person, regulates the body temperature, blood pressure, heart rate, the working of internal organs and gives you the correct information and recommendations that will be helpful for the person [5].

Another example would be bandages which when plastered to a person it stops the blood clotting, prevents the infection to spread and it even quickly heals the infected area. One more example can be of Electroencephalography (EEG), it records the electrical activity of the brain. The recording time would be of about 28-43 minutes [6]. It mainly focuses on the spectral neural oscillations signals that are observed. EEG measures the fluctuations voltage that occurs due to the ionic current flows surrounding the neuron of the brain. Due to this, many diseases of the brain, neuro problems, and diagnosis of epilepsy have been detected. And it also checks whether the patient is corresponding to the therapy or not. All these examples and many other wireless body area sensors help in the correct maintenance of the body. It helps the elderly people, weak people and the people who can't afford costly treatment. It not only detects the patients' health problem but also immediately informs the doctor. And when been informed to the doctor, the patient in response gets a reply. This works even in distant areas. The information can also be transformed through a personal server

such as wireless network like RF, WLAN, Bluetooth or Cellular network. The wireless server before time indicates the upcoming of a person's heart attack. Thus this helps the people in many ways.

III. Wban Applications:

The WBAN has introduced many modern, useful and effectual applications. They have been in use for a couple of years now and have given quite a positive response. For better understanding, they can be categorized into two types [Figure 3]. They are therapeutic and non-therapeutic applications. Let us first look on the therapeutic applications.

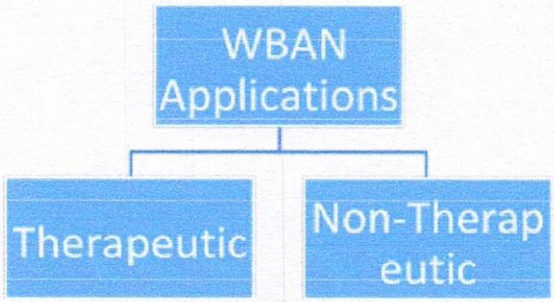

Figure 3: WBAN Applications

A) Therapeutic Application:

The therapeutic applications have helped the people in medical sector. It has improved the doctor-patient relationship. This has made easier for the patients to connect directly to their doctors at the time of emergencies [7]. The therapeutic applications can be divided into various sectors. They are distant health Monitoring, Assisted Support and Telemedicine. Distant health monitoring helps connecting the patients living in isolated areas to their doctors [8]. Through the help of sensors the body organ status can be indicated to the doctors. Assisted Support is another method of therapeutic application, which helps the patient get their treatment in their homes instead of staying in the hospital. Telemedicine is one of the enthralling application fields, which provides medical treatment over a distance through the help of communication technology.

B) Non-Therapeutic Application:

Fitness and Sports Monitoring, military, gaming and for entertainment purpose this non-therapeutic application is used.

IV. Challenges Of Wban:

Despite the fact that WBAN is helping in both therapeutic and non-therapeutic sections, still there are some challenges which are to be taken care of.

Privacy Issues: Privacy is one of the most important challenges of WBAN. The information of patient health is collected that's why WBAN must ensure the privacy of the individual information.

Sensor Authorization: sensor need to validate the data that are being collected. Thus, this will help in the detection of problems in hardware and software designs.

Security: Security is another one of the challenges in WBAN [9]. This is to ensure that one patient's information doesn't mix with other patient information. Attempts are being made to keep the WBAN more secure.

Interference: Interference should be less in the WBAN system of large scale implementation.

Management of Data: A large amount of data is originated in WBAN [10]. Accumulating and organizing this large amount of data is challenging and also important.

V. Conclusion And Future Scope:

WBAN is an arising technology in today's world. WBAN has the capability to provide good and cheap healthcare services and provides more convenience to patients and the society. WBAN improves the standard of life as it enables the person to live a normal life with normal activities rather than staying in a hospital

or near a medical electronic device. WBAN acknowledges the physical, chemical and biological changes in our body and then alarms the person about it. It focuses on prevention and early detection. Context-Sensitive Medicine, a Pre hospital Mobile Database for Emergency Medical Services and Patient Homecare are some of the future applications of WBAN. Thus in this paper we discussed on the architecture of WBAN, its examples, types of WBAN application and the challenges that are faced by WBAN.

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Research paper

S-allyl cysteine as potent anti-gout drug: Insight into the xanthine oxidase inhibition and anti-inflammatory activity

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ABSTRACT

S-allyl cysteine (SAC) is known for its various beneficial effects such as neuroprotection and immunomodulation. The beneficial effect of SAC against gout has not been explored. The present study aims to describe the two roles of SAC: (1) inhibitory effect against xanthine oxidase (XO) enzyme activity; and (2) anti-inflammatory property against MSU crystal-induced gouty inflammation in rat. The inhibitory effect of SAC against bovine XO enzyme activity was determined *in vitro*. *In silico* analysis was carried out to determine the intermolecular interaction between SAC and bovine XO. MSU crystal was injected in the right paw of the rat to induce gouty inflammation. SAC (40 mg/kg body weight) and colchicine (positive control; 1 mg/kg body weight) was given for 3 days. At the end of the treatment, the oxidative stress, antioxidant parameters and mitochondrial function were determined in the ankle joint tissue. The concentration of inflammatory cytokines such as TNF-α and IL-1β was measured in the serum using ELISA. SAC inhibited (IC_{50} value, 33 μg/ml) XO enzyme activity in a competitive mode with corresponding Ki value of 4 μg/ml. *In silico* analysis predicted the interaction of SAC with the amino acids such as Arg880, Phe798, Phe914 and Phe1009 of XO enzyme. The root mean square deviation, root mean square fluctuation and free energy calculation values confirmed the stable SAC-XO interaction. The inhibition of SAC on XO enzyme activity in *in vivo* was further confirmed by silkworm model. SAC through reducing oxidative stress, enhancing antioxidants, protecting mitochondrial function has shown anti-inflammatory effect against MSU crystal-induced gout which was observed as reduced level of inflammatory markers in the serum. The medicinal potential of SAC as a preventive agent through its XO inhibitory property as well as curative agent through its anti-inflammatory property against gout has been understood from the present study.

1. Introduction

Gout is a form of autoinflammatory disease caused by the innate immune response due to the deposition of monosodium urate (MSU) crystals in the joint and periarticular tissues. The deposition of MSU in joints is preceded with hyperuricemia (a condition where serum uric acid/urate levels are above 6.8 mg/dl). Xanthine oxidase (XO) catalyzes the oxidation of xanthine and hypoxanthine into uric acid. In addition, during such oxidation process XO generates reactive oxygen species (ROS) and hydrogen peroxide leading to oxidative stress [1]. The hyperuricemia and oxidative stress caused by XO are considered as important factors that results in gout [2]. MSU formed due to hyperuricemia is engulfed by articular resident macrophages that are present within the joint space and trigger the inflammatory cascade such as nuclear factor kappa-light-chain-enhancer of activated B cells (NF-κB) pathways and nucleotide-binding domain and leucine-rich repeat containing protein 3 (NLRP3) inflammasome. Activation of NLRP3 by MSU leads to the caspase 1-mediated cleavage of pro-interleukin 1β (IL-1β) to the active secreted IL-1β. Besides, contact of MSU crystal with monocytes results in upregulation of tumor necrosis factor-alpha (TNF-α) in gouty tissues *in vivo* [3,4]. Altogether, progressive inflammatory

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0300-9084/

cascade and infiltration of neutrophil due to deposition of MSU crystal in the joints trigger the lysis of membrane which ultimately further lead to bone erosion and damage.

Present anti-gout drugs such as nonsteroidal anti-inflammatory drug, colchicine, and allopurinol are associated with various adverse side effects such as gastrointestinal toxicity and cardiovascular risk [3,4]. Recently, the search for efficient natural compounds with better urate-lowering and anti-inflammatory effect for the treatment of gout has gained importance. The sulphur containing amino acid, s-allyl cysteine (SAC) predominantly present in aged garlic extract has been well known for its antioxidative, anti-inflammatory and neuroprotective property. The presence of sulfur and allyl group in SAC is considered imperative for its antioxidant property. SAC is less toxic and has high bioavailability after oral administration [5]. The effect of SAC against MSU crystal-induced gout model has not been explored. Keeping all these facts in view, in the present study, the efficacy of SAC as multi-target ligand against gout has been delineated. First, the effect of SAC against XO enzyme activity has been determined by *in vitro* and *in silico* analysis. Secondly, the effect of SAC against XO enzyme activity in *in vivo* has been evaluated using silkworm model. Silkworms lacks uricase enzyme and hence the end product of purine metabolism is uric acid which is similar to humans. The cost effectiveness of silkworm model makes it an easy yet efficient model to screen and evaluate gout therapeutic drugs [6]. Thirdly, the antioxidative and anti-inflammatory effect of SAC against MSU-crystal injected rat gout model in *in vivo* has been studied.

2. Materials and methods

2.1. Materials

XO from bovine milk (Grade IV, ammonium sulphate suspension, 0.3 U/mg protein), colchicine, diaminobenzidine and all chemicals used in the mitochondrial oxidative phosphorylation (OXPHOS) complex assay were purchased from Sigma Chemical Co. (St. Louis, MO, USA). SAC was purchased from TCI chemicals, India. Uric acid and protein estimation standard kit were purchased from Coral Clinical systems, Goa, India. ELISA kits to measure IL-1β and TNF-α were purchased from R and D systems (MN, USA). All other chemicals used were procured from Himedia Pvt Limited, Mumbai, India and of the highest purity.

2.2. XO enzyme activity and inhibition study

The enzyme activity of XO was determined by the method as described earlier with slight modifications [7]. The substrate and the enzyme solutions were prepared immediately before use. The assay mixture consisted of 25 mM phosphate buffer (pH 7.5), 75 μM xanthine and 50 μl of XO (0.28 units/ml in the buffer) in a total reaction volume of 1.5 ml. The reaction was initiated by the addition of substrate and the absorbance was measured at 295 nm for 2 min using UV-Vis spectrophotometer (UV-1800, Shimadzu, USA) indicating the formation of uric acid at 25 °C.

The inhibition of XO enzyme activity was determined in the presence of various concentrations (5 μg/ml – 65 μg/ml) of SAC dissolved in 25 mM phosphate buffer. The XO enzyme was incubated with different concentration of SAC at 4 °C for 15 min. After incubation, the XO enzyme activity was determined as given above. A standard drug allopurinol was used as positive control.

2.3. Kinetic analysis of XO enzyme inhibition

Lineweaver and Burk double reciprocal plot and Dixon plot were used to determine the kinetic interaction between SAC and XO [8,9]. Kinetic analysis was studied over a range of xanthine concentrations (0.025 mM-0.100 mM) in the absence and presence of SAC (25, 40, 50, 60 μg/ml). All the enzyme activity assays were carried out in three independent sets of duplicates and data were presented as mean ± SD.

2.4. Molecular docking and molecular dynamics simulation

The X-ray crystal structure of bovine XO (PDB code 1FIQ) was used for the docking studies [10]. The A-chain, B-chain and all small molecules were removed from the protein. The C-chain that contains the active site was used for the docking study. SAC was drawn using chemdraw software and optimized by GAUSSIAN03 package with DFT method using 6-311G** basis set [11]. The molecular docking analysis has been performed using AUTODOCK program [12]. The lowest energy conformer was used to determine the intermolecular interactions between SAC and XO using PyMOL software. The molecular dynamics (MD) simulation of XO-SAC complex was performed using AMBERTOOLS14 package [13].

2.5. In vivo studies

2.5.1. Bombyx mori experimental model

Age matched (from the 2nd to the 8th day of the fifth instar) *Bombyx mori* (silkworm; Dazao strain) model was used in the present study [6]. The *B. mori* was collected during the month of July–August from Krishnagiri, Tamil Nadu, India. *B. mori* were maintained at 25 °C and fed with fresh mulberry until they developed to the fifth instar larva.

On the first day of fifth instar larvae, the silkworms were divided into three groups. Each group consisted of 10 silkworms. The control group was continuously fed on fresh mulberry leaves coated with water. Allopurinol and SAC group was continuously fed on fresh mulberry leaves coated with 5 mg/ml of allopurinol and SAC, respectively. Hemolymph were harvested at 24 h intervals after silkworms were treated with various compounds and stored at −20 °C. The uric acid present in hemolymph was measured in accordance with the manufacturer's instructions.

2.5.2. Rat experimental model

Adult male Sprague-Dawley rats (150–160 g) were used for the gout inflammation study. Rats were maintained at 25 ± 1 °C with light and dark cycle controlled room. Rats were fed with standard pellet diet and water *ad libitum*. The experimental protocol was carried in compliance with the guidelines of the Institutional Animal Ethical Committee, Reg No: (PU/IAEC 1085/PU/OC/07/CPCSEA/Biochem/04/2016) Periyar University, Salem, India. All studies relating to animals were carried out in agreement with the ARRIVE guidelines for reporting tests involving animals [14].

MSU crystal was synthesized as described earlier [3]. MSU crystals were further checked for bacterial contamination, before administration to rat. After acclimatization, about 24 rats were divided into four groups as follows: (1) control group: intra-dermal injection of sterile saline (0.2 ml) into the right hind foot pad; (2) MSU group: intra-dermal injection of endotoxin-free MSU crystal suspension (4 mg in 0.2 ml of sterile saline) into the right hind foot pad; (3) MSU plus SAC group: MSU induction plus intraperitoneal (i.p.) injection of SAC (40 mg/kg bw) prepared in saline; (4) MSU plus colchicine group: MSU induction plus i. p. injection of colchicine (1 mg/kg bw) prepared in saline. The drugs were administered 1 h before the MSU crystal injection (single dose) and then once daily for 3 days. The paw inflammation of rat was quantified by measuring the paw thickness using Vernier scale at different time intervals for 3 days. Animals were sacrificed by cervical dislocation 24 h after administration of the final dose. The ankle joint was

removed and suffused immediately using ice-cold saline (0.9% NaCl). For biochemical analysis, 10% homogenate of ankle joint was prepared using chilled sodium phosphate buffer (0.1 M, pH 7.4) and supernatant was separated by centrifugation at 4 °C at 12,000 × g for 30 min. The supernatant was stored at −80 °C until analyses. Mitochondria from ankle were isolated as described earlier [15] and used for estimation of mitochondrial OXPHOS complex activity.

2.5.3. Determination of oxidative stress and antioxidants

The concentration of reactive oxygen species (ROS) in the tissue homogenate has been determined as a measure of reduction of nitro blue tetrazolium (NBT) at 630 nm [16]. Malondialdehyde (MDA) served as an index of lipid peroxidation (LPO). The MDA present in tissue homogenate reacts with thiobarbituric acid (TBA) to form TBA reactive substance (TBARS) which was measured at 535 nm [17]. The concentration of GSH in the tissue homogenate was measured in the presence of 5,5′-dithiobis (2-nitrobenzoic acid) (DTNB) at 412 nm [18]. The concentration of vitamin C in the tissue homogenate was determined in the presence of DTC reagent (containing 2,4-dinitrophenylhydrazine, thiourea and copper sulfate) and measured at 520 nm [19]. Superoxide dismutase (SOD) activity was measured as described earlier. Briefly, the assay mixture consists of NBT, phenazine methosulfate, reduced nicotinamide adenine dinucleotide (NADH) and tissue homogenate. The inhibition of reduction of NBT in the presence of tissue homogenate was measured at 560 nm [20]. Catalase activity was determined in the presence of hydrogen peroxide (H_2O_2) and tissue homogenate as described earlier. The disappearance of H_2O_2 was determined spectrophotometrically in the presence of potassium dichromate dissolved in acetic acid at 570 nm [21]. Glutathione peroxidase (GPx) activity was determined in the presence of H_2O_2, GSH, DTNB and tissue homogenate as described earlier. The concentration of GSH utilized was measured at 412 nm [22].

2.5.4. Determination of mitochondrial enzyme activities

The activity of complex I (NADH-dehydrogenase) was measured by monitoring the reduction of 2,6-dichloroindophenol indophenol (DCPIP) in the presence of NADH [23]. Briefly, reaction mixture consists of 25 mM potassium phosphate buffer (pH 7.8), 0.35% BSA, 60 μM DCIP, 70 μM decylubiquinone, 1 μM antimycin were prepared. An aliquot of mitochondrial protein was pre-incubated with 960 μl of reaction mixture at 30 °C for 3 min. Then, 20 μl of 10 mM NADH was added to initiate the reaction and the decrease in absorbance was read at 600 nm. The activity of complex III (cytochrome-*c*-reductase) was measured as an increase in absorbance because of the reduction of cytochrome *c* at 550 nm. The reaction mixture consists of 50 mM potassium phosphate buffer (pH 8.0), 100 μM EDTA, 0.2% BSA, 3 mM sodium azide and 60 μM oxidized cytochrome *c* was prepared. An aliquot of mitochondrial protein was pre-incubated with 780 μl of reaction mixture at 30 °C for

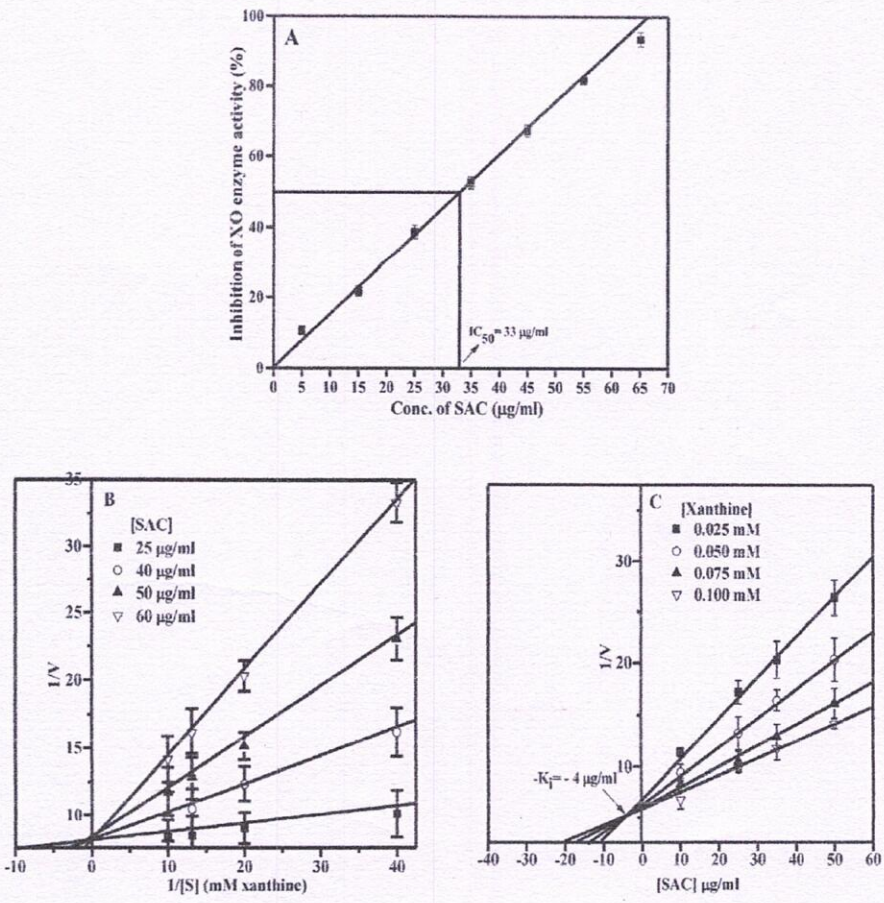

Fig. 1. Effect of SAC on bovine XO enzyme activity. (a) Percentage inhibition of bovine XO enzyme activity by SAC at various concentrations (5 μg/ml to 65 μg/ml) is shown. SAC showed dose-dependent inhibition against bovine XO enzyme activity; (b) LB plot represents the reciprocal of enzyme catalysis (1/V) against reciprocal of different substrate concentrations (1/S) in the presence of various concentrations (25, 40, 50, 60 μg/ml) of SAC for bovine XO enzyme. The plot shows competitive mode of inhibition of SAC against bovine XO enzyme activity; (c) Dixon plot for determining the inhibitor constant of SAC. The plot represents the reciprocal of bovine XO enzyme catalysis (1/V) against their respective different concentrations of SAC (10, 25, 35 and 50 μg/ml) in the presence of various concentrations (0.025, 0.05, 0.075 and 0.1 mM) of the substrate, xanthine. Results are expressed as mean ± SD (n = 3).

3 min. Then, 400 µl of 1 mM decylubiquinol was added to initiate the reaction and the increase in absorbance was read at 550 nm [24].

2.5.5. Assessment of pro-inflammatory mediators

The levels of pro-inflammatory mediators such as IL-1β and TNF-α in serum were measured by ELISA kit in accordance with the manufacturer's instructions.

2.6. Statistical analysis

Results are shown as mean values ± SD of the number of biological replicates indicated in the figure legends. Statistical analysis of the data was performed by one-way analysis of variance (ANOVA) followed by Tukey's multiple range tests for *post-hoc* analysis by using Sigmastat Version-3.5 software. The significance level was set at $P < 0.05$.

3. Results

3.1. Effect of SAC on XO enzyme activity in vitro

The inhibitory effect of SAC on XO enzyme activity is shown in Fig. 1A. SAC showed dose-dependent inhibition of XO activity. The IC_{50} value of SAC for XO activity was found to be 33 µg/ml. Kinetic analysis of enzyme inhibition using Lineweaver-Burk plot revealed that SAC is a competitive inhibitor of XO enzyme activity (Fig. 1B). The corresponding Ki value of SAC against XO enzyme activity is 4 µg/ml as shown by Dixon plot (Fig. 1C).

3.2. In silico prediction of SAC interaction with XO enzyme

The lowest binding energy of SAC with bovine XO enzyme was found to be −1.5 kcal/mol. MD simulation of SAC-XO complex was carried out to confirm the stability and to predict the conformational changes of the complex. The root mean square deviation (RMSD) of SAC-XO complex during MD simulation was in the range of 0.8 Å to 1.6 Å which depicts the stability of backbone atoms throughout the simulation while accommodating the SAC (Fig. 2A). The root mean square fluctuation (RMSF) value shows the fluctuations of residues present in SAC-XO complex during the simulation and confirms the flexibility of active site amino acids (Fig. 2B). The RMSD and RMSF values indicate that SAC is one of the more stable inhibitor of XO enzyme. The intermolecular interaction of SAC with various amino acids present in active site of XO enzyme at 1 ns and 10 ns during MD simulation is shown in Fig. 2C–D, respectively. At 1 ns of MD simulation, SAC showed hydrogen bonding interactions with amino acid Arg880 and water molecules present in the active site pocket (Table 1). Also, SAC showed hydrophobic interactions with amino acids such as Phe798, Phe914 and Phe1009. During 10 ns of MD simulation, SAC showed hydrogen bonding interactions with amino acids (Arg880, Ala1079, Glu802) and water molecule present in the active site. In addition, hydrophobic interaction of SAC with XO enzyme was similar to that of 1 ns time of MD simulation. From 1 ns to 10 ns of MD simulation the intermolecular interactions of SAC with XO enzyme increased. The binding free energy of the SAC-XO complex is shown in Table 2. The gas-phase interactions between protein and ligand are sum of electrostatic (ele) and Vander Waals interaction (vdw) energies; whereas, the solvation free energy $\Delta G_{solvation}$ is the sum of polar (ΔG_{GB}) and non-polar (ΔG_{SA}) contribution. The binding free energy value of SAC is −20.0 ± 3.16 kcal/mol. The contribution of ΔE_{vdw} and

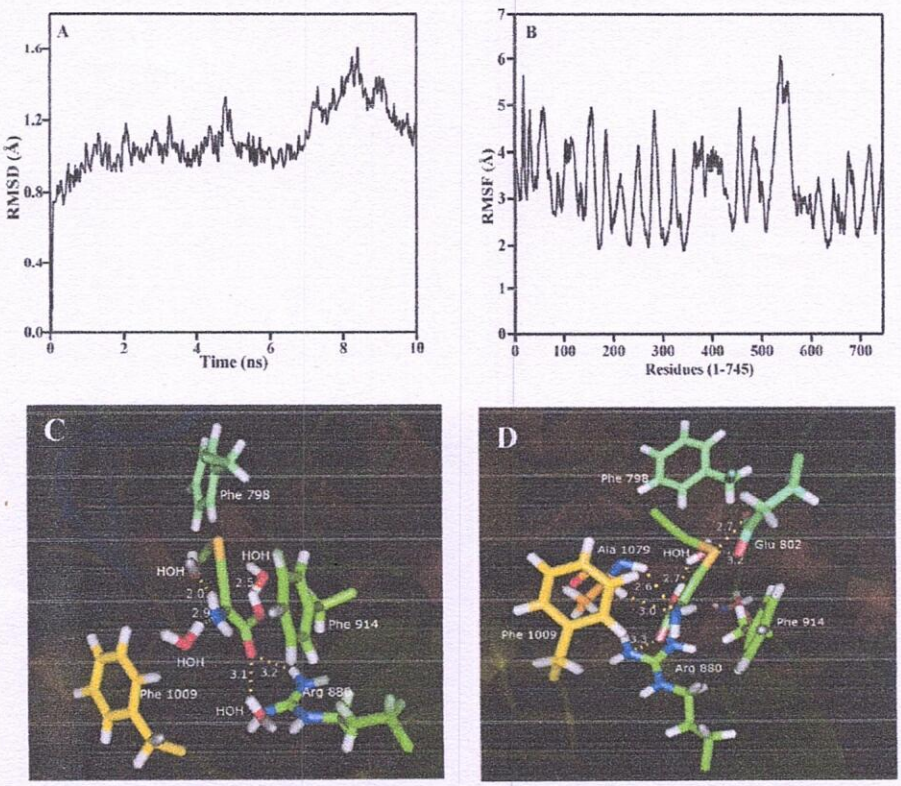

Fig. 2. *In silico* prediction of interaction of SAC with XO enzyme. (A) RMSD of bovine XO protein backbone against the MD simulation time for "SAC-XO complex". (B) RMSF of "SAC-XO complex" during MD simulation. Orientation of SAC near the active site at 1 ns (C) and 10 ns (D) time period during MD simulation.

Table 1
Intermolecular interaction of SAC with XO protein at various time points during molecular dynamics simulation.

Type of interaction	SAC	XO	Distance (Å)	
			1 ns	10 ns
Hydrogen bonding	O2	HH12/Arg880	3.2	3.0
	N1	H/Ala1079	–	2.6
	N1	HA/Ala1079	–	3.0
	S1	OE1/Glu802	–	2.7
	S1	OE2/Glu802	–	3.2
	H1	O/HOH871	2.5	2.7
	H2	O/HOH3091	2.0	–
	O1	H1/HOH9656	3.1	–
	N1	H1/HOH7312	2.9	–
Hydrophobic	C6	Phe798 (Pi-Alkyl … Pi-Orbital)	3.4	4.3
	C1	Phe914 (Pi-Alkyl … Pi-Orbital)	3.9	4.0
	N1	Phe1009(Pi-Alkyl … Pi-Orbital)	5.3	4.4

Table 2
Binding free energy of SAC-XO complex.

Energy Components	Energy (kcal/mol)
Van der Waals Energy	−24.0 ± 2.65
Electrostatic Energy	−71.24 ± 6.35
ΔG_{gas} (Vander Waals + Electrostatic)	−95.24 ± 5.42
ΔG_{GB}	78.75 ± 5.09
ΔG_{SA}	−3.51 ± 0.09
$\Delta G_{Solvation}$ (Polar + non-polar)	75.24 ± 5.10
ΔG (ΔG_{gas} + $\Delta G_{Solvation}$)	−20.00 ± 3.16

ΔE_{ele} is high for the SAC molecule. It indicates that, the SAC molecule forms strong interactions with active site residues and it is stable during the MD simulation.

3.3. Effect of SAC on the activity of XO enzyme and uric acid content in B. mori

The XO enzyme activity in hemolymph decreased significantly ($P < 0.05$) from day 1 to day 5 by SAC treatment in comparison to age-matched control silkworm group (Fig. 3A). The concentration of uric acid which is an end product of purine metabolism decreased significantly ($P < 0.05$) from day 1 to day 5 by SAC treatment in comparison to age-matched control silkworm group (Fig. 3B). The reduction in XO enzyme activity and uric acid concentration by SAC treatment is comparable to that of allopurinol treated group.

3.4. Effect of SAC on MSU crystal injected in vivo rat gout model

3.4.1. Effect of SAC on paw swelling

The inflammation severity caused by MSU crystal injection in the hind leg of rat was measured as swelling of the paw at different time-interval for 3 days (Fig. 4). MSU crystal injection significantly ($P < 0.05$) induced the swelling in the hind leg paw and the inflammation sustained for 72 h compared to control group. However, SAC treatment significantly ($P < 0.05$) reduced the swelling of the paw in ankle circumference induced by MSU in comparison with MSU crystal-injected group. The easing of inflammation by SAC was comparable to that of standard drug colchicine (Fig. S1).

3.4.2. Effect of SAC on oxidative stress to antioxidant status

MSU crystal injection significantly ($P < 0.05$) increased the oxidative stress parameters such as ROS generation and LPO of the joint tissue (Fig. 5A–B). In addition, the activity of enzymatic (SOD, CAT and GPx) antioxidants (Fig. 6A, B and 6C) and concentration of non-enzymatic (GSH and vitamin C) antioxidants (Fig. 6D–E) were decreased in comparison to control. SAC and colchicine treatment significantly subdued the oxidative stress induction by MSU-crystal injection. Also, the level of antioxidant status was sustained close to the control level when compared to MSU crystal-injected rat.

3.4.3. Effect of SAC on ankle mitochondrial OXPHOS activities

The activities of ankle tissue mitochondrial OXPHOS enzymes such as NADH-dehydrogenase (complex I) and cytochrome c-reductase (complex III) in MSU crystal injected rat were significantly decreased in comparison to control group (Fig. 7A–B). SAC treatment maintained the activity of mitochondrial OXPHOS enzymes to control level compared to MSU crystal injected rat. Also, the treatment with SAC was comparable to the colchicine treated group in hindering the reduction of mitochondrial enzyme activity.

3.4.4. Effect of SAC on inflammatory mediators

The level of pro-inflammatory mediators such as cytokines TNF-α and IL-1β significantly increased in the serum of MSU crystal-

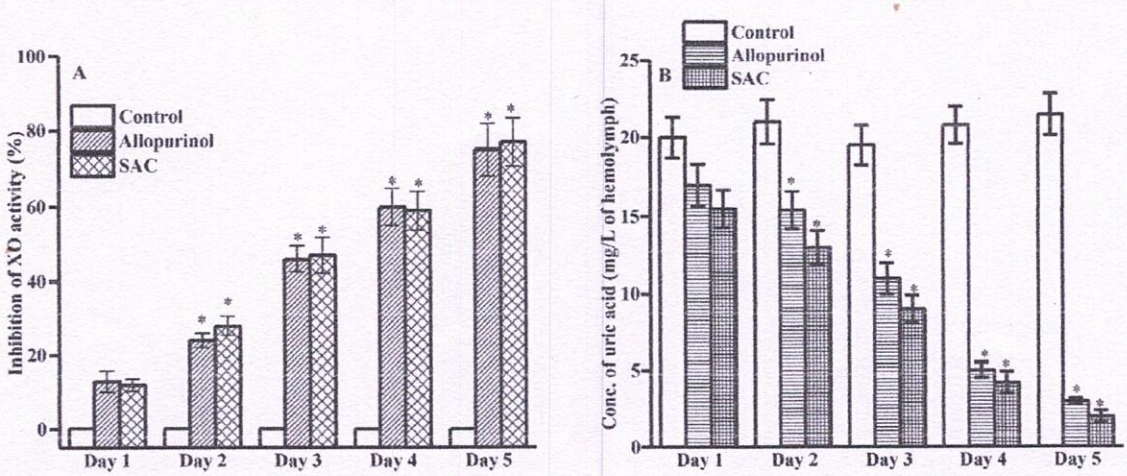

Fig. 3. Effect of SAC on XO enzyme activity (A) and uric acid content (B) in the hemolymph of 5th instar *B. mori*. Allopurinol and SAC group was continuously fed on fresh mulberry leaves coated with 5 mg/ml of allopurinol and SAC, respectively. Hemolymph was harvested at 24 h intervals after silkworms were treated with various compounds. The XO enzyme activity and uric acid content was determined as described in the Materials and Methods section. Results are expressed as mean ± SD (n = 10 per group). *$P < 0.05$ vs. Control.

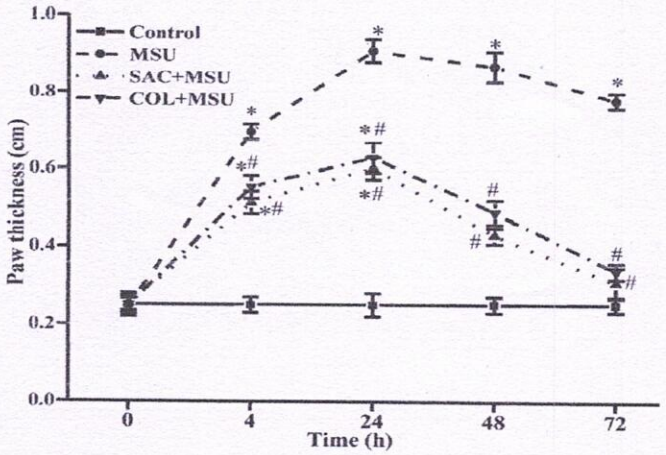

Fig. 4. Effect of SAC on MSU crystal-induced paw edema in rats. Rats in control group were injected with sterile saline (0.2 ml) into the right hind foot pad. Rats in MSU groups were injected with endotoxin-free MSU crystal suspension (4 mg in 0.2 ml of sterile saline) into the right hind foot pad. Rat in MSU plus SAC group were given MSU injection plus i. p. injection of SAC (40 mg/kg bw) prepared in saline. Rat in MSU plus colchicine group were given MSU injection plus i. p. injection of colchicine (1 mg/kg bw) prepared in saline. The drugs were administered 1 h before the MSU crystal injection (single dose) and then once daily for next 3 days. Paw thickness of rats present in various groups was measured at different intervals for 3 days. Results are expressed as mean ± SD (n = 6 per group). *$P < 0.05$ vs. Control; #$P < 0.05$ vs. MSU injected group.

injected rat compared to control rat (Fig. 8A, B and 8C). However, SAC showed potent anti-inflammation property against MSU crystal toxicity by suppressing the marked induction of pro-inflammatory mediators in the serum compared to MSU crystal-injected rat. The anti-inflammatory action of SAC was equivalent to that of colchicine treatment.

4. Discussion

The potential of SAC as a preventive as well as curative agent against gout has been established from the results of the present study. First, SAC inhibited XO enzyme activity which may culminate the hyperuricemic condition thereby prevent the urate crystal deposition in the joint and can prevent the gout. Secondly, SAC showed antioxidative and anti-inflammatory effect against MSU crystal-induced inflammatory cascade thereby can be used as a potential treatment agent against gout.

Hyperuricemia is considered to be the most important risk factor for the onset of gout [2]. Inhibiting XO enzyme activity has been considered promising approach to treat hyperuricemia. SAC competitively inhibited bovine XO enzyme activity with inhibitor constant of 4 μg/ml (23.9 μM). Previous study has shown that allopurinol (standard known XO inhibitor) competitively inhibited bovine XO with inhibitor constant of 15.9 μg/ml (116.8 μM) [25]. These results indicate that SAC inhibited significantly the bovine milk XO activity in comparison to standard drug allopurinol. The kinetic analysis study of SAC inhibition on bovine XO enzyme activity was corroborated by the *in silico* analysis. The SAC oriented in the active site pocket of bovine XO protein and interacted with the amino acid residues present near the molybdenum center (involved in catalysis of the substrate). Arg880 and Glu802 play an important role in the hydroxylation of the XO substrate hypoxanthine. Nevertheless, SAC also formed hydrophobic interaction with amino acid such as Phe914 and Phe1009 which together involved in constraining the substrate to a well-defined plane relative to the molybdenum center [26]. RMSD and RMSF values further confirm the stability of the SAC-bovine XO enzyme complex. Altogether these results indubiously substantiate that SAC might hinder the binding of substrate in the active site pocket of bovine XO enzyme thereby inhibits its catalysis. The *in vitro* XO enzyme inhibitory property of SAC was mirrored in the *in vivo* silkworm model. The results from silkworm model evidently indicated that SAC can prevent gout through its ability to inhibit XO enzyme activity and reduce the uric acid production in *in vivo* conditions.

To further strengthen the therapeutic potential of SAC against gout, the *in vivo* efficacy of SAC against MSU crystal-induced gouty inflammation was determined. SAC treatment reduced the paw swelling induced by MSU crystal injection which indicates that SAC eased the severity of the inflammation which is comparable to that of the standard drug colchicine. ROS generation was considered an imperative factor for the activation of NLRP3 inflammasome by MSU engulfment. Corroborating the previous studies, the MSU crystal injection has increased the ROS and LPO content in the joint tissue of the rat [3,4]. SAC treatment has significantly reduced the

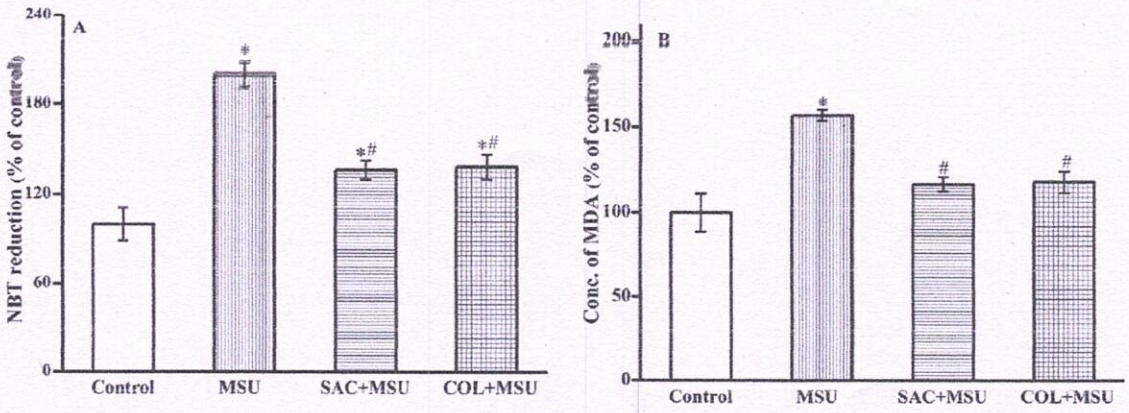

Fig. 5. Effect of SAC on ROS generation (A) and lipid peroxidation (B) in the ankle joints of MSU crystal-injected rat. Rats in control group were injected with sterile saline (0.2 ml) into the right hind foot pad. Rats in MSU groups were injected with endotoxin-free MSU crystal suspension (4 mg in 0.2 ml of sterile saline) into the right hind foot pad. Rat in MSU plus SAC group were given MSU injection plus i. p. injection of SAC (40 mg/kg bw) prepared in saline. Rat in MSU plus colchicine group were given MSU injection plus i. p. injection of colchicine (1 mg/kg bw) prepared in saline. The drugs were administered 1 h before the MSU crystal injection (single dose) and then once daily for next 3 days. At the end of the treatment, the animals from various groups were sacrificed and concentration of oxidative stress parameters (ROS and lipid peroxidation) in the ankle joint has been determined as described in Materials and Methods section. Results are expressed as % variation in comparison to control animals as mean ± SD (n = 6 per group). *$P < 0.05$ vs. Control; #$P < 0.05$ vs. MSU injected group. The 100% value of NBT reduction and MDA concentration corresponds to 0.146 ± 0.024 OD at 630 nm/mg tissue and 0.025 ± 0.006 nM/mg protein, respectively.

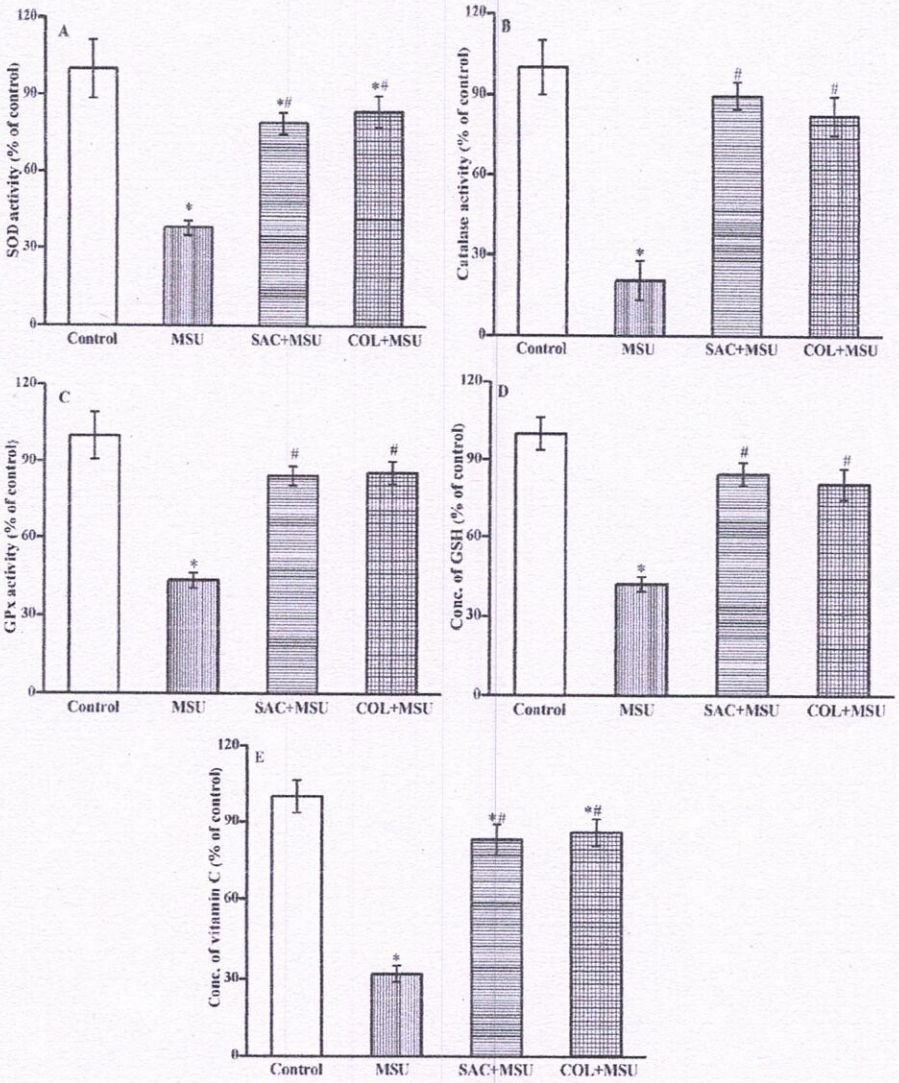

Fig. 6. Effect of SAC on SOD (A), catalase (B), GPx (C), GSH (D) and vitamin C (E) in the ankle joints of MSU crystal-injected rat. Rats in control group were injected with sterile saline (0.2 ml) into the right hind foot pad. Rats in MSU groups were injected with endotoxin-free MSU crystal suspension (4 mg in 0.2 ml of sterile saline) into the right hind foot pad. Rat in MSU plus SAC group were given MSU injection plus i. p. injection of SAC (40 mg/kg bw) prepared in saline. Rat in MSU plus colchicine group were given MSU injection plus i. p. injection of colchicine (1 mg/kg bw) prepared in saline. The drugs were administered 1 h before the MSU crystal injection (single dose) and then once daily for next 3 days. At the end of the treatment, the animals from various groups were sacrificed and concentration of antioxidants (SOD, catalase, GPx, GSH and vitamin C) in the ankle joint has been determined as described in Materials and Methods section. Results are expressed as % variation in comparison to control animals as mean ± SD (n = 6 per group). $^{*}P < 0.05$ vs. Control; $\#P < 0.05$ vs. MSU injected group. The 100% value of enzyme activities of SOD, catalase, GPx corresponds to 3.889 ± 0.361 U/mg of protein, 0.497 ± 0.037 kU/mg of protein and 2.578 ± 0.345 U/mg of protein, respectively. The 100% value of GSH and vitamin C concentration corresponds to 0.547 ± 0.233 nM/mg of protein and 8.363 ± 1.18 μg/mg of protein, respectively.

level of oxidative stress against MSU crystal injection. The nucleophilic nature of thiol group along with presence of allyl group in SAC are considered to be involved in the scavenging of superoxide anion, hydrogen peroxide, hydroxyl radical and peroxynitrite anion [5]. Hence, SAC in turn readily prevent the oxidation of lipid and protein. Further, SAC treatment has protected the level of enzymatic (SOD, catalase and GPx) and non-enzymatic (GSH and vitamin C) antioxidants against the MSU crystal injection. The molecular mechanism for such activity of SAC in MSU crystal-induced gout model has to be further delineated. Internalization of irritant particle has stimulated the mitochondria to produce ROS however the exact mechanism for such production has not been elucidated [27]. The mitochondrial complex I and III are thought to be major sites for the production of ROS [28]. Unprecedented production of ROS at these sites in turn causes oxidative damage to the proteins forming OXPHOS complexes. Hence, the mitochondrial oxidative stress and mitochondrial OXPHOS function are interrelated. MSU crystal injection has reduced the mitochondrial complex I and III activity which might be due to the increased oxidative stress and indirectly shows the damage caused to mitochondria. It is important to consider the fact that oxidized DNA released from damaged mitochondria can directly activate the NLRP3 inflammasome [29]. Mitochondrial dysfunction might be origin or outcome of MSU crystal-induced toxicity. Altogether, oxidative stress, mitochondrial dysfunction and NLRP3 inflammasome activation are interconnected under MSU crystal injection-induced inflammation. On the other hand, SAC treatment has protected the mitochondrial complex activities against MSU crystal-induced dysfunction. Such a protective effect of SAC on mitochondrial function might be related to its antioxidative effect. However, previous studies have shown that SAC has direct positive effect on mitochondrial function and mitochondrial antioxidant

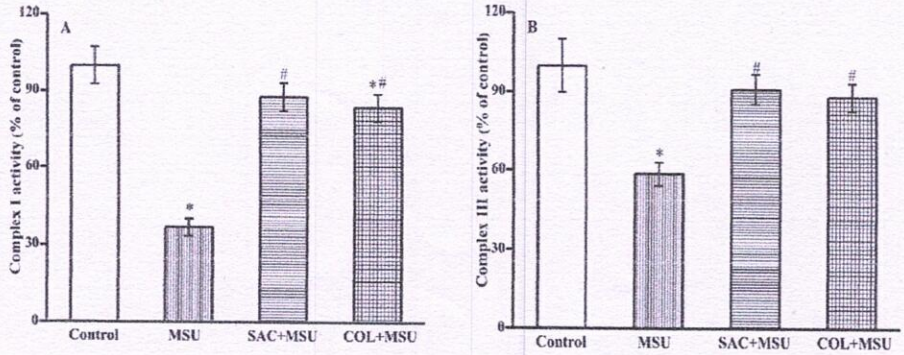

Fig. 7. Effect of SAC on mitochondrial OXPHOS Complex I (A) and Complex III (B) activity in the ankle joints of MSU crystal-injected rat. Rats in control group were injected with sterile saline (0.2 ml) into the right hind foot pad. Rats in MSU groups were injected with endotoxin-free MSU crystal suspension (4 mg in 0.2 ml of sterile saline) into the right hind foot pad. Rat in MSU plus SAC group were given MSU injection plus i. p. injection of SAC (40 mg/kg bw) prepared in saline. Rat in MSU plus colchicine group were given MSU injection plus i. p. injection of colchicine (1 mg/kg bw) prepared in saline. The drugs were administered 1 h before the MSU crystal injection (single dose) and then once daily for next 3 days. At the end of the treatment, the animals from various groups were sacrificed and mitochondrial OXPHOS enzyme activities in the ankle joints have been determined as described in Materials and Methods section. Results are expressed as % variation in comparison to control animals as mean ± SD (n = 6 per group). *$P < 0.05$ vs. Control; #$P < 0.05$ vs. MSU injected group. The 100% value of Complex I and complex III activity corresponds to 0.924 ± 0.051 μM/min/mg and 1.020 ± 0.102 μM/min/mg, respectively.

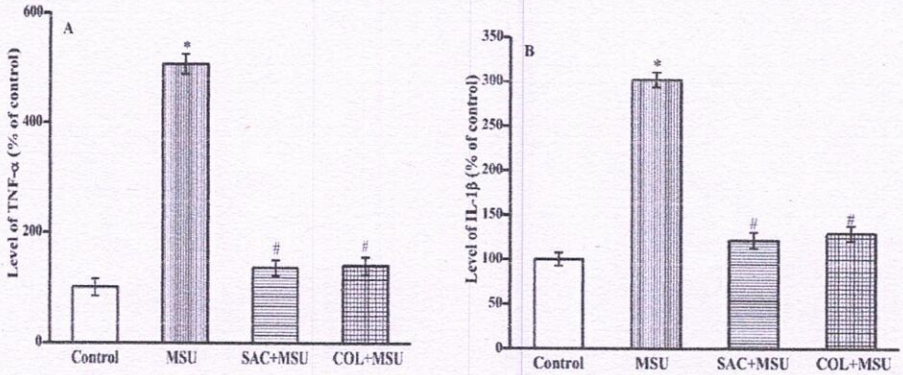

Fig. 8. Effect of SAC on pro-inflammatory mediators such as TNF-α (A) and IL-1β (B) in the serum of MSU crystal-injected rat. Rats in control group were injected with sterile saline (0.2 ml) into the right hind foot pad. Rats in MSU groups were injected with endotoxin-free MSU crystal suspension (4 mg in 0.2 ml of sterile saline) into the right hind foot pad. Rat in MSU plus SAC group were given MSU injection plus i. p. injection of SAC (40 mg/kg bw) prepared in saline. Rat in MSU plus colchicine group were given MSU injection plus i. p. injection of colchicine (1 mg/kg bw) prepared in saline. The drugs were administered 1 h before the MSU crystal injection (single dose) and then once daily for next 3 days. At the end of the treatment, the animals from various groups were sacrificed and concentration of TNF-α and IL-1β in the serum has been determined as described in Materials and Methods section. Results are expressed as % variation in comparison to control animals as mean ± SD (n = 6 per group). *$P < 0.05$ vs. Control; #$P < 0.05$ vs. MSU injected group. The 100% value of TNF-α, and IL-1β corresponds to 0.2475 ± 0.001 and 1.246 ± 0.001 ng/ml, respectively.

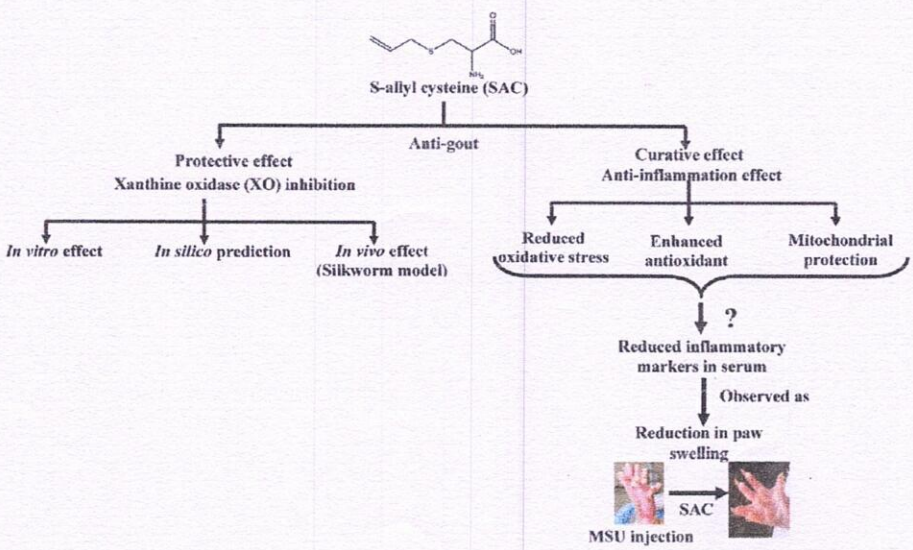

Fig. 9. Scheme showing the effect of SAC against gout. SAC through its XO inhibition property can act as a preventive agent of gout. XO enzyme inhibition by SAC has been established using *in vitro* (Bovine milk XO), *in silico* (Bovine milk XO) and *in vivo* (Silkworm model) analysis. In addition, SAC act as curative agent through reducing inflammatory markers in serum against MSU crystal-induced rat gout model. SAC by reducing oxidative stress, protecting antioxidant status and maintaining the mitochondrial function might have resulted in the reduction of inflammatory markers in the serum against MSU crystal-induced toxicity in rat. The curative effect is observed as reduction in paw swelling in rat against MSU crystal injection. However, the link between antioxidative property, mitochondrial protection and anti-inflammatory effect of SAC against gout model has to be delineated further.

status in myocardium and neuron against various other oxidative stresses. The improvement of antioxidant status and protection of mitochondrial function by SAC has cumulatively resulted in anti-inflammation which is observed as decreased level of TNF-α, and IL-1β in the serum of rats treated with SAC against MSU crystal injection-induced gout. The therapeutic value of SAC in reducing TNF-α and IL-1β level has been well established against various other toxicities such as gastric damage, diabetes-induced kidney damage and hepatotoxicity. SAC inhibits NF-κB transactivation which is a critical regulator of inflammation [5,30]. Such effect of SAC might also be involved in its anti-inflammatory effect against MSU crystal injection-induced gout.

5. Conclusion

The medicinal application of SAC as a preventive agent through its XO inhibitory property as well as curative agent through its anti-inflammatory property against gout has been understood from the present study (Fig. 9). SAC competitively inhibited the bovine XO enzyme activity by binding in the active site pocket of the enzyme *in vitro* which was substantiated by the *in silico* analysis and inhibition of XO enzyme activity *in vivo* in silkworm model. SAC showed antioxidant and mitochondrial function protective property. Also, a marked reduction of inflammatory markers in the serum against MSU crystal-induced rat gout model has been observed by SAC treatment. However, how such antioxidant property of SAC resulted in the reduction of inflammatory markers has to be further elucidated under gout condition. Also, molecular mechanism of SAC for such anti-gout property through modulating NLRP3 inflammasome or NF-κB transactivation has to be further delineated to completely understand the efficacy of the SAC against gout.

Conflicts of interest

All the Authors declare no conflict of interest.

Acknowledgments

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Appendix A. Supplementary data

Supplementary data related to this article can be found at https://doi.org/10.1016/j.biochi.2018.07.015.

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